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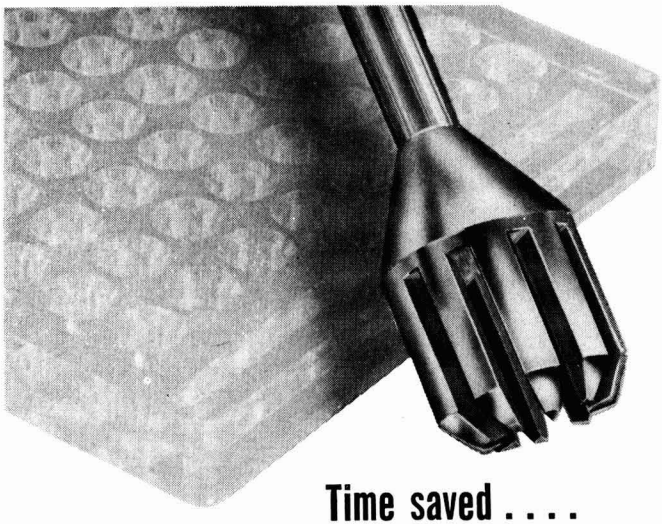

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Briefs

Methods for the Isolation and Characterization of Constituents of Natural Products. II. Separation of Homologous Series of Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone by Thin-Layer Chromatography. D. P. SCHWARTZ AND C. R. BREWINGTON, *Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 20250.*

Quantitative column and qualitative thin-layer chromatographic procedures are described for separating a mixture of the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of primary, secondary, and tertiary aliphatic alcohols into classes. Magnesium oxide is used as the adsorbent in both procedures and separation of the classes follows a similar pattern. The derivatives change color from yellow to violet or blue on the adsorbent. Tertiary alcohol derivatives above butyl show a blue color whereas tertiary butyl and the primary and secondary alcohol derivatives are violet. Isomeric derivatives can also be separated by thin-layer chromatography on aluminum oxide G by using a solvent system containing a strong organic base.

Microchem. J. **12**, 1 (1967).

Analysis of Compounds Containing the *p*-Nitroaniline Phosphor and Analogous Groups by Phosphorimetry and by Room-Temperature and Low-Temperature Fluorimetry. E. SAWICKI AND J. PFAFF, *Laboratory of Engineering and Physical Sciences, Division of Air Pollution, Robert A. Taft Sanitary Engineering Center, Public Health Service, U. S. Department of Health, Education, and Welfare Cincinnati, Ohio 45226.*

Since aromatic nitro compounds usually give poor absorption spectra and do not fluoresce, and since the nitrophenylhydrazines are useful colorimetric reagents for analysis of a wide variety of compounds, the phosphorimetric properties and the room-temperature and low-temperature fluorimetric properties of these types of compounds were investigated. Phosphorimetry and low-temperature and room-temperature fluorimetry are three powerful complementary tools, much more valuable when used together in trace analytical research than when used singly.

Microchem. J. **12**, 7 (1967).

Photometric Titrations of Nickel with Dimethylglyoxime. I. Titration in Colloidal Suspension. II. Titration Using a Two-Phase System. FOUAD G. NASOURI, SALAH A. F. SHAHINE, AND ROBERT J. MAGEE, *Department of Chemistry, The Queen's University, Belfast, Ireland.*

Two procedures have been developed for the photometric determination of nickel by titration with dimethylglyoxime solution. In one procedure, the nickel is titrated

by a colloidal suspension technique while, in the other, the titration is carried out in a two-phase system. Both methods are reproducible, accurate, and nickel can be determined in the presence of a number of associated elements or ions without interference.

Microchem. J. **12**, 26 (1967).

Specific Spectrophotometric Microdetermination of Beryllium. JOHN P. MCCLOSKEY, *Autonetics, A Division of North American Aviation, Inc., Anaheim, California.*

Direct quantitative determination of microgram or nanogram amounts of beryllium, in the presence of specified amounts of other elements, is described. Fluoride interferes to the extent that the method might be used for the determination of fluorine.

Microchem. J. **12**, 32 (1967).

Spectrophotometric Determination of Beryllium in Airborne Dust Samples. JOHN P. MCCLOSKEY, *Autonetics, A Division of North American Aviation, Inc., Anaheim, California.*

Ammonium aurintricarboxylate (Aluminon) is used to determine beryllium contents as low as 0.3 μg.

Microchem. J. **12**, 40 (1967).

Modified Microdetermination of Sulfate Ion: Its Application to Flask Combustion for Organic Sulfur. KEIICHIRO HOZUMI AND KOICHIRO UMEMOTO, *Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.*

Barium perchlorate titration in nonaqueous medium with arsenazo(III) as the indicator is employed together with back titration of excess barium perchlorate with standard sulfuric acid. Halogens (bromine or chlorine) may be determined simultaneously with sulfur by the method described.

Microchem. J. **12**, 46 (1967).

Use of the 1931 C.I.E. System of Color Measurement for the Quantitative Study of Spot Test Reactions. LEROY I. BRADDOCK, THOMAS J. PODLAS, AND NANCY MAREC, *Department of Chemistry, Seton Hall University, South Orange, New Jersey, and Department of Anesthesia, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.*

It has been shown that the 1931 C.I.E. system of color measurement can be used to determine color parameters of spot test precipitates, and these parameters can be related to changes in the reagents' concentrations as long as one also takes into consideration the adsorption isotherms of the reagents and the relationship of reagent concentration to precipitate particle size and, thus, to precipitate hiding power.

Microchem. J. **12**, 55 (1967).

Wet Chemical Analysis of Micro Amounts of Elements in Alloys. CARL TIEDCKE, AND LORNA PATOLSKI, *Laboratory of Microchemistry, Teaneck, New Jersey.*

The wet chemical analysis of micro amounts of elements in various alloys is described. Although this selection of alloys is for obvious reasons a limited one, it yet provides a good representation of those elements most often encountered in alloys as micro amounts. By choosing a course of analysis, which includes the removal of one or more of the main constituents prior to the analysis of the micro amounts, it is shown that the entire analysis can be carried out on a single sample weighing. The percentages obtained by wet chemical analysis are in good agreement with those obtained by physicochemical methods, and that both match the expected figures.

Microchem. J. **12**, 78 (1967).

Micro Measurement of Milk Fat. G. W. MOLNAR AND N. F. POOLE, *Research Laboratory, Veterans Administration Hospital, Kerrville, Texas.*

A modification of the Babcock method for the determination of fat in homogenized milk is presented. This includes the use of diluted sulfuric acid to prevent charring, and of iso-amyl alcohol to facilitate the rise of the fat in the neck of the Babcock bottle.

Microchem. J. **12**, 94 (1967).

Electrochemical Oxidation of Some Aromatic Amines in Acetonitrile Medium. I. *N,N*-dimethylaniline, Triphenylamine, Diphenylamine and Di-4-tolylamine. VLADIMÍR DVORÁK, IVAN NĚMEC, AND JAROSLAV ZÝKA, *Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia.*

The polarographic behavior of *N,N*-dimethylaniline, triphenylamine, diphenylamine, and di-4-tolylamine has been studied in acetonitrile medium on a rotating platinum electrode. It has been found possible to apply the oxidation waves of these substances to their polarographic determination, considering the water content of the solvent, temperature and acidity of the medium. Constant-current coulometric generation has been used to study the number of electrons exchanged in the reactions. The products formed in the electrode reaction are discussed.

Microchem. J. **12**, 99 (1967).

Improved Determination of Carbon and Fluorine in Highly Fluorinated Substances. P. B. OLSON AND R. E. KOLB, *Central Research Laboratories, Minnesota Mining and Manufacturing Company, St. Paul, Minnesota.*

Carbon is determined gravimetrically as carbon dioxide, and fluorine is determined acidimetrically as hydrofluoric acid. Improvements have been made in the method previously described by the senior author.

Microchem. J. **12**, 117 (1967).

Ion Specific Electrochemical Behavior of Macrotetrolides in Membranes. Z. ŠTEFANAC AND W. SIMON, *Department of Organic Chemistry, Swiss Federal Institute of Technology, Zürich, Switzerland.*

Electrochemical cells using macrotetrolides (nonactin homologs) on inert supports as membranes show a specificity for cations comparable to the ion specific behavior observed in the metabolism of rat liver mitochondria.

Microchem. J. **12**, 125 (1967).

MICROCHEMICAL JOURNAL 12, 1-6 (1967)

Methods for the Isolation and Characterization of Constituents of Natural Products. II.

Separation of Homologous Series of Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone by Thin-Layer Chromatography

D. P. SCHWARTZ AND C. R. BREWINGTON

Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C.

Received May 11, 1966

INTRODUCTION

In a previous report from this Laboratory (*1*), the preparation of homologous series of esters of primary, secondary, and tertiary aliphatic alcohols with pyruvic acid 2,6-dinitrophenylhydrazone was described. The present report concerns the separation of homologous series of these esters by thin-layer chromatography (TLC). Partition chromatography, utilizing an ultra-fine grade of Celite as a support, was selected as offering very mild conditions while still possessing high resolving power for the compounds. Both normal- and reversed-phase partition systems are described.

APPARATUS AND REAGENTS

Polyethylene glycol 400 was purchased from the J. T. Baker Company, Phillipsburg, New Jersey; Nujol (a brand of heavy mineral oil) was obtained from a local pharmacy; Micro-Cel T-38, an ultra-fine, synthetic, hydrous calcium silicate with an average particle size of 3.2 μ was obtained from the Johns-Manville Company, Baltimore, Maryland, and was dried 24 hours at 100°C before use; benzene, obtained from the Fisher Scientific Company, Silver Spring, Maryland; *n*-hexane (high purity grade) obtained from the Phillips Oil Company, Bartlesville, Oklahoma, and Acetonitrile (Baker) were redistilled. The TLC spreader was obtained from Research Specialties Company, Richmond, California. The mounting board was homemade, standard size, all aluminum; the de-

veloping tanks were standard, rectangular, high size and were purchased from the Brinkmann Company, Westbury, New York.

EXPERIMENTAL

Normal partition-chromatography. Polyethylene glycol 400 (12.5 ml) is dissolved in 60 ml of absolute alcohol in a 125-ml Erlenmeyer flask, and 15 g of Micro-Cel T-38 is added. The flask is stoppered and shaken vigorously by hand for 3-5 minutes and the slurry is spread over five 8 × 8-inch plates in the usual manner. The solvent is allowed to evaporate at room temperature until the odor of ethanol is absent and the plates are placed in a 100°C oven for an additional 5 minutes. Benzene solutions of the esters are spotted and the plate is developed with the mobile phase (hexane:benzene (85:15) saturated with the stationary phase) in an equilibrated tank lined with filter paper. Development time is approximately 45 minutes. The yellow spots may be changed to violet by placing the finished dry plate in a tank containing a wad of cotton wet with diethylamine. This procedure considerably increases the visual detection of the esters.

Reversed-phase chromatography. The reversed-phase thin-layer plates are prepared by slurrying 15 g of Micro-Cel T-38 in 90 ml of hexane and 5 ml of Nujol. The slurry is shaken for 3-5 minutes by hand and spread over four 8 × 10-inch plates. The plates are ready to use after standing approximately 15 minutes at room temperature. Benzene solutions of the esters are spotted at the origin. Approximately the top inch of the plate is scraped off and the plate is placed in the tank, scraped-side down, to equilibriate for approximately 1 hour. Care should be taken first to remove any Celite adhering to the bottom edge of the glass which might act as a wick conducting solvent from the paper lining the tank onto the main chromatographic surface. The plate is inverted after the equilibration period and developed with the mobile phase (acetonitrile:water (85:15)) for about 45 minutes. It was not found necessary to saturate the mobile phase with Nujol, highly satisfactory chromatograms being obtained without saturation.

RESULTS AND DISCUSSION

Figures 1-3 show the separations achieved for the esters of primary, secondary, and tertiary alcohols, respectively, in the normal partition system. The first 11 members of the primary alcohols are well separated. The C_{12} ester separates from the C_{11} but not from the C_{13} ester and is,

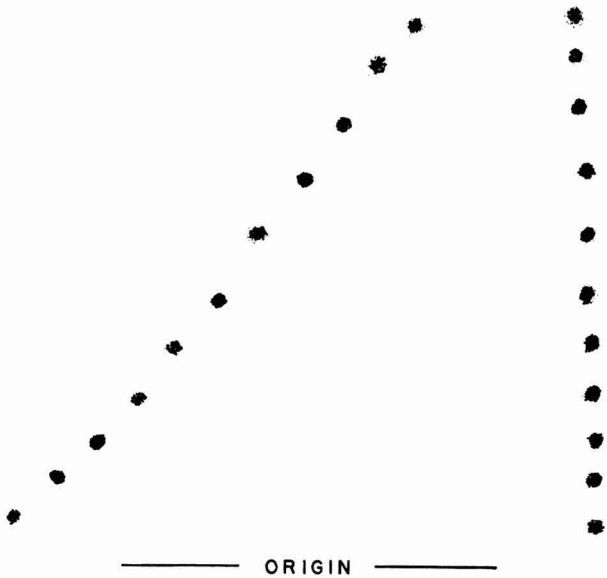

FIG. 1. Thin-layer partition chromatogram of esters of primary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_{11} through C_1 primary alcohols. Column on right represents mixture of all 11 alcohols.

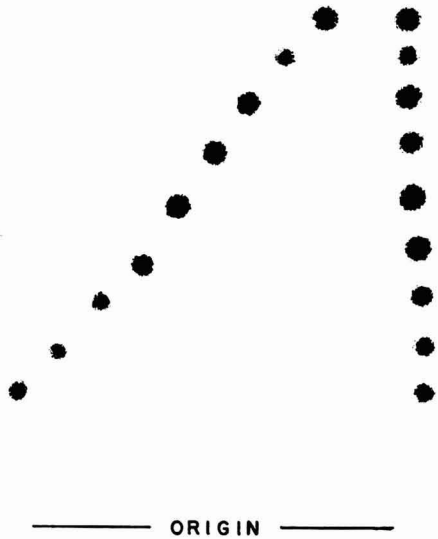

FIG. 2. Thin-layer partition chromatogram of esters of secondary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_{11} through C_3 secondary alcohols. Column on right represents mixture of all nine alcohols.

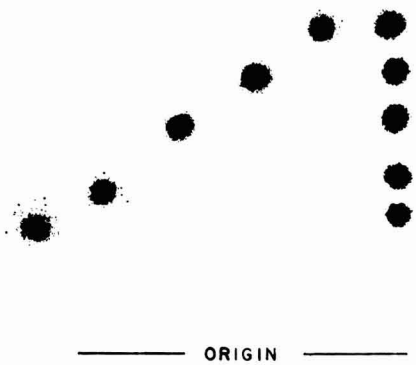

FIG. 3. Thin-layer partition chromatogram of esters of tertiary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_8 through C_4 tertiary alcohols. Column on right represents mixture of all five alcohols.

therefore, not included. The first nine members of the secondary alcohol esters (C_3 through C_{11}) separate satisfactorily as was expected from results with the primary alcohol esters. Only the C_4 through the C_8 tertiary alcohol esters were prepared and good separation of these was made.

Approximately 1.5×10^{-3} μmoles of an ester can be detected when one views the plate during chromatography. Exposure of the dry, finished plate to the vapors of diethylamine gives violet spots and increases the sensitivity about 15 times. Evaporation of the diethylamine from the plate restores the normal yellow color of the spots. This cycle can be repeated at will.

Figures 4 and 5 are reproductions of reversed-phase chromatoplates of the long-chain primary and secondary alcohol derivatives, respectively. The system separates the C_{12} through the C_{19} members and, thus, the normal partition and reversed-phase partition systems described will separate the entire homologous series of the alcohol derivatives prepared thus far in this Laboratory.

The merits of TLC have been well documented. The methods described here have been, in our hands, extremely useful. Plates are ready for use shortly after their preparation; the cost per plate is practically negligible; the layer produced is quite stable even though no binder is used; the stationary phase is uniformly distributed on the plate; the solvent front moves perfectly straight; the spots stay quite compact throughout the development.

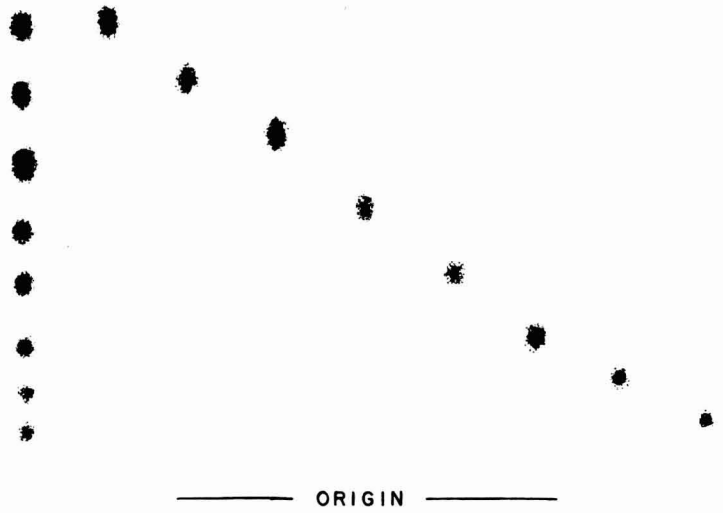

FIG. 4. Thin-layer reversed-phase partition chromatogram of esters of long-chain primary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from right to left C_{12} through C_{19} primary alcohols. Column on left represents mixture of all eight alcohols.

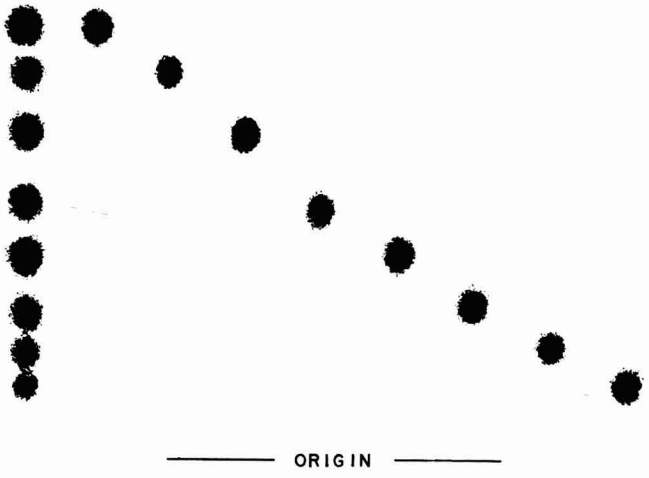

FIG. 5. Thin-layer reversed-phase partition chromatogram of esters of long-chain secondary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_{12} through C_{19} secondary alcohols. Column on left represents mixture of all eight alcohols.

SUMMARY

Esters of homologous series of primary, secondary and tertiary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone have been separated by thin-layer partition chromatography. The C_1 through C_{11} primary, the C_3 through C_{11} secondary and the C_4 through the C_8 tertiary alcohol derivatives are separated in the normal partition system employing polyethylene glycol as the stationary phase and hexane:benzene as the mobile phase. The C_{12} through C_{19} primary and secondary alcohol derivatives are separated by reversed-phase thin-layer chromatography using mineral oil as the stationary phase and acetonitrile-water as the mobile phase. Micro-Cel T-38 is used as the support in both systems. Approximately 1.0×10^{-4} μmoles of an ester can be detected on the plate when exposed to diethylamine vapor.

REFERENCE

1. SCHWARTZ, D. P., AND BREWINGTON, C. R., Methods for the isolation and characterization of constituents of natural products. I. Derivatives of alcohols with pyruvyl chloride 2,6-dinitrophenylhydrazone. *Microchem. J.* **11**, 430-436 (1966).

MICROCHEMICAL JOURNAL 12, 7-25 (1967)

Analysis of Compounds Containing the *p*-Nitroaniline Phosphor and Analogous Groups by Phosphorimetry and by Room-Temperature and Low-Temperature Fluorimetry

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Received May 20, 1966

INTRODUCTION

Spectrophotofluorometry, as compared to absorption spectrophotometry, has the advantage of greater selectivity. A disadvantage, however, is that fewer compounds are amenable to spectral analysis because of the large number of conjugated organic compounds that do not fluoresce. One reason for lack of fluorescence is the presence of electronegative groups in the molecule; another, at least with the highly conjugated molecules, is instrumental limitations. With the phototubes and fluorimeters commercially available fluorescence spectra can be obtained only within the 200 to 650 mμ region. The use of syntheses in fluorimetric analysis is relatively undeveloped because commercial spectrophotofluorometers have only recently become available.

Although low-temperature fluorimetric analysis has not yet been investigated, qualitative work indicates that it has considerable potentiality (*2*).

Very few phosphorimetric spectra, and thus, correlations between structure and spectra, are available, again because commercial phosphorimeters were introduced only within the last few years. Many compounds that fluoresce do not phosphoresce; many compounds absorbing at wavelengths longer than 380 mμ do not seem to phosphoresce. The application of organic syntheses to phosphorimetry is negligible. As in spectrophotofluorometry, phosphorimetric spectra can be obtained on commercial instruments only in the region from 200 to 650 mμ. Since so little is known about what types of compound will phosphoresce and what types will not, a search was conducted for fundamental groupings, common

to many phosphorescent molecules, from which a particular triplet-singlet electronic transition and its resultant phosphorescence emission band would be derived. This type of grouping would be called a phosphorogen.

With some knowledge of the spectral properties of phosphorogens, and of their isomers and derivatives and analogous compounds, methods of phosphorimetric analysis and room-temperature and low-temperature fluorimetric analysis could be more readily developed for compounds of interest to the investigator.

Previous work (*3*) indicated that compounds containing the *p*-nitroaniline group are definitely worth more thorough investigation. Some compounds containing the *p*-nitroaniline group are used as pesticides (*1*). Preliminary qualitative investigations of the reaction products of collected auto exhaust fumes with 4-nitrophenylhydrazine by low-temperature fluorescence and phosphorescence showed that aromatic carbonyl compounds were present. The paper-chromatographic analysis of phenols in automotive exhaust (by means of the derived nitrophenylazophenols, which can be considered at tautomers of nitrophenylhydrazones) indicated that these compounds fluoresce at low temperatures in acid solution. In addition, unknown compounds in the origin of the chromatogram showed this phenomenon even more strongly. Because of these various findings the fluorescence and phosphorescence properties of the 4-nitroaniline grouping were investigated.

REAGENTS AND APPARATUS[1]

Reagents

Most of the chemicals were obtained in the purest form from commercial sources and purified, where necessary, by recrystallization to a constant melting point. A few of the chemicals were synthesized by methods described in the literature and recrystallized from a variety of solvents to a sharp melting point. The solvents were obtained in the purest form and, where necessary, were distilled before use. EPA (ethyl ether-isopentane-ethanol, 5:5:2) was obtained from the Hartman Leddon Company, Philadelphia, Pennsylvania.

Apparatus

Phosphorimetric work was done with an Aminco-Keirs spectrophosphorimeter. Detailed directions are available for obtaining phosphores-

[1] Mention of commercial products does not constitute endorsement by the Public Health Service.

cence spectra directly from paper, glass-fiber paper, or thin-layer chromatograms (*3*). The same instrumentation was used in obtaining low-temperature fluorescence; the rotating shutter was removed, and the slits were arranged in the order 5222232. A phototube RCA type 1P 21 was used in all the fluorimetric and phosphorimetric work. Low-temperature fluorescence and phosphorimetric investigations were done at liquid-nitrogen temperatures.

Low-temperature fluorescence spectra were obtained by the same procedure used in obtaining phosphorescence spectra. The interference of the scatter peaks is a much greater problem than in phosphorimetry. To a large extent this problem arises because the low-temperature excitation and emission spectra are much closer together than they are in phosphorescence spectra. In examination of a solution in a cell, the meter multiplier should be set at readings of 0.03 or larger.

Obtaining low-temperature fluorescence spectra from paper is a little more difficult since the paper creates more scatter. The paper should be kept perpendicular to the light path. Although the sensitivity may be decreased somewhat, the intensity of the scatter peak will be decreased even more. As the distance is increased between the excitation and emission bands used to obtain the emission and excitation spectra, interference from the scatter peak diminishes. The entire excitation and emission spectra can be obtained, even though sensitivity may be decreased somewhat.

An Aminco-Bowman spectrophotofluorimeter with and without the solid-state attachment was used in obtaining fluorescence spectra at room temperature.

RESULTS AND DISCUSSION

Phosphorimetric Analysis of Nitro Compounds and Especially Those Containing the 4-Nitroaniline Phosphor

Table I lists a large group of phosphorescent nitro compounds. Almost all of these compounds can be determined in the nanogram to microgram range, as shown by their low determination limits. Most of these compounds contain the *p*-nitroaniline structure. Vinylogs of 4-nitroaniline, such as 2-amino-6-nitrobenzothiazole and 6-nitroindole, are also phosphorescent, as are the 3-nitrocarbazoles, which could be considered as containing a 4-nitroaniline grouping. In addition, a variety of nitroarenes were also found to be phosphorescent. Probably the most interesting finding was

TABLE I

PHOSPHORIMETRIC SPECTRA OF CONJUGATED NITRO COMPOUNDS IN EPA

Compound	Det. Limit, ng/0.1 ml	Concn., Molarity	Mean Lifetime, sec.	Exc. Spectra[a] λ max	MM.T	Emiss Spectra[a] λ max	MM.T
4-Nitroaniline	2	10^{-6}	0.6	260	0.02	510	0.17
				380	0.17	530	0.14
2-Amino-5-nitrobiphenyl	5	10^{-6}	0.56	275	0.01	520	0.04
				320	0.01	540	0.035
				380	0.04		
4-Nitro-o-toluidine	10	10^{-5}	0.53	255	0.01	520	0.23
				375	0.20	535s	0.20
2,6-Diethyl-4-nitroaniline	19	10^{-5}	0.66	265	0.02	525	0.15
				388	0.15	550s	0.11
2,6-Dichloro-4-nitroaniline	4	10^{-6}	0.47	265	0.01	510	0.10
				368	0.11	525	0.11
N-Methyl-4-nitroaniline	5	10^{-6}	0.5	~260	0.003	522	0.034
				390	0.03	590i	0.025
N,N-Dimethyl-4-nitroaniline	5	10^{-6}	0.54	~265	0.005	525	0.035
				398	0.035	550i	0.027
4-Nitro-1-naphthylamine	6000	10^{-3}		280	0.03	578	0.04
				330	0.02		
				400	0.04		
4-Nitrophenylhydrazine	3	10^{-6}	0.48	260	0.02	520	0.06
				390	0.06	540s	0.04

TABLE I (CONT.)

Compound	Det. Limit, ng/0.1 ml	Concn., Molarity	Mean Lifetime, sec.	Exc. Spectra [a] λ max	MM.T	Emiss Spectra [a] λ max	MM.T
Acetaldehyde 4-nitrophenylhydrazone	10	10^{-5}	0.48	255 390 405i	0.01 0.17 0.16	525 550s	0.22 0.15
Propionaldehyde 4-nitrophenylhydrazone	6	10^{-5}	0.50	255 395 408i	0.03 0.35 0.30	525 550s	0.38 0.28
Acetone 4-nitrophenylhydrazone	10	10^{-5}	0.48	255 392 405i	0.02 0.22 0.20	525 555s	0.23 0.16
Benzophenone 4-nitrophenylhydrazone	200	10^{-4}		250 350i 365	0.01 0.10 0.12	515 530s	0.10 0.09
4-Benzoylbiphenyl 4-nitrophenylhydrazone	40	10^{-5}	0.38	250 370	0.005 0.066	520 540s	0.063 0.06
2-Nitro-N-methylcarbazole	280	4×10^{-5}		260 290 345 400	0.075 0.055 0.17 0.055	530 550s	0.17 0.15
3-Nitro-N-methylcarbazole	20	10^{-5}	0.39	235 275 310 385	0.09 0.37 0.39 0.59	480 510 540s	0.53 0.67 0.33
3-Nitro-N-ethylcarbazole	1	10^{-6}	0.37	235 280 315 385	0.08 0.21 0.20 0.40	475 504 534s	0.18 0.22 0.11

TABLE I (CONT.)

Compound	Det. Limit, ng/0.1 ml	Concn., Molarity	Mean Lifetime, sec.	Exc. Spectra λ max[a]	MM.T	Emiss Spectra λ max[a]	MM.T
2-Amino-7-nitrofluorene	450	10^{-4}	0.38	245	0.01	485	0.12
				340	0.18	520	0.18
						550s	0.08
2-Amino-6-nitro-benzothiazole	10	10^{-5}		265	0.03	515	0.40
				375	0.40	525s	0.39
6-Nitroindole	8	10^{-5}	0.41	255	0.10	498	0.35
				325	0.38	520	0.39
				350	0.37	552i	0.19
				372	0.39		
4-Nitrobiphenyl	20	10^{-5}		240	0.02	480	0.13
				330	0.13	515	0.13
						545s	0.05
2-Nitrofluorene	4	10^{-6}	0.40	245	0.009	485	0.056
				340	0.047	517	0.058
						547s	0.022
1-Nitronaphthalene	150	10^{-4}		255	0.05	520	0.15
				340	0.16	532	0.11
2-Nitronaphthalene	15	10^{-5}	0.36	260	0.23	500	0.22
				308	0.17	538	0.15
				350	0.07	572	0.05
				365i	0.06		
5-Nitroacenaphthene	50	10^{-5}		268	0.034	505	0.024
				310	0.017	540	0.056
				360i	0.045	575	0.028
				380	0.054		
				395i	0.043		

TABLE I (CONT.)

Compound	Det. Limit, ng/0.1 ml	Concn., Molarity	Mean Lifetime, sec.	Exc. Spectra λ max[a]	MM.T	Emiss Spectra λ max[a]	MM.T
9-Nitroanthracene	13	10^{-5}		248	0.19	452	0.10
				270s	0.11	488	0.19
				322	0.10	530	0.08
						565s	0.015
1-Nitroanthraquinone	25	10^{-5}	~0.28	250	0.27	460	0.12
				270	0.15	490	0.28
				325	0.12	530	0.12

[a]Italicized values are emission (excitation) wavelength maxima at which excitation (emission) spectra are obtained.

s=shoulder.

i=inflection.

that 4-nitrophenylhydrazine and its aliphatic aldehyde and ketone derivatives were intensely phosphorescent. Although the few investigated aromatic carbonyl 4-nitrophenylhydrazones appear to be nonphosphorescent (Fig. 1), two benzophenone 4-nitrophenylhydrazones were found to be phosphorescent, apparently because of the steric hindrance at the benzophenone end of the molecule. Extended extra-conjugation at the electron donor end of the 4-nitroaniline molecule apparently destroys the phosphorescence, as shown by the 4-nitrophenylhydrazones of benzaldehyde,

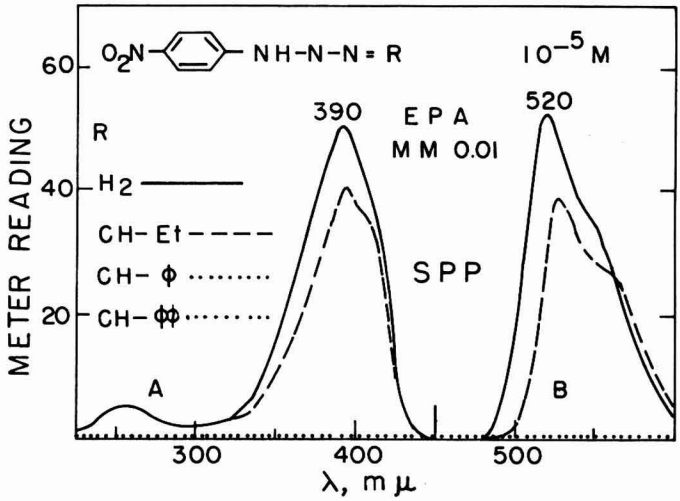

FIG. 1. Phosphorimetric spectra of 4-nitrophenylhydrazine (——) and the 4-nitrophenylhydrazone of propionaldehyde (- - -) in EPA at a concentration of 10^{-5} *M*. The 4-nitrophenylhydrazones of benzaldehyde and 2-naphthaldehyde are nonphosphorescent.

acetophenone, and naphthaldehyde. In the benzophenone 4-nitrophenylhydrazones the extraconjugation of the $C_6H_5C = N$-group is decreased by the steric effect of the extra benzene ring. Thus these benzophenone hydrazones are phosphorescent.

The phosphorescence of 4-nitroaniline was decreased considerably when the amino group was acylated. A few compounds that contain a 4-nitroaniline group substituted with an extra nitro or amino group or that show points of resemblance to this group were nonphosphorescent under the experimental conditions (Table II).

Although much work in the colorimetric analysis of aldehydes, ketones, and acids has been performed with 2,4-dinitrophenylhydrazine, the com-

pounds obtained with 4-nitrophenylhydrazine absorb at longer wavelengths with greater intensity in neutral and alkaline solution. In addition, the 4-nitrophenylhydrazones are phosphorescent or fluorescent. Since the 2,4-dinitrophenylhydrazones do not have the latter properties, the use of 4-nitrophenylhydrazine in the analysis of aldehydes, ketones, and acids is recommended. This reagent appears to be the reagent of choice for investigations of the composition of automotive exhaust fumes and airborne particulates in terms of aromatic carbonyl compounds.

TABLE II

Compounds nonphosphorescent in EPA solution
Acetone 2,4-dinitrophenylhydrazone
Aliphatic aldehyde 2,4-dinitrophenylhydrazones
Aromatic aldehyde 4-nitrophenylhydrazones
Benzaldehyde 2,4-dinitrophenylhydrazone
Benzaldehyde 2-nitrophenylhydrazone
Benzaldehyde 3-nitrophenylhydrazone
N,N-Dimethyl 4-nitrosoaniline
2,3-Dinitroaniline
2,4-Dinitroaniline
2,4-Dinitrophenylhydrazine
o-Nitroaniline
m-Nitroaniline
4-Nitrobenzaldehyde hydrazone
4-Nitrobenzaldehyde phenylhydrazone
Nitro-1,4-phenylenediamine
4-Nitro-1,2-phenylenediamine
2-Nitro-4-toluidine
N-Perfluorobutyryl 4-nitroaniline
4-Phenylazoaniline

The half-life for phosphorescence was also investigated. For most of the compounds containing the 4-nitroaniline grouping the mean lifetime ranged from 0.47 to 0.66 second. For the investigated nitroarenes the mean lifetime was around 0.36 to 0.41 second. The mean lifetime of the 4-nitronanilines is a valuable analytical characteristic in that it differs markedly from the mean lifetime of many other types of compounds. For example, mean lifetimes of the following compounds were strikingly different: 10-thioxanthenone 0.28, 9-xanthenone 0.31, 4′-aminoacetophenone 1.17, 2-naphthylamine 1.7, phenazine 2.2, carbazole 8.6, and triphenylene 15 seconds.

Fluorimetric Analysis of Nitro Compounds

Substitution of a nitro group into a fluorescent molecule usually causes a loss of fluorescence either through a radiationless transition of the absorbed energy to the ground state (i.e., no fluorescence or phosphorescence) or possibly through an intersystem crossing of the absorbed energy from the excited singlet state to the excited triplet state followed by the emission of light energy and the return of the molecule to the ground state (i.e., phosphorescence).

Phosphorescence is shown by compounds such as 4-nitroaniline and 4-nitrophenylhydrazine. However, if extraconjugation is introduced at the electron-donor end of the molecule, as in benzaldehyde 4-nitrophenylhydrazone, phosphorescence is lost and the molecule becomes fluorescent in solvents of low dielectric constant. Examples of 4-nitrophenylhydrazones of this type are given in Table III; these compounds are fluorescent in dioxane and nonfluorescent in alcohol. 2-Naphthaldehyde 4-nitrophenylhydrazone shows this solvent effect. In solvents of high dielectric constant it is nonfluorescent, e.g., formamide-109.5, methanol-32.6, acetone-20.7, *n*-butanol-17.1, and pyridine-12.3. In a solvent of moderate dielectric constant, such as acetic acid-6.2, a light green fluorescence is found; in solvents of low dielectric constant the fluorescence is much more intense, e.g., *o*-dichlorobenzene, 2.40; toluene, 2.38; and dioxane, 2.21. Only a thorough fluorimetric study could determine whether there is a definite relation between fluorescence intensity of these molecules and the dielectric constant of the solvent.

This solvent effect is different from that obtained with aromatic carbonyl compounds, e.g., 1-formylpyrene, which is nonfluorescent in dioxane and fluorescent in alcohol. For these compounds no simple relation can be found between the dielectric constant and the fluorescence intensity, e.g., 1-formylpyrene is nonfluorescent in dimethylformamide and in dioxane but is fluorescent in acetic acid and methanol. Here, intermolecular hydrogen bonding seems to be important. A third type of fluorescent molecule is fluorescent in both dioxane and alcohol, Table III.

A fourth type is also shown in Table III. These are mainly nitrophenylhydrazones that are not fluorescent in dioxane or alcohol: the aliphatic aldehyde and ketone 4-nitrophenylhydrazones, the benzophenone 4-nitrophenylhydrazones, some 2- and 3-nitrophenylhydrazones, the 2,4-dinitrophenylhydrazones and some phenylhydrazones. Strangely enough, although benzaldehyde phenylhydrazone is nonfluorescent in dioxane or alcohol, the

TABLE III

SOLVENT EFFECT ON THE FLUORESCENCE
OF 4-NITROPHENYLHYDRAZONES AND OTHER COMPOUNDS

4-Nitrophenylhydrazones	Fluorescence Color[a] Dioxane	Alcohol
Benzaldehyde	B	
Cinnamaldehyde	G	
N,N-Diethyl-4-aminobenzaldehyde	Y	
3,4-Dimethoxybenzaldehyde	G	
N,N-Dimethyl-4-aminobenzaldehyde	O	
N,N-Dimethyl-4-aminocinnamaldehyde	O	
4-Hydroxy-3-methoxybenzaldehyde	BG	
Indole-3-aldehyde	G	
1-Naphthaldehyde	G	
2-Naphthaldehyde	G	
4-Nitrobenzaldehyde	mG	
Piperonal	G	
Acetophenone	B	
4-Acetylbiphenyl	G	
2-Acetylfluorene	G	
3-Acetylphenanthrene	G	
MISCELLANEOUS COMPOUNDS		
2-Amino-7-nitrofluorene	G	
N,N-Dimethyl-4-amino-4'-nitrostilbene	O	
4-Nitrobenzaldehyde 2-benzothiazolylhydrazone	GY	
2-Nitro-9-methylcarbazole	G	

TABLE III (CONT.)

	Fluorescence Color[a]	
	Dioxane	Alcohol
1-Acetylpyrene	———	B
7H-Benz(de)anthracen-7-one	———	B
1-Benzoylpyrene	———	lB
Dianisalacetone	———	mG
7H-Dibenzo(c,h)xanthen-7-one	———	B
Dipiperonalacetone	———	GY
3-Formyl-9-ethylcarbazole	lB	B
1-Formylpyrene	———	B
1-Hydrazinoanthraquinone	———	GB
Piperonalacetone	———	B
Thiaxanthen-9-one	———	B
7-Acetyl-2-aminofluorene	B	Y
1-Aminoanthracene	BG	YG
2-Aminoanthracene	B	YG
1-Aminoanthraquinone	Y	mRO
7-Aminobenz(a)anthracene	B	B
1-Aminopyrene	B	B
Benz(a)anthracene	B	B
4H-Benzo(def)carbazole	PB	PB
Benzo(a)pyrene	B	B
Dibenz(a,h)acridine	B	B
Dibenz(a,j)acridine	B	B
1-Hydroxyanthracene	B	B
6-Hydroxychrysene	B	B
Pyrenoline	B	B

TABLE III (CONT.)

[a] B=blue; G=green; l=light; m=moderate; P=purple; O=orange; R=red; Y=yellow; and ——— = under long-wavelength ultraviolet light little or no fluorescence visible to the naked eye. The following compounds are, at the most, weakly fluorescent:

1,2-Acenaphthenequinone 4-nitrophenylhydrazone, Acetaldehyde 4-nitrophenylhydrazone, Aliphatic aldehyde 2,4-dinitrophenylhydrazones[b], Benzaldehyde 2,4-dinitrophenylhydrazone, Benzaldehyde 2-nitrophenylhydrazone, Benzaldehyde 3-nitrophenylhydrazone, Benzaldehyde phenylhydrazone, Benzophenone 4-nitrophenylhydrazone, 4-Benzoylbiphenyl 4-nitrophenylhydrazone, 1-Naphthaldehyde 2,4-dinitrophenylhydrazone, 1-Naphthaldehyde 2-nitrophenylhydrazone, 1-Naphthaldehyde 3-nitrophenylhydrazone, 1-Naphthaldehyde phenylhydrazone, 4-(p-Nitrophenylazo)-1-naphthol, 4-(4'-Nitrophenylazo)phenol, 4-Nitrophenylhydrazine, Propionaldehyde 4-nitrophenylhydrazone, and 2-Pyridinealdehyde 4-nitrophenylhydrazone

[b] These include derivatives of formaldehyde, acetaldehyde, propionaldehyde, and butyraldehyde. As a rule most of the aliphatic 4-nitrophenylhydrazones were phosphorescent in solution. The 4-nitrophenylhydrazones that were fluorescent in dioxane solution were usually nonphosphorescent in solution.

substitution of a nitro group para to the NH grouping (e.g., benzaldehyde 4-nitrophenylhydrazone) confers fluorescence on the molecule in dioxane.

Low Temperature Fluorimetric Analysis

It is well-known that decreasing the temperature of measurement can cause intensification of fluorescence and an increase in the spectral fine structure. An example of this phenomenon is seen in the fluorescence emission spectra of 7H-benz(de)anthracen-7-one in Fig. 2. 2-Naphthaldehyde 4-nitrophenylhydrazone shows this phenomenon in both its fluorescence excitation and emission spectra (Fig. 3). Much more fine structure is

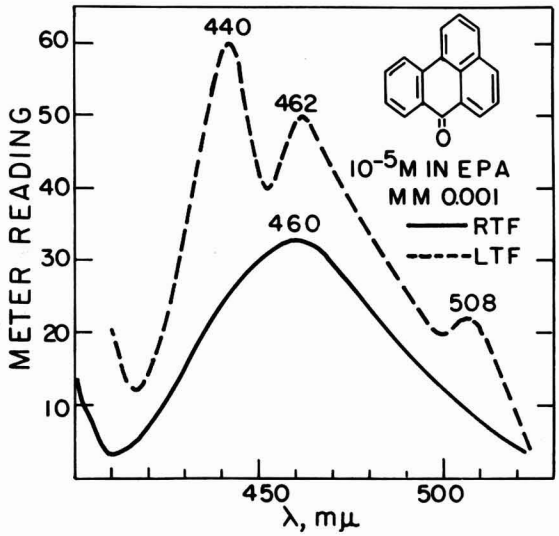

FIG. 2. Fluorimetric emission spectra of 7H-benz(de)anthracen-7-one (10^{-5} *M*) in EPA at room temperature (——) (and at liquid-nitrogen temperatures (– – –).

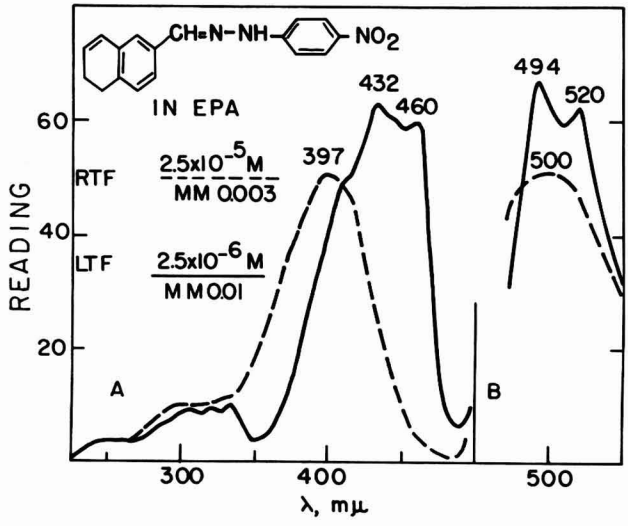

FIG. 3. Fluorescence excitation (A) and emission (B) spectra of 2-naphthaldehyde 4-nitrophenylhydrazone in EPA at room temperature (– – –) and F 397/500 and at liquid-nitrogen temperature and F 432/520 (——).

shown at liquid-nitrogen temperatures. In addition the spectra in liquid nitrogen are much more intense.

An unexplored aspect of low-temperature fluorimetric analysis is that some compounds that are neither fluorescent at room temperature nor phosphorescent at low temperatures can be made to fluoresce at low temperatures. Some examples of this phenomenon are seen in Table IV. Thus, benzaldehyde 4-nitrophenylhydrazone is nonfluorescent and nonphos-

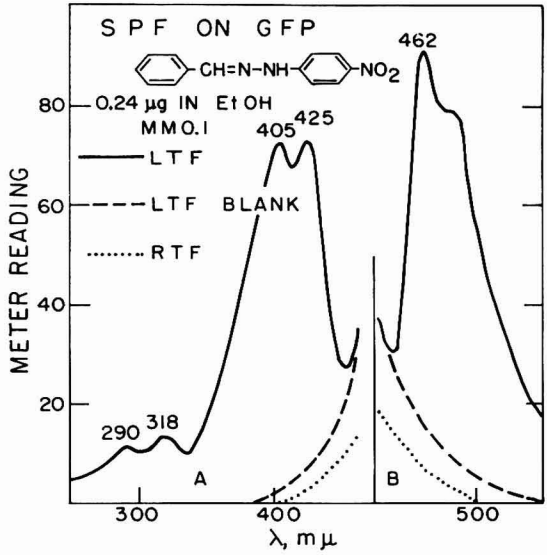

FIG. 4. Fluorimetric excitation (A) and emission (B) spectra of benzaldehyde 4-nitrophenylhydrazone at liquid-nitrogen temperatures (——); at room temperature (···). Concentration: 0.24 µg in 10 µl of 95% ethanol on glass fiber paper. Blank at liquid-nitrogen temperatures (- - -). F 405/480.

phorescent in alcohol, but is fluorescent in alcohol at liquid-nitrogen temperatures, Fig. 4. The type of spectra obtained is characteristic of the benzaldehyde and acetophenone 4-nitrophenylhydrazones.

The azo group resembles the nitro group in its electronegativity and its quenching effect on fluorescence. Examples of azo compounds (of potential value in the analysis of amines and phenols), which neither fluoresce at room temperature nor phosphoresce at low temperatures but do show low-temperature fluorescence, are given in Table IV. 4-Aminoazobenzene has been chosen as a representative of this class of compounds that show low-temperature fluorescence in trifluoroacetic acid solution.

TABLE IV

LOW-TEMPERATURE FLUORIMETRIC SPECTRA OF
4-NITROPHENYLHYDRAZONES AND OTHER COMPOUNDS

Compound[a]	Detm. Limit, ng	Solvent[b]	Concn., µg or (Molarity)	Exc. Spectra λ^cmax	MM.T	Emiss. Spectra λ^cmax	MM.T
Acetophenone 4-nitrophenyl-hydrazone	25	EPA(GFP)	0.26	290	0.40	455	4.0
				310	0.40	490s	3.7
				400	3.5		
				422	3.5		
	25	EPA(Cell)	(10^{-5})	270s	0.24	460	0.4
				290	0.30	480	0.4
				310	0.30	510s	0.2
				405	3.3		
				430	3.2		
	25	A(GFP)	0.26	290	1.8	465	20.
				310	1.8	488	19.
				400	18.		
				420	17.		
	25	D(GFP)	0.13	285	0.1	470	4.3
				315	0.2	490s	4.0
				400	4.0		
				425	4.0		
	25	D(Cell) RTF	(10^{-5})	288	0.02	472	0.2
				390	0.2		
4-Aminoazobenzene	400	EPA-5%TFA (Cell)	(10^{-4})	465	0.65	545	0.72
				490	0.75	580	0.28
				505s	0.64		
Benzaldehyde 2-benzothiazolyl-hydrazone	70	EPA(Cell)	(10^{-5})	250	0.7	410	3.6
				280	0.5	430	3.8
				360	3.7		
				380	3.3		

TABLE IV (CONT.)

Compound[a]	Detm. Limit, ng	Solvent[b]	Concn. μg or (Molarity)	Exc. Spectra λ^cmax	MM.T	Emiss. Spectra λ^cmax	MM.T
Benzaldehyde 4-nitrophenylhydrazone	10	A(GFP)	0.024	270 290 320 405 425	0.1 0.1 0.1 1.6 1.8	460 480	2.2 1.6
	80	EPA(Cell)	(10^{-5})	265 295 325 410 430	0.13 0.24 0.28 3.0 2.9	450 470	3.5 3.0
	80	D(GFP)	0.24	270s 290 325 405 430	0.3 0.6 0.8 5.4 5.2	492	6.2
	60	D(Cell) RTF	(10^{-5})	290s 390[d]	0.004 0.12	465	0.05
Cinnamaldehyde 4-nitrophenylhydrazone	50	EPA(Cell)	(10^{-5})	300 340 355 417s 436	0.4 0.6 0.6 3.8 4.7	470 500s	4.5 3.4
N,N-Dimethyl 4-aminoazobenzene	4000	EPA-5%TFA (Cell)	(10^{-3})	480 525	0.55 <0.9	575 605s	0.95 0.2
2-Naphthaldehyde 4-nitrophenylhydrazone	7	EPA(Cell)	(2.5×10^{-6})	250 285s 305 320 335	0.04 0.07 0.09 0.09 0.09	494 520	0.67 0.62

TABLE IV (CONT.)

Compound[a]	Detm. Limit, ng	Solvent[b]	Concn. μg or (Molarity)	Exc. Spectra λ^cmax	MM.T	Emiss. Spectra λ^cmax	MM.T
				410s	0.50		
				432	0.63		
				460	0.60		
	150	EPA(Cell)	(2.5×10^{-5})	250	0.01	500br[e]	0.15
				300	0.03		
				325s	0.032		
		RTF		400	0.15		
				407s	0.14		
4-(4'-Nitrophenylazo) -1-naphthol	70	EPA-DMF (3:1) (GFP)	1.4	290	1.5	515	29.
				350	5.		
				460	26.		
				480	26.		
9-Phenylazo-10-anthrol	30	EPA(GFP)	(10^{-4})	300	1.1	515	6.6
				350	1.8		
				460	6.5		

[a] These compounds were nonphosphorescent in the described solvents under the described instrumental conditions.

[b] A = 95% ethanol; DMF = dimethylformamide; D = dioxane; EPA = a mixture of diethyl ether, isopentane, and ethanol in volume ratio of 5:5:2; TFA = trifluoracetic acid; Cell = spectra obtained from 0.1-ml solution in a cell; GFP = spectra obtained from 0.01-ml solution on glass-fiber paper; and RTF = room-temperature fluorescence spectra.

[c] Italicized values are emission (excitation) wavelength maxima at which excitation (emission) spectra were obtained.

[d] Wavelength maximum changes with concentration.

[e] br = broad band.

Many phenylazophenols are also fluorescent at liquid-nitrogen temperatures in sulfuric acid solution.

Two examples of another type of nonfluorescent nonphosphorescent azo compound that fluoresces at liquid-nitrogen temperatures are 9-phenylazo-10-anthrol and 4-(4′-nitrophenylazo)-1-naphthol. The latter compound is in equilibrium with its tautomer, 1,4-naphthoquinone 4-nitrophenylhydrazone, in solution.

The data in Table 4 indicate that low-temperature fluorimetric analysis is definitely a useful analytical tool that could complement phosphorimetric analysis and room-temperature fluorimetric analysis.

SUMMARY

Since aromatic nitro compounds usually give poor absorption spectra and do not fluoresce, and since the nitrophenylhydrazines are useful colorimetric reagents for analysis of a wide variety of compounds, the phosphorimetric properties and the room-temperature and low-temperature fluorimetric properties of these types of compounds were investigated. Results showed that 4-nitrophenylhydrazine could be a valuable reagent for the analysis of aromatic carbonyl compounds present in auto exhaust fumes and that low-temperature fluorimetric trace analysis is a tool well worth exploiting.

Most of the aliphatic aldehyde and ketone 4-nitrophenylhydrazones are nonfluorescent but strongly phosphorescent, whereas many of the aromatic aldehyde and ketone 4-nitrophenylhydrazones are nonphosphorescent but strongly fluorescent in solvents of low dielectric constant and are highly fluorescent in all types of solvents at liquid-nitrogen temperatures.

Examples are given of compounds that are neither fluorescent at room temperature nor phosphorescent but are intensely fluorescent at liquid-nitrogen temperatures. Phosphorimetry and low-temperature and room-temperature fluorimetry are three powerful complementary tools, much more valuable when used together in trace analytical research than when used singly.

REFERENCES

1. Roburn, J., Colorimetric determination of 2,6-dichloro-4-nitroaniline in plants and soils. *J. Sci. Food Agr.* **12**, 766-772 (1961).
2. Sawicki, E., and Johnson, H., Characterization of aromatic compounds by low-temperature fluorescence and phosphorescence. Application to air pollution studies. *Microchem. J.* **8**, 85-101 (1964).
3. Sawicki, E., and Pfaff, J., Analysis for aromatic compounds on paper and thin-layer chromatograms by spectrophotophosphorimetry. Application to air pollution. *Anal. Chim. Acta* **32**, 521-534 (1965).

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Photometric Titrations of Nickel with Dimethylglyoxime

I. Titration in Colloidal Suspension
II. Titration Using a Two-Phase System

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INTRODUCTION

In an earlier paper (*1*), two of the above authors described the use of colloidal suspension spectrophotometry for the colorimetric determination of nickel with α-benzildioxime, and recently, the present authors introduced a two-phase photometric method for the titration of Gold (*2*).

Bobtelsky and Welwart (*3, 4*) used dimethylglyoxime for the heterometric titration of nickel. In the present work two methods were developed; one using the colloidal suspension technique, and the second by titrating photometrically in a two-phase system. The investigations carried out and the results obtained are described in the following pages.

EXPERIMENTAL

Apparatus and Reagents

An EEL Photometric Titrator was used, titrations being carried out in cells of 40-ml capacity that were supplied with the titrator.

A 2-ml microburette was employed for titrations.

Chloroform, AnalaR Grade.

Gelatine, 0.25% solution, Gum Arabic, 2% solution and Agar-Agar, 0.25% solution were used, being freshly prepared as required.

Standard Nickel Solution

A 0.05*M* nickel solution was prepared from AnalaR Grade $NiSo_4{}^{aq}$ (Hopkins and Williams, Ltd.) and standardized with EDTA. From this solution 0.005 and 0.0005*M* solutions were prepared by dilution.

Standard Dimethylglyoxime

A 250 ml solution of 0.1*M* dimethylglyoxime were prepared by dissolving the appropriate amount of the AnalaR Grade reagent in 50 ml of 1*M* NaOH and making up to volume with water. From this solution a 0.01*M* solution was prepared by dilution with water and standardized gravimetrically by means of the standard nickel solution.

Procedures

Titration in colloidal suspension. Transfer the sample solution containing 0.06-0.7 mg of nickel to a titration cell. Add 2 ml of 2*M* NH_4Cl and 1 ml of 2*M* NH_4OH, making the pH 8-9. Add 10 ml of suspending agent (gum arabic or gelatine solution), and make up to 30 ml with water. Titrate with the standard dimethylglyoxime, adding the titrant slowly and in small portions with vigorous stirring, and take the reading with a red filter (No. 608) after each addition until finally the optical density becomes steady. From the titer calculate the amount of nickel in the solution.

Titration in a two-phase system. Transfer the sample solution containing 0.06-0.6 mg of nickel to a titration cell. Adjust the pH to 8-9, by adding 2 ml 2*M* NH_4Cl and 1 ml of 2*M* NH_4OH. Dilute to 10 ml with water and add 15 ml of chloroform. Stir vigorously for 2 minutes. Stop the stirring and allow the two phases to separate for 3-4 minutes. Using a violet filter (No. 601), adjust the galvanometer reading to zero. Titrate with dimethylglyoxime adding the titrant in small portions. Stir and after stirring allow the two phases to separate before reading the optical density. Continue the titration until the optical density becomes steady.

RESULTS AND DISCUSSION

As a check on the two procedures 'unknowns,' with and without diverse ions present, were analyzed by one of us (F.G.N.). The results obtained are shown in Table 1 (titration in colloidal suspension) and Table 2 (titration in a two-phase system). Results in all cases are reported in micrograms. Typical titration curves are shown in Figs. 1 and 2.

From the results it will be seen that Fe^{3+}, MoO_4^{2-}, WO_4^{2-} do not interfere up to a ratio of 60:1, providing they are masked with citrate, tartrate or phosphate. Chromium and manganese can be tolerated up to a ratio of 5:1 and can be masked with citrate or tartrate. Copper can be tolerated up to a ratio of 4:1 when citrate or tartrate are used as masking

TABLE I

TITRATION OF NICKEL IN COLLOIDAL SUSPENSION IN THE PRESENCE AND ABSENCE OF DIVERSE IONS

Diverse ions (mg)	Nickel (μg) Taken	Found	Difference	% Error
—	100	99.5	− 0.5	− 0.5
—	100	99.8	− 0.2	− 0.2
—	150	151.0	+ 1.0	+ 0.66
—	150	150.5	+ 0.5	+ 0.33
—	300	300	0.0	0.0
—	300	302.0	+ 2.0	+ 0.66
—	300	297.0	− 3.0	− 1.0
—	500	505.0	+ 5.0	+ 1.0
—	500	503.0	+ 3.0	+ 0.6
Fe^{3+} 1.0	100	99.7	− 0.3	− 0.3
3.0	100	99.5	− 0.5	− 0.5
5.0	100	102.0	+ 2.0	+ 2.0
MoO_4^{2-} 0.5	100	101.0	+ 1.0	+ 1.0
2.5	200	200	0.0	0.0
6.0	200	199	− 1.0	− 0.5
WO_4^{2-} 0.5	200	202	+ 2.0	+ 1.0
3.0	100	100.5	+ 0.5	+ 0.5
6.0	100	102.0	+ 2.0	+ 2.0
Cr^{3+} 0.1	100	100.0	0.0	0.0
0.3	100	101.0	+ 1.0	+ 1.0
0.4	100	99.5	− 0.5	− 0.5
Mn^{2+} 0.2	100	99.0	− 1.0	− 1.0
0.4	200	200.0	0.0	0.0
0.5	100	98.0	− 2.0	− 2.0
Cu^{2+} 0.2	100	100.5	+ 0.5	+ 0.5
0.3	100	101.5	+ 1.5	+ 1.5
0.4	100	102.0	+ 2.0	+ 2.0

agents. Cobalt interferes badly in both methods and should be removed prior to the determination of nickel.

Both methods of determination are reproducible with little to choose between them. The two-phase procedure does not involve a volume correction. Further, it can be adapted to the determination of very small amounts of nickel, if a sensitive galvanometer is used. Such a determina-

TABLE II

TITRATION OF NICKEL IN THE PRESENCE AND ABSENCE OF DIVERSE IONS IN A TWO-PHASE SYSTEM

Diverse ions (mg)	Nickel (μg) Taken	Found	Difference	% Error
—	100	100	0.0	0.0
—	100	100.5	+ 0.5	+ 0.5
—	100	100	0.0	0.0
—	200	201.0	+ 1.0	+ 0.5
—	200	200.5	+ 0.5	+ 0.25
—	200	199.5	— 0.5	— 0.25
—	250	251.0	+ 1.0	+ 0.4
—	250	248.0	— 2.0	— 0.8
—	250	249.0	— 1.0	— 0.4
—	400	402.0	+ 2.0	+ 0.5
—	400	400.0	0.0	0.0
—	400	400.5	+ 0.5	+ 0.12
Fe^{3+} 0.5	100	99.5	— 0.5	— 0.5
2.5	100	99.0	— 1.0	— 1.0
6.0	100	98.0	— 2.0	— 2.0
MoO_4^{2-} 1.0	100	100	0.0	0.0
3.0	100	100.5	+ 0.5	+ 0.5
5.0	100	101.0	+ 1.0	+ 1.0
WO_4^{2-} 1.0	100	100.7	+ 0.7	+ 0.7
2.5	200	200.0	0.0	0.0
5.0	200	202.0	+ 2.0	+ 1.0
Cr^{3+} 0.2	100	99.7	— 0.3	— 0.3
0.4	100	99.0	— 1.0	— 1.0
0.4	200	202.0	+ 1.0	+ 0.5
Mn^{2+} 0.2	100	100	0.0	0.0
0.3	100	99.3	— 0.7	— 0.7
0.5	100	98.5	— 1.5	— 1.5
Cu^{2+} 0.1	100	100.5	+ 0.5	+ 0.5
0.2	100	102.0	+ 2.0	+ 2.0
0.4	200	202.5	+ 2.5	+ 1.25

tion is not possible in aqueous medium, as very small amounts of nickel give a yellow solution with dimethylglyoxime (*5*).

Note. At the beginning of the titration, after the addition of 0.05 ml of

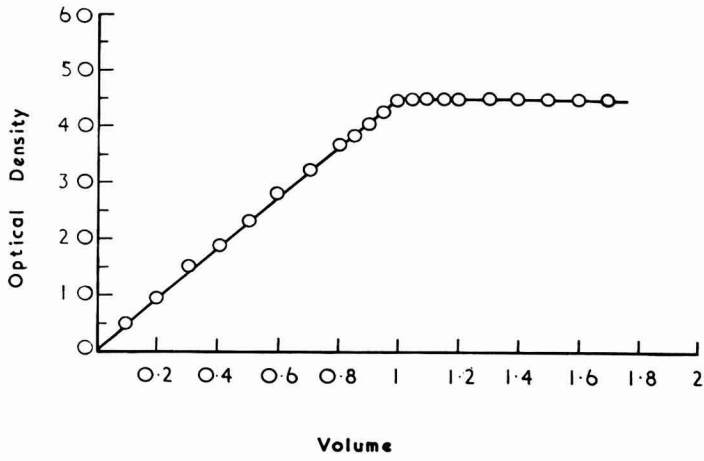

FIG. 1. Titration curve of 10 ml of nickel (0.0005 *M*) with dimethylglyoxime (0.01 *M*) in colloidal suspension (after volume correction).

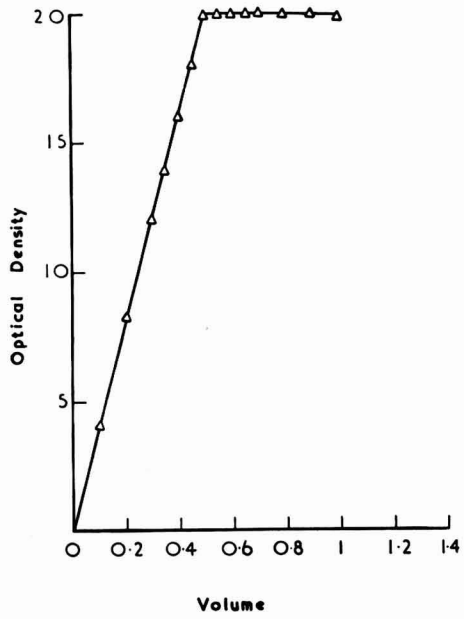

FIG. 2. Titration curve of 5 ml of nickel (0.0005 *M*) with dimethylglyoxime (0.01 *M*) in a two-phase system.

0.01*M* dimethylglyoxime, the solution is yellow in color. However, in the presence of a suspending agent, a red color is produced in 2-3 minutes (*5*).

SUMMARY

Two procedures have been developed for the photometric determination of nickel by titration with dimethylglyoxime solution. In one procedure, the nickel is titrated by a colloidal suspension technique, while, in the other, the titration is carried out in a two-phase system. Both methods are reproducible, accurate, and nickel can be determined in the presence of a number of associated elements or ions without interference.

REFERENCES

1. MAGEE, R. J., AND NASOURI, F. G., Determination of small amounts of nickel in the presence of large amounts of associated elements by colloidal absorption spectrophotometry. *Microchem. J.* **9**, 324-333 (1965).
2. NASOURI, F. G., SHAHINE, S. A. F., AND MAGEE, R. J., The spectrophotometric and titrimetric determination of gold with ferroin as reagent. *Anal. Chim. Acta*, in press.
3. BOBTELSKY, M., AND WELWART, Y., Heterometric microtitration of nickel with dimethylglyoxime. *Anal. Chim. Acta* **9**, 281-286 (1953).
4. BOBTELSKY, M., AND WELWART, Y., Direct heterometric microtitration of nickel with dimethylglyoxime in the presence of other metals. *Anal. Chim. Acta* **9**, 374-383 (1953).
5. MAGEE, R. J., AND GORDON, L., Studies on nickel(II) dioximes of analytical importance. *Proc. S.A.C. Conf.*, Nottingham, England, July 19-23, 1965. Paper No. 37.

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Specific Spectrophotometric Microdetermination of Beryllium

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INTRODUCTION

The highly specific spectrophotometric analytical procedure for beryllium described in this paper, with "Aluminon" (ammonium aurintricarboxylate) reagent used for color reaction, was developed primarily for the rapid determination of beryllium in air-borne dust samples for hygienic controls (*4*). The "Zenia" (*p*-nitrophenylazoorcinol) method (*1*) commonly used for this analysis is time consuming, requires the use of platinumware, and is not as sensitive or specific as desirable. The fluorometric "Morin" (a tetrahydroxy flavonol) method (*6*) also used, is extremely sensitive but lacks adequate specificity and requires the use of a fluorometer not always available for measurement.

During the past decade, numerous colorimetric procedures for beryllium have been developed by researchers using a wide variety of organic reagents for color formation. Although none of the reagents used were found to be entirely specific for beryllium, satisfactory colorimetric procedures have been developed with over 25 different organic chemicals. Aluminon reagent was selected from this wide variety of organic chemicals for development of the present procedure. The reagent is highly sensitive for beryllium, forms a stable color with the element, and the reagent solutions are stable for long periods of time. Also, the chemical is readily available and does not require further purification for the present analysis. Yoe and Hill (*7*) were the first to report that aluminon reagent reacts with beryllium to form a red complex similar to that with aluminon. Kosel and Neuman (*2*) developed a colorimetric procedure for beryllium with the reagent, but no complexing agents were used and the method

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therefore lacks specificity. Luke and Campbell (*3*) improved on the specificity by employing Na_2EDTA for complexing a great many interferences without significantly affecting the sensitivity of the test. The present procedure further enhances the specificity by including calculated amounts of triethanolamine and cyanide with the Na_2EDTA prescribed by previous investigators.

The composite effects of the additional complexing agents are to prevent interference from elements only weakly complexed by the Na_2EDTA and also to increase the tolerance limits of those elements already partly tied up by this versatile chelating agent. Tests conducted to determine the degree of specificity achieved show that the complexing mixture prevents interference from varying amounts of all elements tested, except fluoride, which interferes in all concentrations.

EXPERIMENTAL

Instruments

1. Buret, Ultra-Precision Micro, 1 Div. = 0.01 μl, accuracy ± 0.04%, Laboratory Supplies, Inc.
2. pH Meter, Beckman Zeromatic.
3. Spectrophotometer, Bausch and Lomb Spectronic 20.
4. Ultramicrospectrophotometer, Beckman Model 151.

Materials

1. Beryllium metal standard, NBL Standard No. 88, Atomic Energy Commission, New Brunswick Area Office, P. O. Box 150.
2. Triethanolamine (*2, 2,′ 2,″* nitrilotriethanol) MP20-22C.
3. Na_2EDTA (Disodium ethylenediaminetetraacetate) Reagent grade.
4. "Aluminon" (ammonium aurintricarboxylate) Practical grade.
5. Acetic acid (99.7%), MP16.0-16.6 C.
6. Benzoic acid, ACS grade, primary standard.
7. Methyl alcohol, ACS grade.
8. Sodium hydroxide, ACS grade pellets.

PREPARED SOLUTIONS

Aluminon Reagent

Dissolve 8.0 g of benzoic acid in 400 ml of methyl alcohol in a large beaker. Add about 200 ml of water and 10 ml of glacial acetic acid. Stir to mix. Adjust pH to 4.8 with approximately 5*N* sodium hydroxide using

a pH meter for measurement. Add exactly 1.00 g of aluminon reagent and stir with mechanical stirrer until completely dissolved. Filter through a Whatman No. 42 paper using a Buchner funnel with suction. Wash the filter well with pure water. Transfer the filtrate to a 2-liter volumetric flask and dilute to the mark with water. Stopper flask, then mix well by inverting. The solution is stable if stored in Pyrex.

Buffer-Complex Reagent

Weigh out 100 ± 1 g of Na_2EDTA in a large beaker. Add about 500 ml of pure water and stir until completely dissolved. In another beaker dissolve 20 ± 1 g of KCN in about 100 ml of water. Add this solution slowly while stirring to the solution in the large beaker. Adjust the pH to 4.8 (under a hood) with glacial acetic acid (about 80 ml) using a pH meter for measurement. Transfer to a 2-liter volumetric flask and dilute to the mark with water. Stopper flask, then mix well by inverting. The solution is stable if stored in Pyrex.

PROCEDURES

Micro

Place an aliquot of the slightly acid sample containing preferably between 1 and 4 μg of beryllium in a 10-ml volumetric flask. Add 1 ml of buffer-complex reagent and swirl flask to mix. Add 1 ml of aluminon reagent solution and dilute to 10 ml with water. Stopper flask and invert to mix. Allow 60 min for maximum color development, then determine the percent transmittance or absorbance against a reagent blank at a wavelength of 530 mμ using the 1-cm light path comparison cells. Determine the micrograms of beryllium in the sample by reference to a standard curve such as Fig. 1, constructed for this purpose using known amounts of beryllium metal as a standard substance.

Ultramicro

The following ultramicro spectrophotometric analytical procedure has been developed specifically for use with a Beckman Model 151 Ultramicro Spectrophotometer or similar apparatus. This instrument enables transmittance or absorbance measurements to be made on samples as small as 0.1 ml in volume.

Place an aliquot of the slightly acid sample containing up to 1 mg of beryllium in a small glass stoppered weighing bottle (about 1.0 ml size).

Determine the volume of the sample by weighing to the nearest 0.1 mg, assuming a specific gravity of 1.0 for the sample solution. Add a volume of buffer-complex solution equal to 12.5% of the sample volume, then swirl to mix.[2] Add the same volume of aluminon reagent solution and again

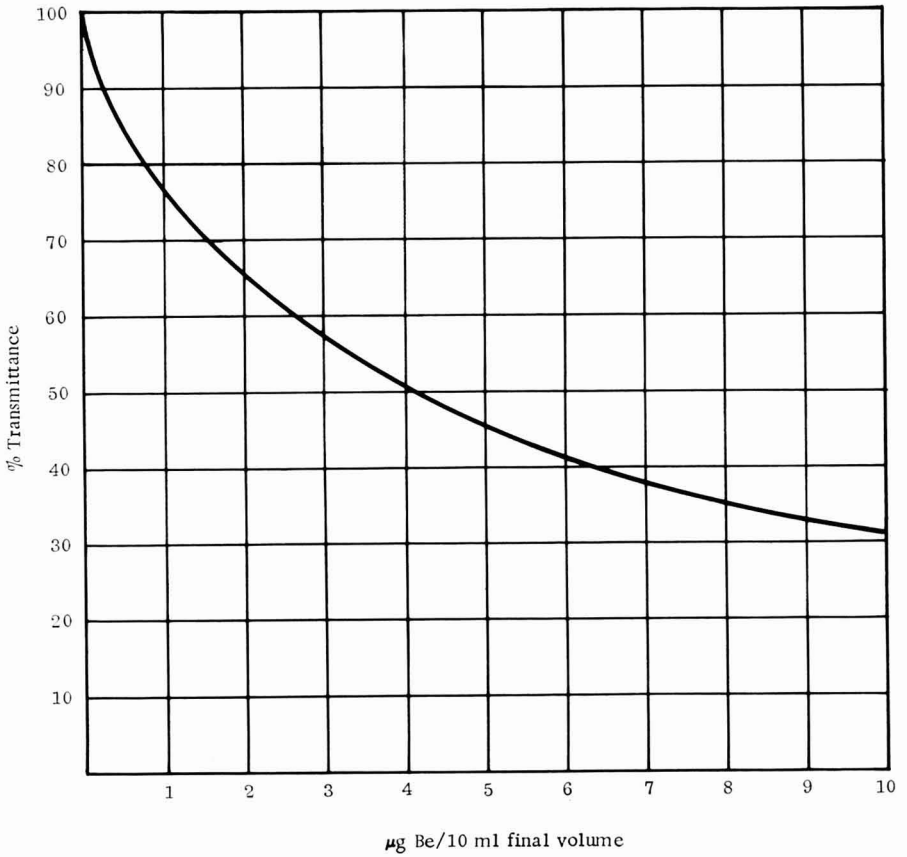

FIG. 1. Standard curve for beryllium. Spectronic 20, 530 mμ, 1-cm square cuvettes, 60 min.

swirl to mix. Allow 60 min for maximum color development and then determine the transmittance at 530 mμ against a reagent blank. A macro-size reagent blank may be used for convenience by using the same pro-

[2] For example, add 20 μl for a 0.16-ml sample. An R. G. Ultra Precision Micrometer Buret was used for developing the present procedure. This Buret delivers solution increments of as little as 0.01 μl with an accuracy of ± 0.04%.

portions of reagents used in conducting the ultramicro test. Determine the nanograms of beryllium in the sample by reference to a standard curve such as Fig. 2, constructed for this purpose using known amounts of beryllium metal as a standard substance.

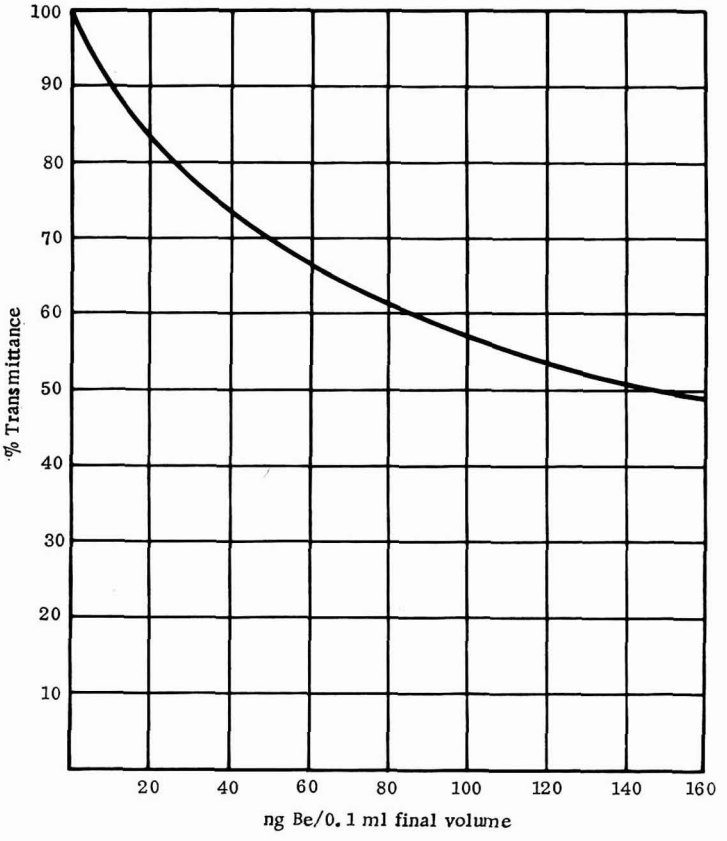

FIG. 2. Standard curve for beryllium. Beckman Model 150 ultramicrospectrophotometer, 530 mμ, 60 min.

RESULTS AND DISCUSSION

Beers Law

Test results obtained with standard beryllium solutions indicate conformance of the red beryllium-aluminon complex to Beers's law in the concentration range of 10-40 ppm beryllium.

Rate of Color Formation

Table 1 shows the results of tests conducted to determine the time required for maximum color development. The results indicate that maximum color and complete color stability are achieved after 60 min. Color development is 95% complete in about 12 min. No change in transmittance was observed after 7 days in another test run.

TABLE 1
RATE OF COLOR FORMATION

Time after mixing (min)	Percent transmittance[a]
1	56.2
6	54.2
12	53.4
16	53.1
33	52.7
45	51.7
60	51.0
20 hours	51.0

[a] The transmittance readings were obtained at a wavelength of 530 mμ, using 1-cm cuvettes and a concentration of beryllium of 40 μg/100 ml final volume.

Effect of pH

Reference to Table 2 shows no measurable change in percent transmittance of the beryllium-aluminon complex in the pH range of 4.5-5.0 A pH value of 4.8 was selected as satisfactory for the present procedure.

TABLE 2
EFFECT OF pH ON PERCENT TRANSMITTANCE

pH[a]	Percent transmittance[b]
3.0	78.5
4.0	72.5
4.5	71.5
5.0	71.5
5.5	73.5
6.0	78.5
7.0	91.5

[a] The pH was adjusted with sodium hydroxide or acetic acid as required under a hood.

[b] Transmittance measurements made at 530 mμ, using 1-cm cuvettes, with a beryllium concentration of 14 μg/100 ml final volume.

Effect of Wavelength

Table 3 shows the effects of various wavelengths on the transmittance values obtained. The test solution contained 25 μg of beryllium in a final volume of 100 ml. The readings were made after allowing 60 min

TABLE 3
EFFECT OF WAVELENGTH ON PERCENT TRANSMITTANCE

Wavelength	Percent transmittance (60 min)
400	96.0
450	82.5
500	73.5
505	70.5
510	68.0
515	66.0
520	63.5
525	62.5
530	61.5
535	61.5
540	62.5
550	67.5
575	87.5
600	97.0

for maximum color development. The results show a minimum transmittance value at between 530 and 535 mμ.

Sensitivity

The sensitivity of the procedure, as determined in accordance with the method of Sandell (*5*), is 0.01 μg per square centimeter based on an absorbance of 0.01.

Interference Limits

The tolerable limits of cations and anions were determined by a Bausch and Lomb Spectronic 20 spectrophotometer for transmittance measurements. The acceptable tolerance limit is defined as that concentration of foreign ion that influences the absorbance of the system by less than $\pm$ 2%. The following elements may be tolerated in the amounts indicated:

2000 fold: Cl^{-1}, SO_4^{-2}, NO_3^{-1}, CO_3^{-2}, $C_2H_3O_2^{-1}$
1000 fold: PO_4^{-3}
100 fold: Cu^{+2}, Fe^{+3}, Ni^{+2}, Co^{+2}, Mg^{+2}, Al^{+3}, Bi^{+3}, Pb^{+2}, Hg^{+2}, Ce^{+4}, Li^{+1}, Na^{+1}, K^{+1}, Rb^{+1}, Cs^{+1}, Ca^{+2}, Ba^{+2}, Sr^{+2}, Th^{+4}, Pd^{+4}, Ge^{+3}, Sb^{+3}, Cd^{+2}, Nb^{+5}, Os^{+4}, Pt^{+4}, Tl^{+1}, Ag^{+1}, Mn^{+2}, Zn^{+2}, CrO_4^{-2}, WO_4^{-2}, AsO_4^{-2}, MoO_4^{-2}, SiO_3^{-2}, Br^{-1}, I^{-1}.
50 fold: U^{+6}, In^{+3}, Ti^{+4}, Au^{+3}, Bo_3^{-3}
25 fold: Zr^{+4}

Fluoride ion interferes in all concentrations.

SUMMARY

A specific spectrophotometric microprocedure for beryllium has been developed which permits the direct quantitative determination of microgram or nanogram amounts of the element in the presence of specified amounts of all of the elements tested, except fluoride, which interferes in all concentrations. The high degree of specificity achieved is of especial consequence in ultramicro analysis. It enables direct beryllium determinations without the need for prior separations which may be very difficult or sometimes even impractical. The interference of fluoride suggests the use of the method for development of a highly specific and sensitive procedure for fluoride since parts per million amounts cause measurable changes in the transmittance values.

REFERENCES

1. Hiser, R. A., Donaldson, H. M., and Swenzfeier, C. M., A rapid analytical method for the determination of beryllium in air samples. *Am. Ind. Hyg. J.* **22**, 280-285 (1961).
2. Kosel, G. E., and Neuman, W. F., Color reaction between beryllium and aurin tricarboxylic acid. *Anal. Chem.* **22**, 936-939 (1950).
3. Luke, C. L., and Campbell, M. E., Photometric determination of beryllium in beryllium-copper alloys. *Anal. Chem.* **24**, 1056-1057 (1952).
4. McCloskey, J. P., Spectrophotometric determination of beryllium in airborne dust samples. *Microchem. J.* **12**, 40-45 (1967).
5. Sandell, E. B., "Colorimetric Determination of Traces of Metals." 3rd ed. Wiley (Interscience), New York, 1959.
6. Walkley, J., A study of the Morin method for the determination of beryllium in air samples. *Am. Ind. Hyg. J.* **20**, 241 (1959).
7. Yoe, J. H., and Hill, W. L., An investigation of the reaction of aluminium with the ammonium salt of aurin tricarboxylic acid under different experimental conditions, and its application to the colorimetric determination of aluminum in water. *J. Am. Chem. Soc.* **49**, 2395 (1927).

MICROCHEMICAL JOURNAL 12, 40-45 (1967)

Spectrophotometric Determination of Beryllium in Airborne Dust Samples

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INTRODUCTION

Beryllium has certain unique physical properties contributing to its use in the design of critical components of aerospace vehicles and electronic guidance systems. The machining of the metal for fabrication purposes creates a dangerous health environment due to the highly toxic nature of the dust particles. As a safeguard for hygienic controls, measured volumes of air in fabrication work areas are filtered through small 2.4 cm Whatman No. 41 filter papers and submitted to the laboratory for routine analysis of the beryllium contents. Beryllium values found usually lie between 0.1 and 10 μg per filter for the 4- to 5-cubic meters of air sampled. However, sample filters of larger volumes of air may sometimes contain in excess of 50 μg of beryllium per filter. The present procedure is designed for the analysis of sample filters containing between 0.1 and 100 μg of beryllium.

The analytical procedure commonly used for determining beryllium in airborne dust is known as the Zenia (*p*-nitrophenylazoorcinol) method (*1*). This procedure is time consuming, requires the use of platinumware, and is not as sensitive or specific as desirable. In view of the large number of analyses required, a study was initiated to develop an improved colorimetric procedure for beryllium. This objective has subsequently been accomplished and the general spectrophotometric procedure published in this issue (*2*). The method has been adapted to the determination of beryllium in airborne dust samples and used for the past 3 years with satisfactory results. The techniques of the procedure used are described in this paper.

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EXPERIMENTAL

Apparatus

1. Spectrophotometer; Bausch and Lomb, Spectronic 20.
2. Buret, automatic, reservoir type, gravity fill, Scientific Glass Apparatus Co., Item #JB 6700.
3. Quartz beakers, Microchemical Specialties Co.
4. pH meter, Beckman, Zeromatic.

Materials

1. Beryllium metal, NBL Standard No. 88, Atomic Energy Commission, New Brunswick Area Office, P. O. Box 150.
2. Triethanolamine (2, 2′, 2″ nitrilotriethanol) MP 20-22°C.
3. Na_2EDTA (Disodium ethylenediaminetetraacetate), Reagent grade.
4. Aluminon (ammonium aurintricarboxylate) Practical grade.
5. Acetic acid (99.7%), MP 16.0-16.6°C.
6. Benzoic acid, ACS grade, primary standard.
7. Methyl alcohol, ACS grade.
8. Sodium hydroxide, ACS grade pellets.

Prepared Reagents

Buffer Complex–Strong. Weigh out 100 ± 2 g of triethanolamine and 40 ± 1 g of Na_2 EDTA in a large beaker. Add about 500 ml of pure water and stir to dissolve. In another beaker dissolve 20 ± 1 g of potassium cyanide in about 100 ml of water. Add this solution slowly (under a hood) while stirring, to the contents of the large beaker. Adjust the pH of the mixture to 4.8 (under a hood) with glacial acetic acid (about 80 ml) using a pH meter for measurement. Transfer the contents of the beaker to a 2-liter volumetric flask and dilute to the mark with water. Stopper flask and mix well by inverting. Transfer the prepared solution to a "Pyrex" bottle for storage or preferably to a 2-liter reservoir type gravity fill automatic buret for both storage and use. The solution is stable if stored in Pyrex.

Buffer Complex–Dilute. Add 223 ml of the Buffer Complex–Strong solution to a 2-liter volumetric flask and dilute to the mark with water. Stopper flask and mix well by inverting. Transfer to a Pyrex bottle for storage or preferably to a 2-liter reservoir type gravity fill automatic buret for both storage and use. The reagent is stable if stored in Pyrex.

Aluminon Reagent

Dissolve 8.0 g of benzoic acid in 400 ml of methyl alcohol in a large beaker. Add about 200 ml of water and 10 ml of glacial acetic acid. Adjust the pH to 4.8 with approximately 5*N* sodium hydroxide, using a pH meter for measurement. Add exactly 1.00 g of aluminon reagent and stir with magnetic stirrer until completely dissolved. Filter through a Whatman No. 42 filter paper, using a Buchner funnel and vacuum. Wash filter well with pure water. Transfer filtrate to a 2-liter volumetric flask and dilute to the mark with water. Stopper flask and mix well by inverting. Transfer to a Pyrex bottle for storage or preferably to a 2-liter reservoir type gravity fill automatic buret for both storage and use. The reagent is stable if stored in Pyrex.

Procedure

Fold the filter paper containing the beryllium dust sample and place in the bottom of a 10-ml quartz beaker. Place the beaker in a muffle furnace and heat slowly at first to char paper, then heat to 700°C until paper is completely destroyed. Cool, add 1 ml of 1*N* sulfuric acid and wet sides of beaker with the acid, using a micro stirring rod. Leave the rod in the beaker. Place the beaker on a cold-to-warm hot plate and gradually increase heat until the sulfuric acid is completely volatilized. The evaporation rate should be regulated to take no less than 30 min. It is considered advisable to place a metal shield about 6 inches in height in front of the beakers to ensure complete removal of the acid. This prevents condensation of the acid on the upper sides of the beakers due to the cooling action from the draft of the hood. The sulfuric acid treatment converts the beryllium to either the hydrated or anhydrous beryllium sulfate, depending upon the final temperature of heating. Both forms are soluble, however, in the Buffer Complex solution added in the next step during the 30-min dissolution time allowed. Cool, add exactly 9.0 ml of the Buffer Complex–Dilute reagent and stir with the stirring rod left in the beaker from the sulfuric acid treatment. Allow at least 30 min for dissolution, then add 1.0 ml of the Aluminon reagent and stir to mix.

If the color that develops at once indicates that the beryllium content of the sample is below 10 μg, proceed as follows:

Allow 60 min or longer for maximum color development, then determine the percent transmittance at 530 mμ using 1-cm light path cuvettes and a reagent blank for comparison. Determine the beryllium content of the

sample by reference to a standard curve, such as Fig. 1, prepared for this purpose using beryllium metal as a standard substance.

If the color that develops at once indicates that the beryllium content of the sample is more than 10 μg, proceed as follows:

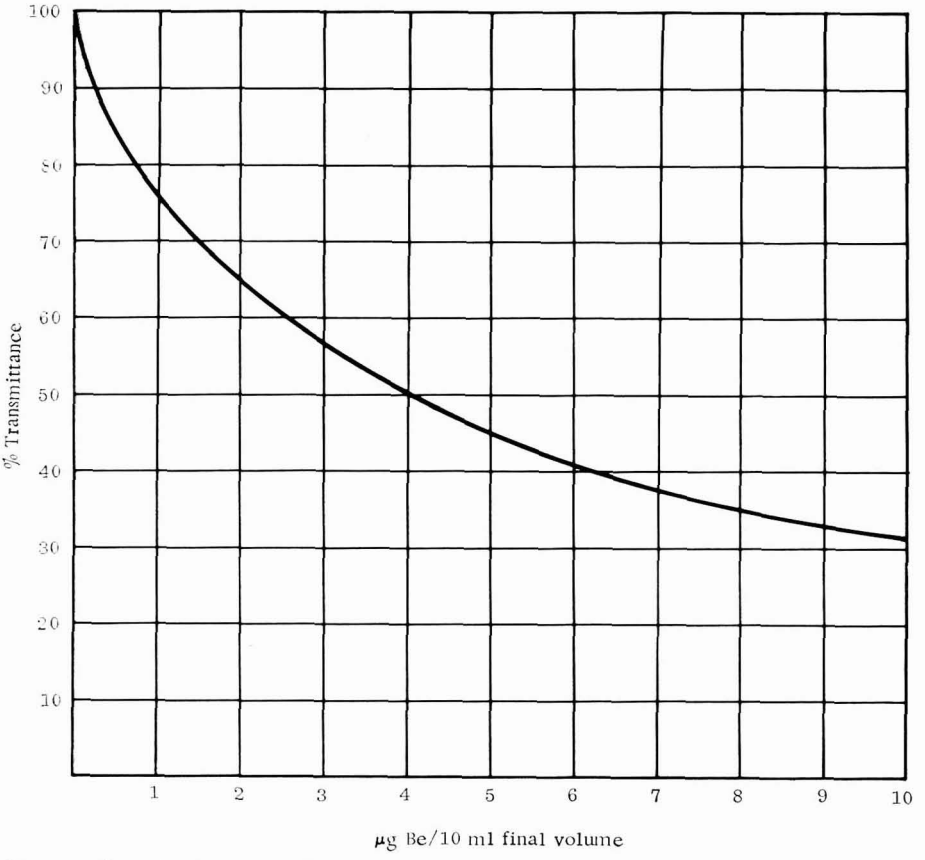

FIG. 1. Standard curve for beryllium. Spectronic 20, 530 mμ, 1-cm square cuvettes, 60 minutes.

Immediately transfer the contents of the 10-ml quartz beaker to a 100-ml volumetric flask. Add 9.0 ml of the Buffer Complex–Strong solution; then swirl flask to mix. Add 9.0 ml of the Aluminon reagent and dilute to 100 ml with water. Stopper flask, invert to mix, then allow 60 min for maximum color development. Determine the percent transmittance at 530 mμ, using 1-cm light path cuvettes and a reagent blank for com-

parison. Determine the beryllium content of the sample by reference to the same curve used for the low range of beryllium such as Fig. 1. To find the actual amount of beryllium in the sample, multiply the value obtained from the graph by ten, since a 10-fold dilution is involved.

Recovery of Known Amounts of Beryllium

In order to evaluate the accuracy of the method, known amounts of dissolved beryllium were added to small 2.4-cm Whatman No. 41 filter papers. The prepared papers were then subjected to the same techniques used for determining the beryllium contents of actual samples. The results obtained are shown in Tables 1 and 2. Statistical calculations show a

TABLE 1
ANALYSIS OF KNOWN BERYLLIUM SAMPLES (LOW RANGE)

Be added (μg)	1.0	2.0	4.0	7.0
	1.03	1.97	4.08	7.18
	1.03	1.90	4.02	6.98
Be found (μg)	1.00	2.00	3.98	6.88
	.96	2.04	4.08	6.80
Final volume 10 ml	1.00	1.98	3.92	6.88
	1.02	1.88	3.77	6.86
	1.00	1.98	4.02	6.88
	1.00	2.05	4.02	7.00
	.96	2.02	3.98	6.88
	.97	1.98	4.02	6.88
Av. Be found (μg)	.997	1.98	3.99	6.92

TABLE 2
ANALYSIS OF KNOWN BERYLLIUM SAMPLES (HIGH RANGE)

Be added (μg)	10	20	30	40	60	100
	10.10	19.8	30.2	41.0	59.0	97.0
	9.40	19.8	33.0	39.0	58.5	98.0
Be found (μg)	10.10	20.5	29.0	39.0	58.5	98.0
	9.90	20.0	30.0	37.8	60.0	98.0
Final volume 100 ml	9.70	20.3	29.2	39.2	58.5	102.0
	10.00	20.5	30.0	39.4	60.0	100.0
	9.90	20.5	28.5	39.0	58.2	98.0
	9.90	20.2	28.5	37.2	60.2	98.5
	10.10	19.6	29.8	37.6	59.0	99.5
	9.30	19.8	29.8	37.4	59.2	99.0
Av. Be found (μg)	9.84	20.1	29.8	38.7	59.1	98.9

standard deviation of 2.3% or 95% confidence limits of 4.6% for the results in Table 1, and a standard deviation of 2.5% or 95% confidence limits for the values shown in Table 2. This is a marked improvement over the Zenia method (*1*), with 95% confidence limits of 16.8% for the 2.5-45 μg range.

SUMMARY

A simple, rapid, and accurate spectrophotometric procedure for the determination of beryllium in airborne dust samples has been developed, with aluminon reagent used for color formation. The use of a complexing buffer solution containing one complexing and two chelating agents improves on the specificity of previous methods. In contrast to the Zenia method which has a lower limit of 2.5 μg, the present procedure is much more sensitive and affords accurate results for beryllium contents as low as 0.3 μg. Also, the procedure is rapid, as many as 40 samples having been analyzed in a normal 8-hr work period by a single analyst.

REFERENCES

1. Hiser, R. A., Donaldson, H. M., and Swenzfeier, C. M., A rapid analytical method for the determination of beryllium in air samples. *Am. Ind. Hyg. J.*, **22**, 280-285 (1961).
2. McCloskey, J. P., Specific spectrophotometric microdetermination of beryllium. *Microchem. J.*, **12**, 32-39 (1967).

MICROCHEMICAL JOURNAL 12, 46-54 (1967)

Modified Microdetermination of Sulfate Ion: Its Application to Flask Combustion for Organic Sulfur

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INTRODUCTION

Microtitration of sulfate ion involving barium perchlorate and thorin was first investigated by Fritz and Yamamura (*5*) in a nonaqueous medium. The method has been widely used since then in numerous laboratories because of its extreme simplicity and proper sensitivity for dilute solutions. The flask combustion for organic sulfur is currently finished, therefore, by this method (*1, 6, 11*). Untrained operators sometime cite the indistinct color change at the end point as a minor shortcoming of the thorin method. Skilled operators find no such flaw. The difference in the two opinions is probably due to the orange and pale pink colors, which thorin gives before and after the end point, being closely related, therefore resulting in a faint contrast of the color change.

Recently arsenazo(III) (*7*) and sulfonazo(III) (*2*) have been introduced in place of thorin for the same purpose but with stronger contrasts of the color changes at the end point. A comparison of the reproducibilities of the titrations with these three indicators has been investigated in the authors' laboratory, and arsenazo(III) has been suggested as the best selection. Another shortcoming has been experienced, however, in that the blue color just after the end point lasted for a short time but rapidly returned back to the original red color. Consequently, a higher end point was obtained with smaller increments of the titrant, and a lower end point was with larger increments of the same titrant. The present authors have therefore investigated a back titration of excess barium perchlorate with sulfuric acid solution and found an excellent reproducibility with arsenazo(III). The back titration has been successfully applied to the

flask combustion of organic sulfur and its extension to the simultaneous determination of organic halogens and sulfur has been attempted.

EXPERIMENTAL

Reagents

*0.01*N *barium perchlorate solution.* Approximately 1.95 g of $Ba(ClO_4)_2 \cdot 3\ H_2O$ was dissolved in 200 ml of distilled water with an addition of a few drops of perchloric acid. Reagent grade isopropyl alcohol was added to make up 1 liter.

*0.01*N *sulfuric acid solution.* A standard solution of 0.1*N* sulfuric acid was diluted exactly tenfold by distilled water.

Indicator solutions. Commercially available thorin, arsenazo(III), and sulfonazo(III) of 0.2 g were dissolved in 100 ml of distilled water, respectively.

*0.1*N *nitric acid solution.*

*0.1*N *ammonia solution.*

Optimum Conditions for Titrations

A screening test was carried out to investigate the optimum conditions for obtaining the most distinct color change at the end point. Five ml of 0.01*N* sulfuric acid solution was pipetted into a beaker in which isopropyl alcohol was further added to make 50 ml. Titrations with 0.01*N* barium perchlorate solution were proceeded with different amounts of the respective indicators at different pH, the latter being adjusted by 0.1*N* nitric acid and ammonia solutions. The relative distinctnesses are illustrated in Table 1, where the number of +'s represent the psychological grades of the color changes at the end point, while — indicates insufficient color change for the determinations.

The optimum amounts of the indicator solutions under the suggested conditions were found at 0.20 ml for thorin and 0.15 ml for arsenazo(III) and sulfonazo(III). The pH should be adjusted at 6 for sulfonazo(III), while pH 4 was recommended for the others.

Visible absorption spectra before and after the respective end points are illustrated in Fig. 1. The full lines, in which an absorption cell of 1 cm thickness was used, indicate the spectra at 0.5 ml before the end points. Broken lines are at 0.5 ml after the end points. Reference solution was freshly prepared at every test in the same manner as the sample solution, except that the indicator was eliminated. Better isolations of spectral

TABLE 1
RELATIVE DISTINCTNESSES OF COLOR CHANGES AT END POINTS UNDER DIFFERENT CONDITIONS

Indicator: Thorin; orange → pink

Vol. of indicator solution (ml)	pH 3	4	5	6	8
0.10	+	++	+	—	—
0.15	++	++	++	—	—
0.20	+++	+++	++	—	—
0.30	++	+++	+	—	—
0.40	—	—	—	—	—

Indicator: Arsenazo(III); red → blue

Vol. of indicator solution (ml)	pH 3	4	5	6	8
0.10	+	++	++	+	—
0.15	++	+++++	+++	+	—
0.20	++	++++	+++	+	—
0.30	—	+	+	—	—
0.40	—	—	—	—	—

Indicator: Sulfonazo(III); purple → blue

Vol. of indicator solution (ml)	pH 3	4	5	6	8
0.10	—	—	+	+	—
0.15	—	+	++++	+++++	—
0.20	—	+	++	+++	—
0.30	—	—	+	++	—
0.40	—	—	—	—	—

patterns were observed with arsenazo(III) and sulfonazo(III) than with thorin.

Reproducibilities of End Points

A series of titrations of 5 ml of standard 0.01*N* sulfuric acid solution with 0.01*N* barium perchlorate solution under different indicators are listed in Table 2. The titrations proceeded with Metrohm's 10-ml piston burette, with the graduation board of the burette sheltered by a screen so that the operator could not see the volumes of the titrants during the titrations.

The mean volumes of the titrants with thorin and arsenazo(III) gave

a good approximation of each other, while sulfonazo(III) gave a slightly smaller mean value of the titrants. Regarding the reproducibilities with the three indicators, arsenazo(III) was best recommended, having the minimum standard deviation of ± 11 μl.

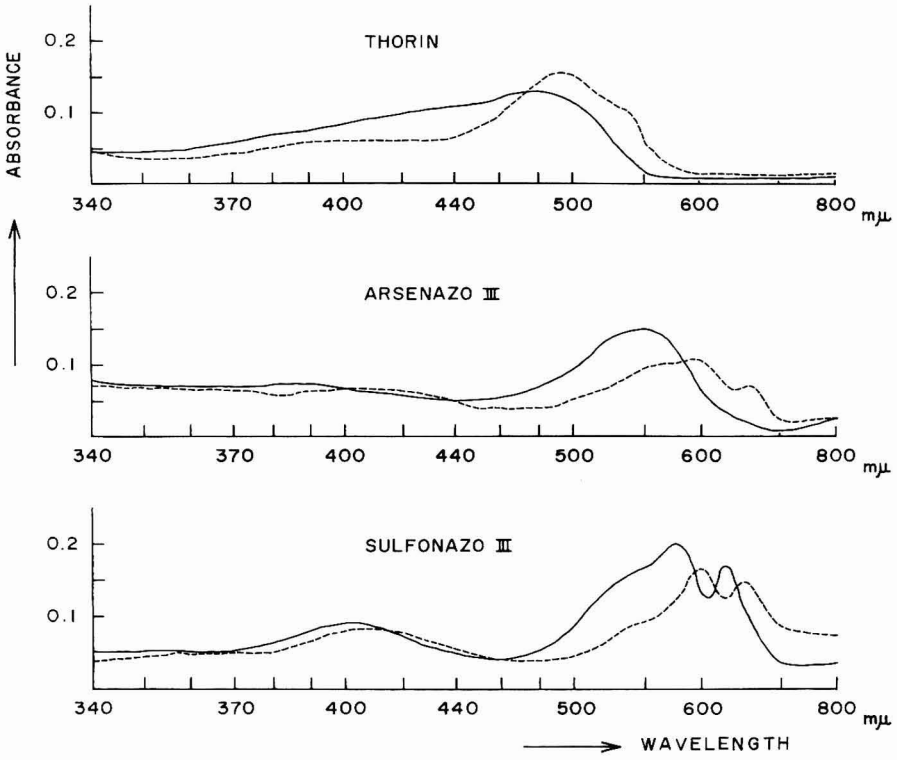

FIG. 1. Absorption spectra of titration solutions. —— 0.5 ml before end point; - - - 0.5 ml after end point.

A further investigation was carried out to improve the reproducibility with arsenazo(III). With the normal increments, red color sharply changed at the end point to blue but a short time later it was found that the latter color returned back to the former. It is known that such a behavior is usually harmful with the reproducibility of the titration unless the increment of the titrant will be strictly regulated at the same rate. As a matter of fact, a very low concentration of the sulfate ion just before the end point slowly forms barium sulfate so that charged barium ion will be chelated temporarily by the indicator. To eliminate this disad-

TABLE 2

SERIES OF TITRATIONS OF 5 ml 0.01*N* SULFURIC ACID WITH 0.01*N* BARIUM PERCHLORATE SOLUTION

Indicator	Vol. of barium perchlorate solution (ml)					
Thorin	4.970	4.923	4.981	4.957	4.936	5.013
	4.970	4.919	4.975	4.971	4.960	4.943
	4.950	4.932	4.954	4.920	4.947	4.925
	4.944	4.945	4.975	4.958	4.979	4.927
	4.967	4.946	4.947	4.940	4.962	4.970
			Mean vol. = 4.954 ml		*SD* = ± 22 μl	
Arsenazo(III)	4.956	4.981	4.966	4.976	4.989	4.978
	4.970	4.972	4.958	4.977	4.994	4.974
	4.986	4.999	4.975	4.969	4.973	4.987
	4.959	4.973	4.966	4.967	4.991	4.979
	4.965	4.959	4.978	4.991	4.978	4.965
			Mean vol. = 4.975 ml		*SD* = ± 11 μl	
Sulfonazo(III)	4.818	4.850	4.805	4.837	4.845	4.799
	4.842	4.830	4.802	4.817	4.825	4.847
	4.802	4.833	4.823	4.848	4.828	4.809
	4.831	4.824	4.826	4.798	4.853	4.818
	4.785	4.824	4.811	4.816	4.821	4.852
			Mean vol. = 4.824 ml		*SD* = ± 18 μl	

vantage, a back titration of excess barium perchlorate with sulfuric acid solution was considered.

Five ml of 0.01*N* barium perchlorate solution was titrated by 0.01*N* standard sulfuric acid solution with 0.15 ml of arsenazo(III) solution. pH at the end point coincides approximately with the optimum value. A series of titrations is tabulated in Table 3 where a significant improvement of reproducibility with a standard deviation of ± 6 μl has been attained.

TABLE 3

TITRATIONS OF 5 ml 0.01*N* BARIUM PERCHLORATE WITH 0.01*N* SULFURIC ACID SOLUTION USING ARSENAZO(III) AS INDICATOR

Vol. of sulfuric acid solution (ml)						Mean vol. (ml)	SD (μl)
4.933	4.937	4.934	4.939	4.922	4.924		
4.932	4.930	4.938	4.924	4.924	4.938		
4.925	4.939	4.946	4.930	4.937	4.937	4.933	± 6
4.934	4.937	4.928	4.933	4.941	4.929		
4.939	4.929	4.937	4.942	4.930	4.936		

APPLICATION TO FLASK COMBUSTION FOR ORGANIC SULFUR

Reagents

*0.01*N *barium perchlorate solution.*

*0.01*N *sulfuric acid solution.*

Arsenazo(III) solution.

30% hydrogen peroxide solution.

Diphenylcarbazone solution. A 0.1 g of diphenylcarbazone was dissolved in 100 ml of isopropyl alcohol.

*0.005*N *mercuric nitrate solution.* A 0.8 g of $Hg(NO_3)_2 \cdot \frac{1}{2} H_2O$ was dissolved in 20 ml of distilled water with an addition of a few drops of nitric acid and diluted to make up 1 liter. The solution was standardized against standard 0.005*N* potassium bromide solution.

*0.005*N *potassium bromide solution.*

Procedure

Organic sample was weighed on a filter paper and folded by the conventional manner (8-10). A combustion flask of 300 ml charged with 5 ml of distilled water and 0.5 ml of 30% hydrogen peroxide solution was filled by oxygen and the sample was burned instantaneously in the closed flask. The flask was shaken vigorously and kept standing for 30 min. The absorption liquid was transferred perfectly into a small beaker by means of 50 ml of isopropyl alcohol. Five or 10 ml of 0.01*N* barium perchlorate solution was pipetted into the beaker with an addition of 0.15 ml of arsenazo(III) solution. Back titration with 0.01*N* sulfuric acid solution was carried out until blue color turned to red.

Calculation and Results of Analysis

The percentage composition of sulfur in the sample is calculated as follows:

$$\% \text{ S} = \frac{(0.01N \text{ Ba}(\text{ClO}_4)_2 \text{ ml} \times \text{F} - 0.01N \text{ H}_2\text{SO}_4 \text{ ml}) \times 0.1603}{\text{Sample weight (mg)}} \times 100,$$

where F is a normality factor against standard 0.01*N* sulfuric acid solution.

A series of analyses involving different types of organic compounds are listed in Table 4, where an excellent reproducibility with a standard deviation of $\pm$ 0.04% from the analytical errors has been observed.

Blank value with an ash free filter paper was practically negligible, but otherwise it should be subtracted from the volume of barium perchlorate solution in the above equation.

TABLE 4
DETERMINATIONS OF SULFUR WITH DIFFERENT TYPES OF ORGANIC COMPOUNDS

Sample	Sample wt. (mg)	0.01N $Ba(ClO_4)_2$ — 0.01N H_2SO_4 (ml)	S(%) Found	S(%) Deviation
Bromthymol blue	7.403	2.386	5.17	+ 0.03
S = 5.14%	6.010	1.941	5.18	+ 0.04
	7.420	2.407	5.20	+ 0.06
	7.831	2.503	5.12	— 0.02
sym-Diphenylthiourea	5.157	4.511	14.02	— 0.02
S = 14.04%	4.833	4.240	14.06	+ 0.02
	4.865	4.265	14.05	+ 0.01
	4.980	4.355	14.02	— 0.02
l-Cystine hydrochloride	5.295	6.739	20.40	— 0.03
S = 20.43%	4.678	5.983	20.50	+ 0.07
	3.795	4.853	20.50	+ 0.07
	4.445	5.686	20.51	+ 0.08
Sulfathiazole	5.385	8.474	25.23	+ 0.11
S = 25.12%	5.280	8.288	25.16	+ 0.04
	5.320	8.345	25.14	+ 0.02
	5.205	8.171	25.16	+ 0.04
Thiourea	3.345	8.796	42.15	+ 0.03
S = 42.12%	3.500	9.183	42.06	— 0.06
	3.860	10.151	42.16	+ 0.04
	3.320	8.736	42.18	+ 0.06
			SD of analytical errors = ± 0.04%	

Simultaneous Determinations of Halogens and Sulfur

A further investigation was made for the simultaneous determination of chlorine or bromine with sulfur. The absorption liquid in the combustion flask was transferred into a beaker with 50 ml of isopropyl alcohol and a few drops of 0.1% diphenylcarbazone solution was added. Titration with 0.005N mercuric nitrate solution was proceeded until pale yellow color turned to reddish purple (*3, 4, 12*). A few drops of 0.005N potassium bromide solution was then added to take back the former pale yellow color and the titration of sulfur was succeeded with the above mentioned

method. Analytical results with a few organic compounds are listed in Table 5, where practically the same accuracy of sulfur values as the exclusive sulfur determination has been indicated.

TABLE 5

SIMULTANEOUS DETERMINATIONS OF HALOGENS AND SULFUR IN ORGANIC COMPOUNDS

Sample	Sample wt. (mg)	X(%)		S(%)	
		Found	Deviation	Found	Deviation
Bromthymol blue	5.325	25.82	+ 0.22	5.12	— 0.02
Br = 25.60%	5.460	25.80	+ 0.20	5.16	+ 0.02
S = 5.14%	5.235	25.39	— 0.21	5.06	— 0.08
	4.750	25.32	— 0.28	5.04	— 0.10
l-Cystine hydrochloride	4.290	22.36	— 0.28	20.50	+ 0.07
Cl = 22.64%	3.610	22.41	— 0.23	20.40	— 0.03
S = 20.43%	3.430	22.42	— 0.22	20.50	+ 0.07
	4.295	22.53	— 0.11	20.53	+ 0.10

SUMMARY

An accurate titration method has been introduced for the determination of sulfate ion in connection with the microdetermination of organic sulfur using the flask combustion. Arsenazo(III) has shown superior reproducibility to that of thorin or sulfonazo(III) as it was used as an indicator during the titration with barium perchlorate solution in a nonaqueous medium. A further improvement of the reproducibility has been attained by a back titration of excess barium perchlorate with standard sulfuric acid solution. Application to the flask combustion of organic sulfur has been successfully achieved with an excellent accuracy of a standard deviation of ± 0.04% from a series of analytical data. Simultaneous determination of halogens and sulfur has been also accepted with the method.

REFERENCES

1. ALICINO, J. F., The determination of sulfur in organic compounds. *Microchem. J.* **2**, 83-90 (1958).
2. BUDESINSKY, B., Modification of flask method of sulfur determination. *Anal. Chem.* **37**, 1159 (1965).
3. CHENG, F. W., A rapid method for microdetermination of halogens in organic compounds. *Microchem. J.* **3**, 537-542 (1959).
4. COOK, W., A modified method for the microdetermination of halogens in organic compounds. *Microchem. J.* **5**, 67-71 (1961).
5. FRITZ, J. S., AND YAMAMURA, S. S., Rapid microtitration of sulfate. *Anal. Chem.* **27**, 1461-1464 (1955).
6. INGRAM, G., "Methods of Organic Elemental Analysis." Chapman and Hall, London, 1962.

7. SAVVIN, S. B., DEDKOV, Y. M., AND MAKAROVA, Y. P., New metalloindicators for barium ions: Determination of sulfates. *Zh. Anal. Khim.* **17**, 43-47 (1962).
8. SCHÖNIGER, W., Eine mikroanalytische Schnellbestimmung von Halogen in organischen Verbindungen. *Mikrochim. Acta* **1955**, 123-129.
9. SCHÖNIGER, W., Die mikroanalytische Schnellbestimmung von Halogenen und Schwefel in organischen Verbindungen. *Mikrochim. Acta* **1956**, 869-876.
10. STEYERMARK, AL, "Quantitative Organic Microanalysis." 2nd ed. Academic Press, New York, 1961.
11. WAGNER, H., Beitrag zur Mikrobestimmung des Schwefels in organischen Substanzen. *Mikrochim. Acta* **1957**, 19-23.
12. WHITE, D. C., Microdetermination of chlorine and bromine in organic compounds. *Mikrochim. Acta* **1961**, 449-456.

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Use of the 1931 C.I.E. System of Color Measurement for the Quantitative Study of Spot Test Reactions[1, 2]

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The use of classical spot-test techniques is quite common in biomedical research for the identification and semi-quantitative estimation of components separated by paper or thin-layer chromatographic procedures, and in environmental health programs for the estimation and detection of air pollutants. The lower limit of identification of such methods can usually be established with considerable precision, but further quantitative results depend on the visual or instrumental estimation of color. Visual comparisons involve a subjective estimate of color intensity, and can be adversely affected by changes in the hue and saturation of the color (*11*, *18*). Instrumental estimates are generally made from a reflectance spectrum. Although Ingle and Menshall (*5*) seemed to obtain adequate results with direct, single-reading photometry, much of the data in the literature on the reproducibility of such measurements is not encouraging (*11*, *13*, *17*, *19*). And with a copper-pyridine-barbiturate complex system, Braddock and Marec (*1*) were not able to show any significant improvement in accuracy and precision in the comparison of instrumental techniques to visual estimates. Any improved use of spot-tests must depend on a more thorough knowledge of the effects of variables such as substrate properties and reagent levels which affect adsorption and migration; reaction kinetics and

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equilibria; precipitate size; external lighting; etc.; and the relationship between these and the color formed and/or observed. In this paper, we are presenting the results of an investigation into the use of the C.I.E. system of color measurement (*14*, *18*) as a quantitative tool for such a study of spot-test systems.

The choice of the potassium chromate-silver chromate system for investigation was due to several factors. The reactants, first of all, were readily available and in a high state of purity, enabling precise control of concentration levels to be attained and unfavorable side reaction to be avoided. Secondly, the reaction, being an old and sensitive microchemical test for silver (*3*, *10*), was a familiar one to all chemists. In addition, silver chromate-impregnated substrates have been used for the qualitative detection of many anions (*12*), and have had particular semi-quantitative uses in the clinical measurement of sweat chloride in children (*8*, *15*), thus providing a diagnostic tool in the study of cystic fibrosis. We therefore felt that the study of this system would not only satisfy our immediate needs for the problem at hand, i.e., the effect of changes in reaction variables on the color observed with spot-test techniques, but also might yield data of wider applicability to the analytical and clinical chemist.

MATERIALS AND METHODS

Instrumentation and calculations. Reflectance spectra of samples were obtained with a Beckman DK-2 spectrophotometer equipped with a reflectance attachment. All readings were taken relative to a magnesium carbonate standard. The instrument required a sample size of about 1 inch square. Relative reflectance readings were taken from the recorded trace every 5 mμ from 380-700 mμ, and the chromaticity coordinates and tristimulus values were calculated with a programmed IBM 1620 computer. The statistical handling of the data was similar to that in a previous publication (*1*). A Bausch & Lomb L-1 polarizing microscope was used for optical crystallographic estimates and particle size determinations.

Silver and potassium chromate immersion spots. Whatman #120 papers, 1 inch square and 2 inches square in area, were totally immersed in aqueous potassium chromate solutions (0.05 and 0.15*N*) for a predetermined time (1, 5, 10, 30, 60, 120 minutes and 24 hours), rinsed with distilled water, and dried under an infrared lamp. The gain in weight was obtained with a Mettler H-15 balance. Some samples were rinsed with alcohol prior to drying.

Another series of immersions were made in different strengths of potas-

sium chromate solutions with square papers 1.5 in. on an edge for a constant time of 10 min. Concentrations were varied from 0.05 to 6.4*N*, and the gain in weight as a function of concentration was observed. A similar set of data were collected using various concentration levels (0.1 to 7.2*N*) of aqueous silver nitrate.

A series of papers were immersed in various potassium chromate solutions (0.05, 1.0, 5.0*N*), followed by immersion in various silver nitrate solutions (0.01, 0.1, 1.0*N*). For half the runs, the papers were not dried between the two immersions. All combinations of the different solute levels were studied qualitatively for appearance and uniformity of the colored precipitate.

Eight papers were immersed in 0.05*N* potassium chromate, dried, and weighed. Four were then immersed in 0.1*N* silver nitrate and four in 1.0*N* silver nitrate, and the dried paper was weighed again. The experiment was repeated with eight other papers which were first immersed in 5.0*N* potassium chromate.

Papers of equal area were next immersed in different potassium chromate solutions (0.05, 0.01, 0.50, 1.00, 2.00*N*), dried, and the reflectance spectrum for the obverse and reverse side obtained for each sample preparation. The papers were then immersed in various silver nitrate solutions (0.05, 0.1, 0.5, 1.0, 2.0*N*), dried, and the obverse and reverse reflectance spectrum again obtained for each paper. From the reflectance spectra it was possible to calculate the chromaticity coordinates and the *Y* tristimulus values for each side of each paper for all three standard light sources. Sixteen papers were used, and the order of immersion and data collection were randomized. Ten minute immersions were used.

Several of the silver chromate immersion spots were stored in the dark and several stored without any precaution against light exposure. The reflectance spectra were obtained for these at intervals and the color coordinates calculated.

All solutions were prepared, at 25°C, from reagent grade chemicals by weight and dilution to volume with distilled water.

Particle size studies. Copper sulfate pentahydrate and potassium ferricyanide were chosen as solid materials for the study because they both show significant color changes with particle size, and they represent colors at two spectral extremes. Crystalline potassium ferricyanide was graded with a set of standard sieves into four classifications, less than 88 μ, 88-125 μ, 125-590 μ, and greater than 590 μ. The copper sulfate pentahydrate was ground and similarly sieved into the following groups: less than 62 μ,

88-125 μ, and greater than 590 μ. Actual sizes and distributions were then determined microscopically with Martin's diameter (*2*). Optical crystallographic measurements indicated no change in composition had occurred with size reduction.

The "spots" were prepared by mixing the solid with a transparent, liquid binder and then applying the mixture to a paper substrate. The paste was allowed to dry and harden on the paper before reflectance measurements were made. From these spectra, it was possible to compute the chromaticity coordinates and Y tristimulus value as before.

The experimental design was a randomized block with two weight levels (a 2:1 ratio on a bulk volume basis) per particle size level, and two replicates per block. Absolute weights per "spot" were not determined, and were probably different for the two compounds studied.

Similar preparations were made for varying particle sizes of the following compounds: copper acetate monohydrate, potassium chromium sulfate 24 hydrate (65, 125, 285 μ), potassium dichromate (50, 105, 140, 315, 855 μ), and potassium chromate (90, 130 160 210, 330, 725 μ), all crystalline, as well as for ground potassium dichromate (45, 77, 210 μ) and potassium ferricyanide (35 and 55μ).

For each of the above preparations, the color coordinates were also determined from reflectance measurements made with the Bausch & Lomb Spectronic 20 as outlined in a previous paper (*1*). A similar check was made using the Beckman DU and its reflectance attachment.

Hiding power. A paper with a red hue and one with a yellow hue were both studied by cutting a series of circular holes in the paper, backing it with a white paper, and observing the change in color parameters with the increase in the number of holes. Each hole represented about 3% of the total area used for reflectance measurements. The reflectance measurements were made with a Bausch & Lomb Spectronic 20, equipped with a reflectance attachment, at ten selected ordinates as outlined in a previous publication (*1*).

Calculation of 95% confidence ellipses. The method of computing the 95% confidence ellipses for the chromaticity coordinates was based on the graphical construction reported by Mandell and Linnig (*9*). By using their nomenclature, the equations for the points necessary to construct the hexagon approximations are:

$$\pm L_x = S_x \sqrt{2F};\ d_x = S_x \sqrt{2F(1-r^2)} = L_x \sqrt{1-r^2} = \frac{L_x}{L_y} d_y$$

$$\pm L_y = S_y \sqrt{2F};\ d_y = S_y \sqrt{2F(1-r^2)} = L_y \sqrt{1-r^2}.$$

The final ellipses were inscribed within the constructed hexagon. The equation for the ellipse is:

$$\frac{(x-\bar{x})^2}{S_x^2} - \frac{2r}{S_x S_y}(x-\bar{x})(y-\bar{y}) + \frac{(y-\bar{y})^2}{S_y^2} = \chi^2(1-r^2),$$

where x and y are the two chromaticity coordinates, $\bar{x}$ and $\bar{y}$ are the means of their respective x and y sets, S_i is the standard deviation of each set, and r is the correlation coefficient for the set of x,y pairs. The χ^2 value is chosen for the number of degrees of freedom held by the standard deviation. The F value is based on the tabulated column for 2 degrees of freedom in the numerator and a denominator value equal to that assigned the standard deviation.

RESULTS

Adsorption properties for Whatman #120 papers. The amount of potassium chromate or silver nitrate adsorbed from aqueous solution followed a typical logarithmic curve (Figs. 1 and 2). The relative weight gain was independent of the substrate area and, between 1 minute and 2 hours, not significantly dependent on immersion time. From 2 hours to 24 hours, the relative weight increase was still slight, the 24-hour value being only 10% higher than the 2-hour value. Rinsing of the immersed paper in distilled water prior to drying had negligible effect on the total weight gain. The same observation was made when ethanol was used as a rinse, but here the nature of the surface was changed so that it became more brittle. For the remainder of the work, a 10-minute immersion time was chosen arbitrarily, and the use of organic solvents to enhance drying was avoided.

Regardless of the amount of potassium chromate adsorbed on the paper substrate, the lower limit of silver ion concentration for the formation of a detectable, colored precipitate of silver chromate by total immersion in aqueous silver nitrate was between 0.01 and 0.05N. When the chromate solution used for impregnation was 2 to 5N, silver nitrate solutions stronger than 1N resulted in the formation of a precipitate paste, some of which occasionally failed to adhere to the substrate surface. Between these extremes, it was possible to obtain uniform "spots" by the immersion technique. Reversal of the immersion procedure, i.e., initial impregnation in aqueous silver nitrate followed by drying and immersion in aqueous potassium chromate, appeared to have no significant effect on the weight changes or on the quality of the color produced; but this procedure was not used for the remainder of the study. It should be kept in mind that no quantita-

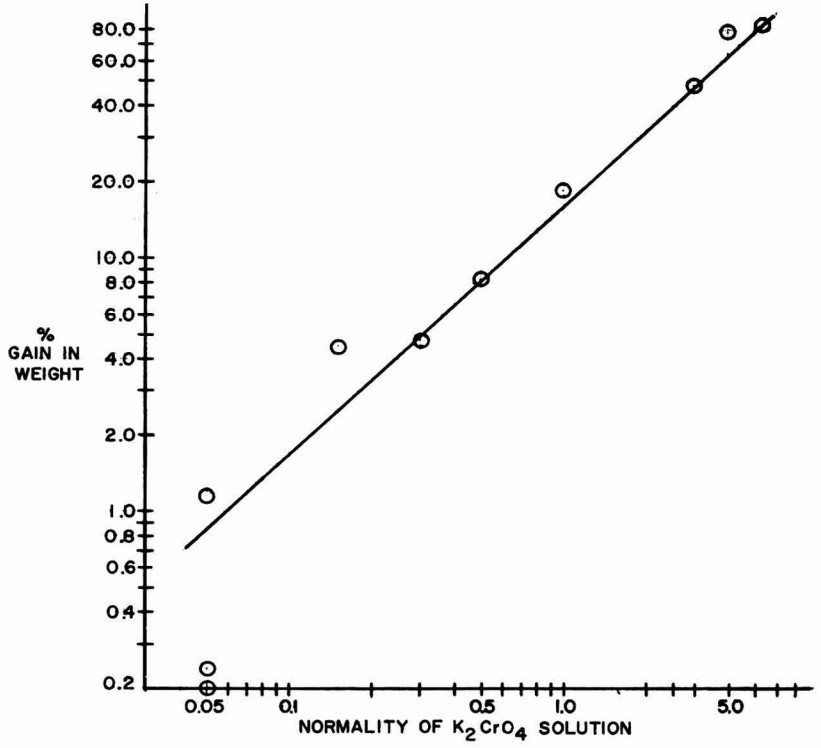

FIG. 1. Log-log plot of the relative gain in weight of Whatman #120 paper upon total immersion in aqueous potassium chromate as a function of the concentration of the immersion solution.

tive study of the color was attempted at this stage. Had it been, an effect would probably have been detected similar to those found in the remainder of the study.

The total weight increases obtained with the potassium chromate immersion and silver nitrate reimmersion have been reported in Table 1. In the case of the papers impregnated with 0.05*N* potassium chromate, the weight gain upon immersion in silver nitrate is far in excess of the stoichiometric increase estimated, but it is in good agreement with the weight increase expected from silver nitrate adsorption (in this case, 13.5 mg for the 0.1*N* solution and 125 mg for the 1.0*N* solution). For the papers impregnated with 5.0*N* potassium chromate solution, the adverse effect noted qualitatively above, is shown here quantitatively. The final observed weights are lower than anticipated, either from stoichiometry or from ad-

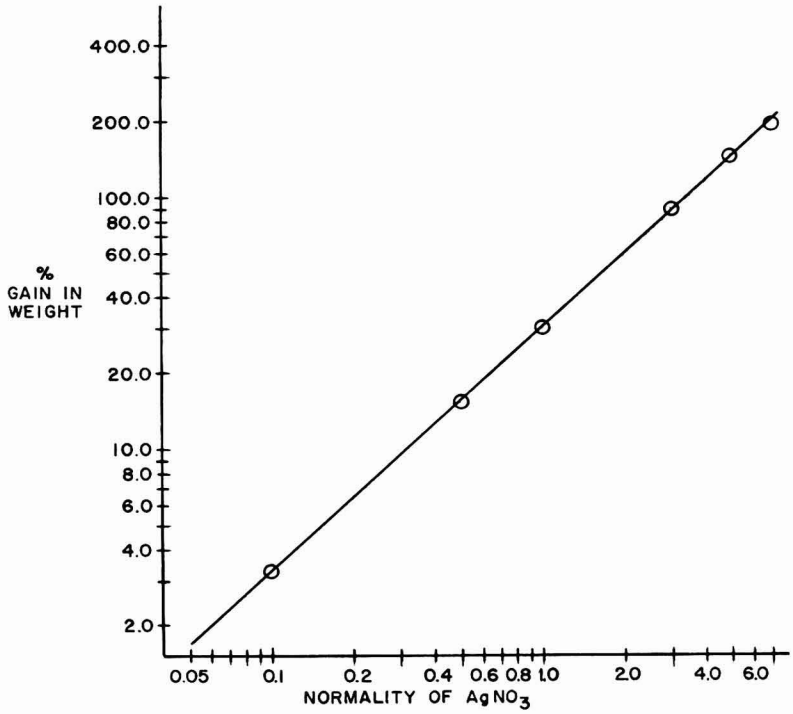

FIG. 2. Log-log plot of the relative gain in weight of Whatman #120 paper upon total immersion in aqueous silver nitrate as a function of the concentration of the immersion solution.

sorption considerations. The final weight for the paper immersed in 0.1*N* silver nitrate is close to the value of 21 mg, estimated with the assumption that the silver nitrate adsorption was typical, the amount adsorbed reacted

TABLE 1

TOTAL WEIGHT INCREASES FOR WHATMAN #120 PAPERS OF UNIFORM AREA IMMERSED IN AQUEOUS POTASSIUM CHROMATE SOLUTIONS FOLLOWED BY IMMERSION IN AQUEOUS SILVER NITRATE SOLUTIONS[a]

Concentration of potassium chromate soln.	Wt. of potassium chromate adsorbed (mg)	Conc. of silver nitrate soln. and total wt. increase above initial wt. of paper (mg)	
		0.1 N $AgNO_3$	1.0 N $AgNO_3$
0.05*N*	0.9 ± 0.4	13.9 ± 0.7	126.3 ± 3.5
5.0*N*	345.0 ± 7.8	27.8 ± 1.5	438.4 ± 29.6

[a] Precision Indices represent the 95% confidence limits for the reported mean, eight replicates for each chromate weight and four replicates for each of the others.

stoichiometrically with the potassium chromate present, the excess chromate redissolved, and the potassium nitrate formed did not. For the immersion in 1.0*N* silver nitrate, less chromate was lost. At any rate, it seemed apparent that a high chromate level on the substrate resulted in a considerable dissolution upon reimmersion.

Colorimetric properties of potassium chromate impregnated papers. The effect of light source and reagent concentration on the chromaticity coordinates is illustrated in Fig. 3. The tendency was for both coordinates to

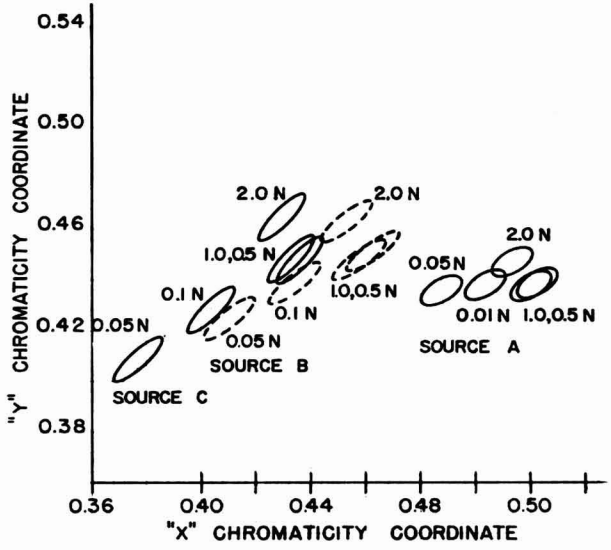

FIG. 3. Chromaticity ellipses of various Whatman #120 papers impregnated with potassium chromate by total immersion in aqueous solutions of different strengths and viewed with different standard light sources. Source A—incandescent lighting; source B—sunlight; source C—daylight.

increase with concentration in such a manner that the dominant wavelength remained essentially unchanged and the colorimetric purity became greater with increasing concentration. Actually, the dominant wavelength did tend to move slightly towards the red as the solution concentration was increased to about 0.5*N*. From 0.5*N* to 1.0*N* there was no apparent change in color. Beyond this range, the dominant wavelength began to shift towards the green with a further increase in purity as evidenced by the ellipse for the 2.0*N* solution.

The main effect of the light source was to cause a gross shift of the x

coordinate to larger values. With source A there was also a noticeable decrease in the magnitude of the concentration effect.

The *Y* tristimulus value also varied with concentration level as shown in Fig. 4. At low concentration levels, it tended to decrease rapidly with an increase in the chromate content, soon leveling off for a wide range, and then showing a tendency to increase again at higher levels. The effect of a change in light source was to cause an absolute shift in the tristimulus value. This shift was most noticeable over the flat portion of the curve.

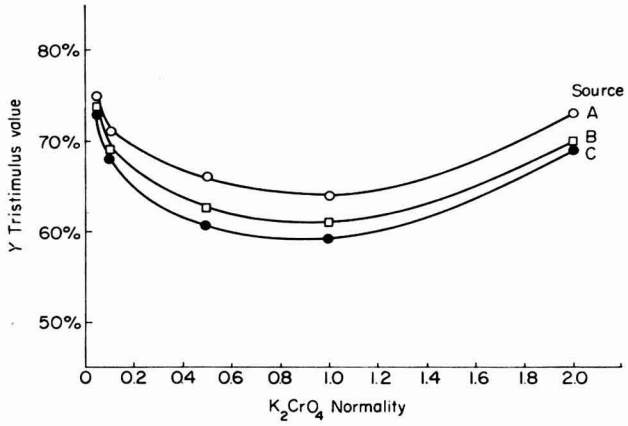

FIG. 4. The effect of immersion solution concentration and light source on the *Y* Tristimulus value for Whatman #120 papers impregnated with potassium chromate by total immersion.

No significant obverse/reverse differences could be found for either the chromaticity coordinates or the *Y* tristimulus values for the potassium chromate impregnated papers.

Colorimetric properties of silver chromate immersion "spots." The most noticeable effect was an increase in variance for the chromaticity coordinates and in relative variance for the *Y* tristimulus values (Table 2 and Fig. 5). This was probably a result of the inability to control the precipitation phase as closely as the adsorption phase of the experiment. The other predominant differentiating effect was the sign change in the correlation coefficient for source A chromaticity data (Table 2, and Fig. 6 in relation to Figs. 7 and 8). We have no explanation for this.

With the silver chromate-impregnated papers, a significant obverse/reverse effect was obtained for the *Y* tristimulus value at low chromate levels regardless of the concentration level of the silver nitrate solution

used for immersion (Figs. 9 and 10). This effect was also noticeable with high chromate levels but low silver nitrate concentration (Fig. 9). Figures 9 and 10 also indicate a relationship between reagent concentration and Y tristimulus value which is similar to that for potassium chromate impregnation in Fig. 4, but more complex because of interactions within the system. Changing the light source was found to have no appreciable effect on

TABLE 2

STANDARD DEVIATIONS AND CORRELATION COEFFICIENTS FOR CHROMATICITY COORDINATES AND TRISTIMULUS VALUES CALCULATED FOR PAPERS IMPREGNATED WITH POTASSIUM CHROMATE AND SILVER CHROMATE BY IMMERSION TECHNIQUES[a]

	Papers impregnated with potassium chromate			
	Standard deviation for chromaticity coordinates		Correlation coefficient	Standard deviation
Light source	x	y	x vs. y	Y tristimulus
A	0.0023 (30)	0.0014 (30)	0.250	0.022 (15)
B	0.0029 (30)	0.0021 (30)	0.862	0.029 (15)
C	0.0031 (30)[b]	0.0027 (30)	0.914	0.029 (15)
	Papers impregnated with silver chromate			
	Standard deviation for chromaticity coordinates		Correlation coefficient	Standard deviation
Light source	x	y	x vs. y	Y tristimulus
A	0.026 (4)	0.013 (5)	—0.775	0.0050 (10)
B	0.034 (4)	0.017 (5)	0.027	0.0068 (19)
C	0.035 (4)	0.024 (5)	0.235	0.0061 (28)

[a] Degrees of freedom in parentheses. Variances homogeneous at 5% and 1% level of significance.

[b] Significance level for homogeniety between 5% and 1%. Effect on confidence ellipse calculation is negligible.

the observed tristimulus value, possibly because of the low, absolute reflectances involved.

With the chromaticity coordinates, a slight obverse/reverse effect was noticeable only at the lowest levels of both reagents. This was also independent of the light source (Figs. 6-8). These graphs also illustrate the smallness of the change in chromaticity with changes in reagent concentration levels. One interesting observation is the reversal of the 2.0N silver ellipse in relation to the position of the ellipse for the lower concentration level as the chromate level of the substrate was increased from an immer-

sion solution strength of 0.5N to one of 1.0N. This effect was also independent of light source.

Particle size studies. Good linear coorelation was observed between the x and y chromaticity coordinates, and 95% confidence ellipses were drawn for the copper sulfate pentahydrate and potassium ferricyanide sets of data. These ellipses showed a movement with particle size similar to that

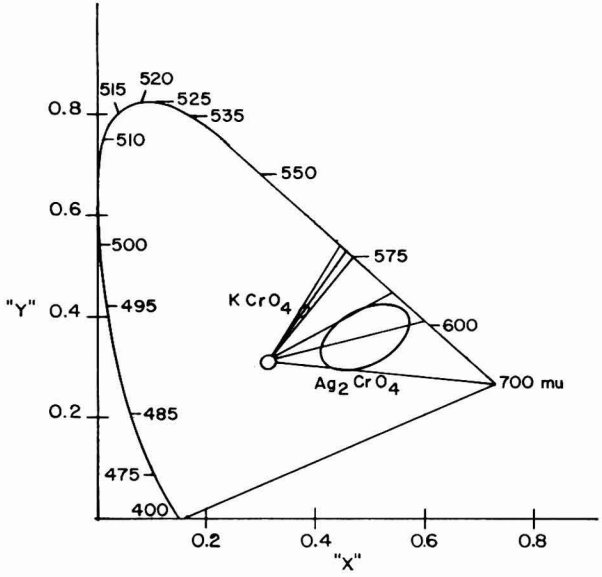

FIG. 5. Position of chromaticity ellipses for Whatman #120 papers impregnated with potassium chromate and silver chromate by total immersion techniques. Potassium chromate solution, 0.05N; silver nitrate solution 0.1N; source C. Lines through midpoint of ellipses provide estimates of color purity and dominant wavelength. Lines tangent to ellipses provide precision indices (95% confidence limits) for this wavelength.

observed for changes in concentration levels in the impregnation experiments already described. The conclusions drawn from these ellipses for particle size effects are summarized below.

For copper sulfate pentahydrate, the color purity decreased significantly (i.e., the chromaticity coordinates shifted towards the white point) with decreasing particle size, and the dominant wavelength shifted towards the green (Table 3). The Y tristimulus value tended to increase slightly with decreasing particle size (Table 5). The amount of copper sulfate present

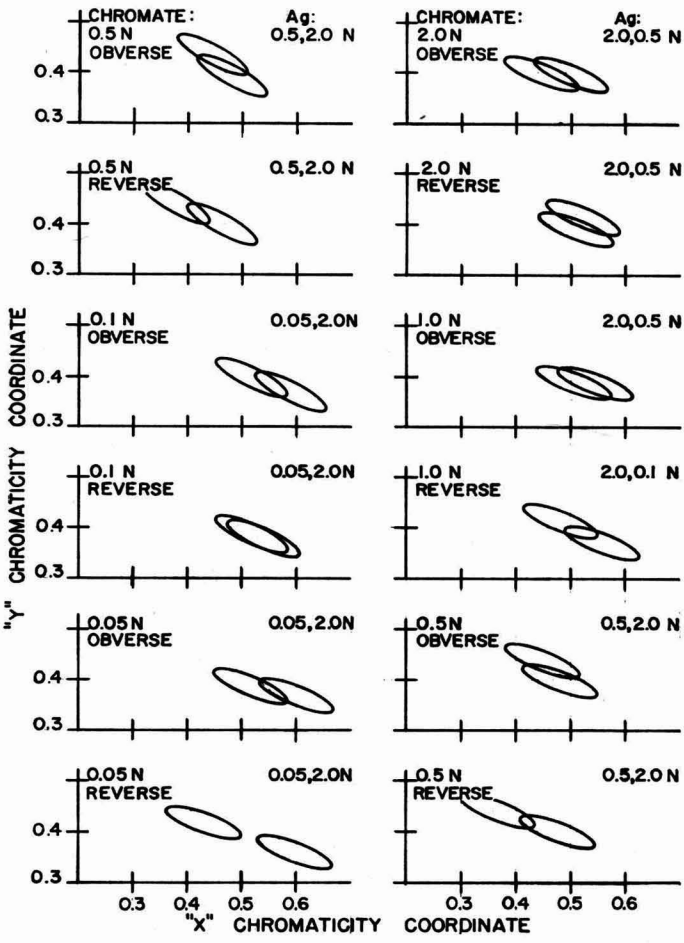

FIG. 6. Chromaticity ellipses of Whatman #120 papers after immersion impregnation with potassium chromate and subsequent precipitation of silver chromate by immersion in aqueous silver nitrate. Concentration levels refer to the immersion solutions. Source A.

had no effect on the chromaticity coordinates. For the Y tristimulus values, there also were no significant changes with the amount of material until the particle size was reduced to between 50 and 100 μ; at which point a rather sharp rise occurred with the increased weight of material used.

For potassium ferricyanide, the color purity was observed to increase with reduced particle size, but the dominant wavelength shift was still

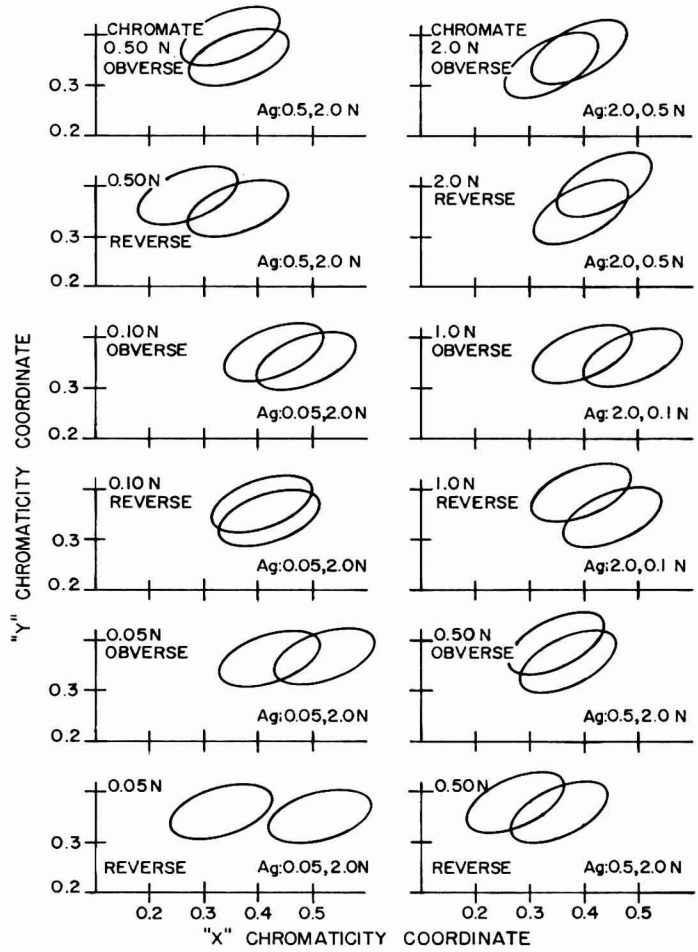

FIG. 7. Chromaticity ellipses of Whatman #120 paper after immersion impregnation with potassium chromate and subsequent precipitation of silver chromate by immersion in aqueous silver nitrate. Concentration levels refer to the immersion solutions. Source B.

towards the green (Table 4). The chromaticity coordinates were not affected significantly by the amount of material present. The Y tristimulus values (Table 6) tended to increase as before with a reduction in particle size, the increase being more apparent the greater the amount of material present. In this system, it was not possible to show any apparent effect at constant particle size for the amount of material present.

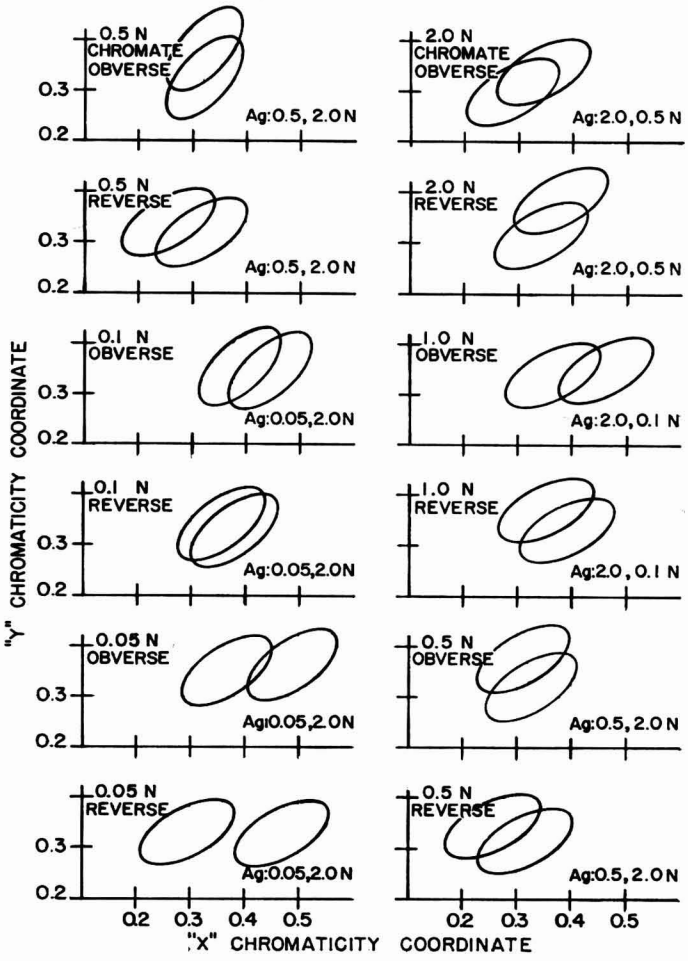

FIG. 8. Chromaticity ellipses of Whatman #120 paper after immersion impregnation with potassiom chromate and subsequent precipitation of silver chromate by immersion in aqueous silver nitrate. Concentration levels refer to the immersion solutions. Source C.

The data obtained for several other compounds are presented in Table 7. In some of the systems, the color parameters showed definite changes with particle size; in other systems, the changes were very slight. The effect of ground vs. crystalline material was studied for both potassium dichromate (Table 7) and potassium ferricyanide (Table 8). Both showed

minimal changes as far as chromaticity coordinates were concerned, the major difference being in the *Y* tristimulus value.

Hiding power. The data collected (Table 9) were not extensive, and the effect of coverage on the chromaticity coordinates seemed to follow no pattern other than a gross decrease in color purity with decreased coverage. This extreme variability could have been due, in part, to the rather large shifts observed in the *Y* tristimulus values between specimens. These tri-

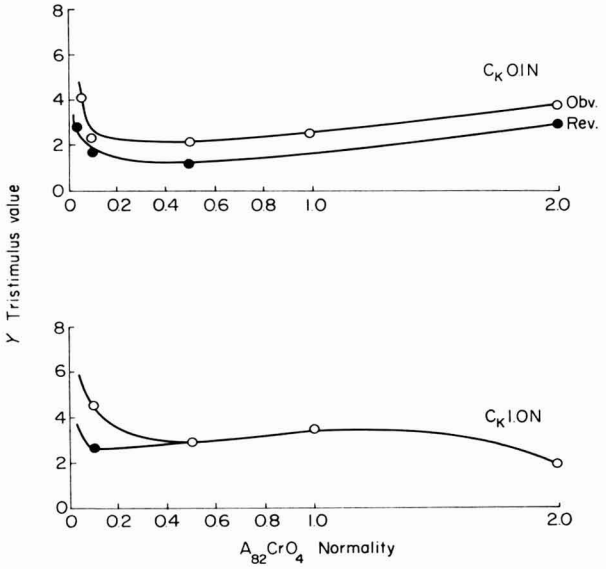

FIG. 9. The effect of reagent concentrations on the *Y* Tristimulus value as %, for Whatman #120 paper impregnated with silver chromate by total immersion techniques. Source C. ⊗ refers to reverse side of paper when a difference occurs.

stimulus values, when graphed with the fraction coverage as the abscissa, yielded a curve similar to the tristimulus vs. concentration curve in Fig. 4.

Effect of aging. Slight shifts in the values of the color parameters were observed with the age of the samples of silver chromate. The magnitude of the shifts were small, however, and the time required sufficiently long to make the changes of no practical importance in the study. The changes appeared to be a function of time rather than of exposure or lack of exposure to light. This observation has also been reported for this compound in the early literature (*10*).

Instrument variations. The absolute values of the color parameters were

found to depend on the number of ordinates chosen and the spectrophotometric equipment used for the reflectance measurements. The relative values, within one instrumental setup, however, were not affected and were generally comparable between instrumental systems.

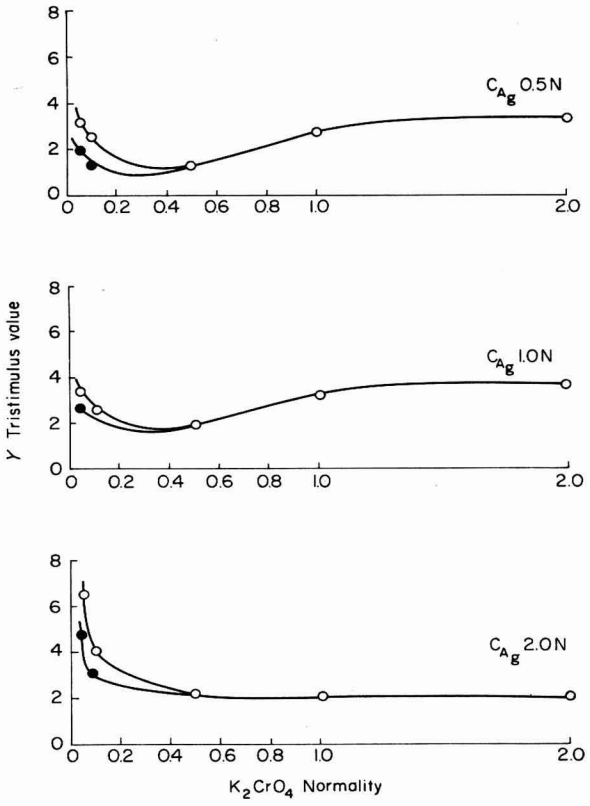

FIG. 10. The effect of reagent concentrations on the *Y* Tristimulus value as %, for Whatman #120 paper impregnated with silver chromate by total immersion techniques. Source C. ⊗ refers to reverse side of paper when a difference occurs.

DISCUSSION

It is clear, from an examination of the computational procedure for the chromaticity coordinates (*14*), that these x and y values are not independent of each other. Regression analyses of y on x calculated for a particluar system generally yielded a significant linear correlation coefficient. In those cases in which this coefficient was not significantly different from

TABLE 3

EFFECT OF PARTICLE SIZE ON CHROMATICITY COORDINATES FOR COPPER SULFATE PENTAHYDRATE

Particle size (μ)	Chromaticity coordinates	
	x	y
50 ± 5	0.233	0.287
110 ± 10	0.226	0.285
160 ± 10	0.207	0.268
370 ± 60	0.200	0.251
1000 ± 90	0.186	0.224

TABLE 4

EFFECT OF PARTICLE SIZE ON CHROMATICITY COORDINATES FOR POTASSIUM FERRICYANIDE CRYSTALS

Particle size (μ)	Chromaticity coordinates	
	x	y
70 ± 15	0.50	0.40
120 ± 10	0.50	0.40
415 ± 35	0.48	0.35
715 ± 20	0.48	0.35

TABLE 5

EFFECT OF PARTICLE SIZE ON Y TRISTIMULUS VALUE FOR COPPER SULFATE PENTAHYDRATE

Particle size (μ)	Y Tristimulus value	
	Amt. W.	Amt. 2 W.
50 ± 5	0.29	0.36
110 ± 10	0.31	0.34
160 ± 10	0.30	0.32
370 ± 60	0.27	0.26
1000 ± 90	0.24	0.24

TABLE 6

EFFECT OF PARTICLE SIZE ON Y TRISTIMULUS VALUE FOR POTASSIUM FERRICYANIDE CRYSTALS

Particle size (μ)	Y Tristimulus value	
	Amt. W.	Amt. 2 W.
70 ± 15	0.21	0.24
120 ± 10	0.21	0.21
415 ± 35	0.15	0.15
715 ± 20	0.15	0.13

TABLE 7

EFFECT OF PARTICLE SIZE ON VARIOUS COLOR PARAMETERS FOR DIFFERENT SUBSTANCES

Substance	Particle size (μ)	Chromaticity coordinates		Dominant wavelength	Purity	*Y* Tristimulus value
		x	*y*	mu		
$Cu(CH_3COO)_2 \cdot H_2O$	65	0.128	0.263	487	72%	0.044
	110	0.145	0.291	489	63	0.044
	145	0.145	0.257	487	66	0.033
	1070	0.229	0.293	487	32	0.034
$Cr_2(SO_4)_3 \cdot K_2SO_4 \cdot 24\ H_2O$	65	0.291	0.268	566[a]	16%	0.20
	120	0.292	0.276	567[a]	13	0.23
	285	0.298	0.282	565[a]	12	0.24
$K_2Cr_2O_7$ (crystalline)	50	0.564	0.379	596	85%	0.21
	105	0.560	0.375	597	83	0.21
	140	0.570	0.373	598	85	0.20
	315	0.585	0.360	601	85	0.22
	850	0.590	0.346	605	83	0.17
$K_2Cr_2O_7$ (ground)	45	0.555	0.397	593	88%	0.28
	75	0.551	0.391	594	85	0.26
	210	0.572	0.372	598	85	0.24
K_2CrO_4	90	0.465	0.486	576	87%	0.59
	130	0.466	0.487	576	88	0.63
	160	0.468	0.482	577	87	0.64
	210	0.462	0.484	576	86	0.66
	330	0.498	0.482	578	90	0.57
	725	0.480	0.484	578	91	0.60

[a] Complimentary wavelength.

TABLE 8

EFFECT OF PARTICLE SIZE ON CHROMATICITY COORDINATES AND *Y* TRISTIMULUS VALUES FOR GROUND FERRICYANIDE FRAGMENTS

Particle size (μ)	Amt. of solid in preparation	Chromaticity coordinates		*Y* Tristimulus value
		x	*y*	
34 ± 5	W	0.41	0.39	0.097
	2W	0.44	0.40	0.095
53 ± 10	W	0.48	0.40	0.098
	2W	0.47	0.40	0.13

TABLE 9

EFFECT OF AREA COVERED BY THE COLORED MATERIAL ON THE CHROMATICITY COORDINATES AND THE *Y* TRISTIMULUS VALUES

% Area not covered	Hue: Red			Hue: Yellow		
	Chromaticity coordinates			Chromaticity coordinates		
	x	*y*	*Y* Tristimulus	*x*	*y*	*Y* Tristimulus
0 (all red or yellow)	0.444	0.301	0.21	0.397	0.408	0.63
3	0.312	0.307	0.71	0.343	0.368	0.81
6	0.315	0.277	0.42	0.368	0.376	0.68
8	0.391	0.306	0.31	0.376	0.385	0.68
17	0.396	0.325	0.39	0.375	0.386	0.67
22	—	—	—	0.305	0.319	0.78
100 (all white)	0.307	0.313	0.88	0.307	0.313	0.88

zero, an inspection of the data usually indicated the presence of a straight line relationship parallel to one of the coordinate axes. The possibility of treating the chromaticity coordinates as a normal, bivariant population was thus strongly suggested, and this approach, used here and in a previous publication (*1*), proved to be quite fruitful. Not only did it provide a comparison of two or more sets of coordinate points within a known level of confidence, but it also provided a means of assigning definite confidence limits to graphical estimates of color purity and dominant wavelength (Fig. 5). Furthermore, from the viewpoint of their relative positions, relative axial ratios, and angles of their major axis with the abscissa, these confidence ellipses bore a striking resemblance to those obtained by MacAdam (*7*) in his study of the sensitivity of a single observer toward discernable chromaticity differences (Table 10). The overall larger size of the ellipses obtained was simply a reflection of the difficulty in controlling the adsorption and precipitation processes involved. This handling of the chromaticity coordinates, together with the treatment of the *Y* tristimulus values as a typically normal population, provided a useful measuring tool to investigate further the effect of reaction variables on the nature of the color observed in a spot-test procedure. The remainder of the data in the paper serves as an illustration of this use.

From the weight data shown in Table 1 and Figs. 2 and 3, it would appear that the adsorption isotherms of the two reactants played a primary

role in the precipitation process. For example, the adsortion of silver nitrate seemed to proceed independently of any prior impregnation of the paper substrate with potassium chromate. From the premise of adsorption control of the precipitation process, it is possible to construct a mechanistic model which is, in turn, reflected by the observed changes in color parameters with changes in reagent concentrations.

TABLE 10

COMPARISON OF CONFIDENCE ELLIPSES FOR SILVER CHROMATE SUBSTRATES FORMED FROM 2.0*N* SOLUTIONS WITH THRESHOLD DIFFERENCE ELLIPSES OBTAINED FOR SINGLE OBSERVERS BY MAC ADAM (*7*)

Origin of data	Light source	Ellipse Center x	Ellipse Center y	Length of axes in Chromaticity U. Major	Length of axes in Chromaticity U. Minor	Angle of major axis with abscissa	*Y* Tristimulus value
MacAdam	C	0.475	0.300	0.00289	0.00099	29°	—
Silver chromate	C	0.291	0.302	0.192	0.096	31°	2.31%
	B	0.330	0.338	0.180	0.090	23°	2.29%
	A	0.440	0.395	0.144	0.040	158°	2.27%

Beginning with low levels of chromate impregnation, one would expect the amount of silver chromate formed to be a function of the amount of silver nitrate taken up from the solution by the substrate, since the potassium chromate level remains fixed. The amount of precipitate, and the color parameters given by it, would then be an indirect function of the concentration of the silver nitrate solution used. Such a relationship should hold until the silver nitrate concentration became sufficiently high to yield a quantitatively complete reaction with the chromate present. A further increase in silver nitrate concentration would add to the weight increase of the substrate but could produce no more precipitate and, thus, no further change in the color parameters. On the other hand, an increase in the amount of potassium chromate impregnated on the substrate would be expected to extend one's ability to differentiate between silver nitrate concentration at higher levels. A close study of the graphs relating to the chromaticity coordinates and *Y* tristimulus values shows a trend in the movement of these color parameters with reagent concentration changes which is consistent with the model so far presented.

As the reagent concentration levels continue to be increased, however, other effects would be exected to appear. Junge (*6*), for example, has shown that the von Weimarn (*16*) relationship of concentration to precipi-

tate particle size occurs at a solid/liquid interface as well as in solution. Gulbranson and co-workers (*4*), in an electron microscopic study of the silver chromate precipitation process involving diffusion of the silver nitrate through a membrane, have shown the expected decrease in particle size with an increase in silver nitrate concentration in the concentration ranges used in this study. Such changes in particle size could affect the color parameters directly, as shown by the data in Tables 3-8, or indirectly by a change in the hiding power of the precipitate. The color parameter data obtained by us showed the presence of some type of change at these higher concentrations, but was not of the kind which could delineate the exact nature of the change.

Another effect of increased concentration, however, would be the eventual saturation of the substrate and the loss of an obverse/reverse surface effect. Such an effect would be anticipated at the lower concentration levels because the adsorptive properties of most paper surfaces are found to be different. With low chromate levels, this effect should be observable at all silver nitrate levels, since adsorption of the latter reagent in excess of the chromate level would have no effect on the amount of material precipitated. The absolute values of the color parameters might be expected to level off beyond a certain silver nitrate level, but the surface differentiation should remain (Fig. 9).

At higher chromate levels, there was indirect evidence to support the contention that the surfaces had been saturated and additional material had been adsorbed to the primary chromate layer rather than the substrate itself. The weight loss observed upon reimmersion of papers with high levels of chromate impregnation, Table 2, and the poor adhesion properties of the silver chromate precipitates formed under these conditions, regardless of the silver nitrate concentration used for reimmersion, were interpreted in this manner.

If this interpretation were true, one would expect some obverse/reverse effect to occur, because of preferential adsorption with low levels of silver nitrate, but to disappear as the amount of silver nitrate adsorbed was increased. The presence of both types of obverse/reverse effect is shown in the behavior of the Y tristimulus value in Figs. 9 and 10, and to a lesser extent, in the diagrams of the behavior of the chromaticity coordinates.

The data collected for particle size variations did show, in addition, the possibility of mistaking a particle size shift for a change in the amount of precipitate formed. All three color attributes changed with particle size in a manner analogous to that observed for changes in reagent concentration

levels. This was particularly well illustrated by the data for copper sulfate and potassium ferricyanide. A possible dependence of Y tristimulus value with amount of product was also found for low particle sizes of copper sulfate. Precipitates of silver chromate formed in this study were around 2-5 mμ (*4*), which is well below the size for which data were collected, so that the results obtained probably reflect an interaction between amount and size.

Of greater interest was the close resemblance of the Y tristimulus behavior with hiding power to that observed with changes in reagent concentration levels, particularly those for potassium chromate impregnation in Fig. 4. The possible relationship between hiding power and particle size, and thus concentration, has already been alluded to.

The effect of light source was of minimal value. A shift in the absolute values was observed, as anticipated, but no particular effect on relative changes was noticed.

In summary, therefore, it has been shown that the 1931 C.I.E. system of color measurement (*14*) can be used to determine color parameters of spot test precipitates, and these parameters can be related to changes in the reagents' concentrations as long as one also takes into consideration the adsorption isotherms of the reagents and the relationship of reagent concentration to precipitate particle size and, thus, to precipitate hiding power. The use of the chromaticity coordinates can be enhanced by treating them as a normal, bivariant population. Any attempt to improve the precision of spot test procedures must, of necessity, involve a knowledge of the topographical nature of the reaction and an attempt to control reagent adsorption, surface migration, and precipitate particle size as well as reaction stoichiometry.

REFERENCES

1. Braddock, L. I., and Marec, N., Use of reflectance spectra for the estimation of barbiturates by spot test techniques. *Microchem. J.* **11**, 121-138 (1966).
2. Chamot, E. M., and Mason, C. W., "Handbook of Chemical Microscopy," Vol. 1, 2nd ed. Wiley, New York, 1938.
3. Feigl, F., "Qualitative Analysis of Spot Tests," p. 13. Nordemann, New York, 1937.
4. Gulbransen, E. A., Phelps, R. T., and Langer, A., A use of the electron microscope in chemical microscopy. *Ind. Eng. Chem., Anal. Ed.*, **17**, 646-652 (1945).
5. Ingle, R. B., and Menshall, E., Qualitative estimation of paper chromatograms by direct photometry, I and II. *J. Chromatog.* **8**, 369-385 (1962).
6. Junge, A. E., unpublished data, Pittsburgh Plate Glass Co., Harmarville, Pennsylvania.

7. MacAdam, D. L., Visual sensitivities to color differences in daylight. *J. Opt. Soc. Am.* **32**, 247-249 (1942).
8. McFarlane, J. C., Norman, A. P., and Stroud, C. E., Fingerprint sweat test in fibrocystic disease of the pancreas; preliminary communication, *Brit. Med. J.*, **1957** (ii), 274-275.
9. Mandell, J., and Linnig, F. J., Accuracy in chemical analysis by using linear calibration curves. *Anal. Chem.* **29**, 743 (1957).
10. Mellor, J. W., "A Comprehensive Treatise on Inorganic Chemistry." Wiley, New York, 1937.
11. Pazdera, H. J., and McMullen, W. H., Paper chromatography. *In* "Treatise on Analytical Chemistry" (Kolthoff, I. M., and Elving, P. J., eds.) Part I, Vol. III, p. 1630. Wiley (Interscience), New York, 1961.
12. Sabo, J. G., Detection of anions with silver chromate-impregnated paper. *Chemist-Analyst* **54**, 110-111 (1965).
13. Sendroy, J., and Granville, W. C., Application of reflectance spectrophotometry to quantitative microanalysis. *Anal. Chem.* **19**, 500-501 (1947).
14. Smith, R., and Guild, J., C. I. E. colorimetric standards and their use. *Trans. Opt. Soc.* **33**, 73 (1932).
15. Swachman, H., and Gahm, N., Studies in cystic fibrosis of pancreas; simple test for detection of excessive chloride on skin. *New Engl. J. Med.* **255**, 999-1001 (1956).
16. Von Weimarn, P. P., "Die Allgemeinheit des Kolloidzustandes." Steinkopf, Dresden, 1925.
17. Winslow, E. H., and Liebhafsky, H. A., Spectrophotometric study of spot tests. *Anal. Chem.* **21**, 1338-1342 (1947).
18. Wright, W. D., "The Measurement of Color," 2nd ed. Higler and Watts, London, 1958.
19. Zeitlin, H., and Nimato, A., Comparison of transmittance and reflectance spectra of the 2.4-Dinitrophenylhydrazones of acetone and 4-methyl-2-pentanone. *Anal. Chem.* **31**, 1167-1170 (1959).

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Wet Chemical Analysis of Micro Amounts of Elements in Alloys

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The great advances made in aeronautics during the last 25 years and the advent of astronautics have led to a growing demand for special alloys to meet specific requirements with regard to resistance to extremely high temperatures, extraordinary stress, and corrosion without sacrifice of other desirable properties related to hardness, tensile strength, malleability, and ductility. As a result, a large variety of alloys has come into being. The composition of an alloy can be made up of three different amounts of elements: macro amounts, micro amounts, and trace amounts. The delineation of their exact range may be subject to opinion. For this report they shall be defined as follows: macro amounts are those of 2% and more; less than 2% down to 0.01% are micro amounts; and trace amounts are defined as less than 0.01%. All alloys contain of course macro amounts and some are composed of macro amounts only. However, most alloys contain also micro amounts and/or trace amounts. These are either intentionally added or have found their way into the alloy through ores or scrap used in the process of manufacture.

Prior to the introduction of modern procedures based on optical, electrical, and electromagnetic principles, respectively, the chemist had, aside from colorimetric methods, only few means at his disposal to analyze quantitatively for trace amounts. Today their analysis has become not only routine, but these modern methods have to a large extent replaced gravimetric, volumetric, and colorimetric procedures for the determination of micro amounts as well. They have proven to give good results with much less effort in much less time than wet chemical methods. And yet these latter methods still have their merits, as shall be demonstrated in this report which will be confined to the determination of micro amounts only. Although theoretically it should also be possible to apply wet chemical procedures to the determination of trace amounts by starting

out with a very large weighing (100 g and more), the handling and manipulations of such large amounts and the correspondingly large precipitates would render the analysis not only extremely cumbersome and time consuming but would also be detrimental to obtaining accurate results. We have never attempted to analyze trace amounts by wet chemical analysis.

Physicochemical methods for the determination of trace amounts, such as spectrographic and X-ray spectroscopic procedures, have their flaws which are principally caused by too many lines in the spectrum and their overlapping when a large number of elements (five and more) is present in an alloy as trace amounts. The same holds true with regard to micro

TABLE 1

THEORETICAL WEIGHTS OF ANALYTICAL END PRODUCTS FOR VARIOUS ELEMENTS

Alloy contains (in %)	End product is (in mg)
0.061 silicon	6.548 silica
0.015 sulfur	5.430 barium sulfate
0.012 phosphorus	43.700 ammonium phosphomolybdate
0.031 lead	2.310 lead sulfate
0.033 molybdenum	2.500 molybdenum trioxide
0.014 nickel	3.600 nickel dimethylglyoxime
0.017 chromium	4.180 barium chromate
0.039 titanium	3.250 titanium oxide

amounts, though to a lesser degree. For the analysis of micro amounts of various elements in a great variety of alloys, we have found wet chemical methods to be equal in performance to physicochemical methods, in some cases even better. It is of course quite possible that modern methods with still more improved instruments may in the future entirely replace wet chemical analysis for the determination of micro amounts.

By starting out with a large sample weighing (from 1 to 10 g) the weight of the end product in the course of an analysis will always amount to at least 2 mg, which can be easily and accurately weighed out on a microanalytical balance or analyzed by a volumetric procedure. To give some examples a simple calculation shows that on the basis of a 5-g sample the end products listed in Table 1 can be expected theoretically.

In our way of analyzing micro amounts of elements, two features stand out. The analysis of micro amounts of elements present in an alloy, regardless of the number of elements present as macro amounts, is carried out in a single sample weighing, except for carbon determination which

requires a separate weighed portion. Furthermore one or more of the main constituents whenever feasible and appropriate are removed by precipitation or ether extraction prior to the analysis of the micro amounts. The separation and removal of silicon, whether present in macro amounts, micro amounts, or trace amounts, is already carried out in the process of dissolving the sample. Wet chemical analysis of micro amounts in an alloy, and for that matter in any material, can by no means be looked upon as mere routine work. To assure success it requires not only special skill and strict attention to details but also the planning of an ingenious course of analysis which must be well adapted for each special case.

The analysis of eight alloys, from among the many which have been analyzed in our laboratory, will be reported. Their selection has been made with the purpose of demonstrating the analysis of those elements which are most often present in alloys as micro amounts in various combination with macro amounts. Among these eight are four which contain trace amounts of various elements as well. While these may interfere in the analysis of micro amounts by physicochemical methods, their interference in wet chemical analysis is negligible. Hence their presence can be and has been ignored. The fact that none of these alloys contain carbon in micro amounts explains the absence of carbon analysis from this report.

GENERAL REMARKS

Whenever possible the analysis has been carried out on a sample in powder form. If such was not available, drillings or chips have been used, cut into pieces as small as possible to facilitate their dissolution. To get an alloy into solution either acid treatment or fusion or a combination of both has been applied. No single reagent will act as a solvent for all alloys. While nitric acid, hydrochloric acid, and sulfuric acid, each alone or in proper combination, dissolve at elevated or boiling temperatures quite a number of alloys, others require in addition an oxidizing agent. Among these $KClO_3$ has been found to be one of the most effective and universal. Ferro-alloys containing among others large amounts of silicon, chromium, vanadium, and titanium, respectively, are known to resist attack by acids to such a degree that dissolution remains incomplete even when the acid is used in combination with an oxidizing agent. In such cases we have always applied a combination of acid treatment and fusion rather than the latter alone, because fusion of large amounts of an alloy is not easy to accomplish. Besides a combination treatment requires a much smaller amount of fusion agent. The alloy is first treated with an acid to dissolve

as much as possible, and after the mixture has been evaporated to dryness the residue is subjected to fusion. This has been mostly carried out with sodium peroxide which is an excellent fusion agent for many alloys. In getting the alloy into solution care must of course be taken to prevent volatilization of constituents such as phosphorus and boron. All evaporations to dryness have been carried out in two steps by applying heat to the surface with the aid of an electric bulb. In the first step the solution is evaporated to near dryness and then to complete dryness with less heat to prevent baking of the residue. The Fisher radiator with built-in rheostat has proven to be very effective for this purpose. All filtrations have been carried out by decantation as complete as possible without allowing more than a very small amount of precipitate to go over into the filtering device. A precipitate has been transferred to the filter only after it has received a thorough washing with the respective agent. As told above we have made it a practice to remove whenever possible the main constituents prior to the analysis of the micro amounts. Such a procedure entails, of course, rather large amounts of precipitates. Experience has shown that even so large a precipitate of NiS as results from a 5-g sample containing about 70% of nickel can be washed clean by boiling with 50 ml of water five times in succession and decanting. In other words a precipitate, in particular a large one, can be rid quantitatively of by-products in this manner in much less time than by washing it in the filter. Besides, the filtration with only a clear solution passing through the filter is allowed to proceed much quicker. For those precipitates which are of no further interest anyway folded filter papers have been used exclusively. For the filtration of precipitates which require ultimate ignition Neubauer crucibles have been used which have proven to be superior to all other filtering devices. Precipitates which require ultimate drying have been filtered through filter tubes with a sintered glass filter plate. The large filtrates obtained from large precipitates have been reduced to a small volume by boiling in a flask prior to final evaporation to dryness in a dish. All determinations have been carried out in duplicate on two separate sample weighings. The percentages in the tables represent the mean of two such analyses. The percentages presented in parenthesis after each element are approximate figures. They are given merely to indicate the composition of the alloy. Finally, it should be noted that all alloys under discussion are in reference to their micro amounts "man made" alloys, which means that each micro amount has been added intentionally in a known amount to impart specific properties.

ALLOY No. 1

This heat resisting alloy is composed of iron (80%), chromium (15%), aluminum (4%), and micro amounts of nickel, silicon, and phosphorus. To 3 g of fine chips in a 500-ml Erlenmeyer flask 1 g of $KClO_3$ and 150 ml of concentrated HCl are added. The mixture is gently heated until the reaction has ceased. The solution is now boiled for 30 minutes, then transferred to a dish and evaporated to dryness. The residue is digested with 50 ml of concentrated HCl at 80°C under stirring and the mixture is again evaporated to dryness. Digestion and evaporation are repeated. Finally, the residue is washed with a total of 100 ml of concentrated HCl into a flask, the transfer being aided by scrubbing the wall and the bottom of the dish with a rubber policeman. The solution is now boiled for 15 minutes. The repeated manipulations of evaporating to dryness and dissolving the residue have the effect of rendering the silica practically insoluble in acid and practically 100% dehydrated and pure. Consequently, there is no need for treatment of the silica with hydrofluoric acid as it is described in text books (*5*), which besides has the disadvantage of transforming the determination of silicon into an indirect method. The settled SiO_2 is transferred to a weighed Neubauer crucible after it has been washed with 30 ml of cold water three times and the water decanted. The silica is then dried and ignited for 15 minutes, and finally after cooling it is weighed. From the filtrate after its evaporation to a volumen of about 100 ml the ferric chloride is removed by ether extraction with the aid of a separatory funnel (*5*). Three to four extractions each time with 50 ml of ether are sufficient to effect practically total removal of the iron chloride. The solution to be extracted must not contain substances such as free chlorine or nitric acid which tend to decompose ether and it must be kept cool during the extraction procedure to prevent the formation of ferrous chloride which would remain in the acid layer. The acid solution is evaporated to dryness and the residue is quantitatively washed into a flask with a total of 50 ml of hot water and then boiled until the solution becomes clear. Ten milliliters of a 20% solution of tartaric acid are added to prevent precipitation of aluminum and to this solution, which is heated to near boiling, a slight excess of an alcoholic 1% solution of dimethylglyoxime (*2, 8*) is added slowly under stirring, followed carefully by diluted ammonium hydroxide until the solution smells slightly of it. After the precipitate has settled, it is filtered through a sintered glass filter tube by decantation, washed with hot water, then dried at 120°C for 30 minutes under gentle suction and finally weighed. The filtrate is evaporated to

dryness and the residue is rinsed into a 150-ml flask with ground-in stopper with enough of boiling 10% HNO_3 to effect total transfer. The solution is boiled for 10 minutes to destroy residual organic matter. Five milliliters of 10% ammonium nitrate are added and after warming to about 80°C, 10 ml of ammonium molybdate solution are dropwise added under constant stirring (*3*, *6*). The flask is closed and the mixture thoroughly shaken. After removal of the stopper it is again warmed at 80°C for 15 minutes and the precipitate is then allowed to stand for 24 hours.

TABLE 2
PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 1

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Nickel	0.158	0.155	0.150
Phosphorus	0.085	0.088	0.090
Silicon	0.044	0.048	0.050

It is filtered through a sintered glass filter tube by decantation, thoroughly washed with a hot 2% ammonium nitrate solution, followed by hot water and finally with alcohol and ether. The filter tube is dried at 40°C in a drying block under slight suction for 30 minutes and weighed after cooling.

ALLOY No. 2

Composition. Nickel (80%), copper (15%), aluminum (4%), micro amounts of cobalt, silicon, and sulphur, and trace amounts of iron, phosphorus, and carbon. (It has a high degree of malleability and ductility.) Into solution is brought 5 g of the sample in powder form in the same manner as described under alloy No. 1. The settled silica is filtered through a Neubauer crucible by decantation and washed, dried, and ignited. The filtrate is evaporated to dryness and the residue washed into a 500-ml conical flask with a total of 200 ml of hot 1% nitric acid. It is boiled for 10 to 20 minutes until the solution is clear. After 20 ml of 5% NH_4Cl have been added a slight excess of $(NH_4)_2S$ is slowly poured into the solution under constant stirring. Boiling is continued for 10 minutes. The precipitate of nickel sulfide, cobalt sulfide, and aluminum hydroxide is allowed to settle and a few more milliliters of precipitant are added to assure total precipitation. The precipitate is transferred to a folded filter paper after it has been washed clean five times in succession with boiling water, each time with 50 ml and separating the

precipitate from the supernatant liquid by careful decantation. The filtrate (No. 1) is reserved for the sulfur determination. The precipitate is rinsed quantitatively into a 500-ml beaker with a total of 200 ml of aqua regia and the mixture is boiled until the solution is complete which takes about 30 minutes in which time the sulfur formed in the process of dissolving is gradually oxidized to sulfuric acid. The solution is evaporated to dryness and the residue dissolved in 100 ml of hot 1% HCl. Two milliliters of perhydrol are added and then just enough of 5% NaOH to cause the formation of $Co(OH)_3$. After this precipitate has been dissolved with a few drops of glacial acetic acid a slight excess of α-nitro-β-naphtol reagent (a 1% solution of the compound in 15% acetic acid) is dropwise added. When the precipitate has settled it is transferred to a sintered glass

TABLE 3
PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 2

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Cobalt	0.935	0.952	1.000
Sulfur	0.058	0.052	0.050
Silicon	0.054	0.047	0.050

filter tube after a thorough washing with diluted acetic acid and hot water in the flask to be followed each time by decantation. It is dried at 130°C for 40 minutes with gentle suction and after cooling weighed.

Prior to its evaporation to dryness in a dish the large filtrate No. 1 is boiled for 20 minutes to reduce its volumen and at the same time to expel the excess of $(NH_4)_2S$. The residue, now considerably smaller in size is dissolved in 50 ml of boiling 10% nitric acid. To the hot solution a slight excess of 5% barium chloride is dropwise added. The settled barium sulfate is transferred to a Neubauer crucible after a thorough washing with boiling water. It is first carefully dried, then ignited to dull red for 10 minutes, and finally weighed as $BaSO_4$.

ALLOY No. 3

The composition of this alloy is: cobalt (80%), nickel (10%), chromium (5%), iron (3%), and micro amounts of tungsten and molybdenum. (It is used in high speed metal turning as it can become red hot without losing its hardness.

To 3 g of small drillings in a 500-ml Erlenmeyer flask, 1 g of $KClO_3$

and 100 ml of concentrated HNO_3 are added. The mixture is gently heated until reaction starts. When the latter has ceased the solution is boiled for 20 to 30 minutes until the alloy is completely dissolved and the oxides of nitrogen have been expelled. After the solution has been evaporated to dryness the residue is rinsed into a 500-ml flask with a total of about 200 ml of hot 1% HNO_3, the transfer being aided by scrubbing the wall and the bottom of the dish with a rubber policeman. Ten milliliters of 5% NH_4Cl are added, and into the boiling solution a slight excess of $(NH_4)_2S$ is slowly poured with constant stirring. Boiling is continued for 10 minutes. The precipitate is allowed to settle and to assure total precipitation a few more milliliters of precipitant are added to be followed by 10 more minutes of boiling. The precipitate of cobalt

TABLE 4
PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 3

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Tungsten	0.957	0.980	1.00
Molybdenum	0.508	0.479	0.50

sulfide, nickel sulfide, and ferric hydroxide is transferred to a folded filter paper after it has been washed clean with boiling water. The filtrate is evaporated to dryness and the residue, now considerably reduced in size, is dissolved with a total of 100 ml of boiling water into a 250-ml conical flask. The solution is evaporated to a volume of about 20 ml and after cooling 10 ml of 50% ammonium formate and 10 ml of 30% tartaric acid are added and hydrogen sulfide is passed for 15 minutes. The mixture is then heated to about 60°C and kept at this temperature for 30 minutes. After the precipitate of MoS_3 has settled it is filtered through a Neubauer crucible by decantation and thoroughly washed with a 2% solution of ammonium formate. The precipitate is carefully dried and the crucible then heated in an electric oven at 550°C to constant weight, whereby the molybdenum sulfide is converted into the oxide which is finally weighed.

The filtrate is evaporated to a few milliliters, and after transfer with 50 ml of 10% HNO_3 into a flask the solution is boiled for 10 minutes to destroy the ammonium salts and residual organic matter. Five milliliters of cinchonine hydrochloride are added and the solution is kept at 80°C for 30 minutes. After 1 hour when the yellow precipitate of tungstic

acid has settled it is transferred to a Neubauer crucible after it has received a thorough washing with a 1% solution of the precipitant. The tungstic acid is then carefully dried and the crucible is placed into an electric oven which is slowly heated up to about 750°C and kept at this temperature for 30 minutes. The formed WoO_3 is weighed after cooling (*9*).

ALLOY No. 4

This ferrosilicon is composed of silicon (50%), iron (45%), micro amounts of copper, nickel, chromium, and trace amounts of manganese, aluminum, carbon, sulfur, and phosphorus.

One gram of $KClO_3$ and 150 ml of aqua regia are added to 5.000 g of the alloy in powder form. The mixture is first gently heated until the main reaction has abated and then boiled for 30 minutes whereby the larger part of the alloy gets into solution. The mixture is evaporated to dryness and baked for 5 minutes. The residue is quantitatively transferred to an iron crucible of 100-ml volume. The transfer is aided by scrubbing the bottom and the wall of the dish with a rubber policeman which has been moistened with diluted HCl. The residue is dried again and then thoroughly mixed with enough of sodium peroxide to fill half of the crucible. The mixture is carefully fused over a low flame by revolving the crucible around the outer edge of the flame until the contents have melted down. When the fusion is molten the heat is increased gradually to bright redness and maintained at it for 10 minutes. When the melt has solidified the bottom of the crucible, while still warm, is tapped several times on an iron plate in order to loosen the fused mass as a solid cake. The cake is transferred into a 500-ml beaker and 100 ml of cold water are cautiously added. When the reaction has ceased the remaining parts of melt in the crucible are washed with water into the beaker. The solution is cooled, and after 200 ml of concentrated HCl have been added it is boiled until reduced to a volume of about 50 ml, which after transfer to a dish is evaporated to dryness. The residue is rinsed with a total of 100 ml of concentrated HCl into a flask and boiled for 30 minutes. Evaporation and dissolving are repeated twice. Finally the settled silica is transferred to a folded filter paper after five washings with cold water under agitation, each time using 50 ml to be followed by decantation. The filtrate is evaporated to a volume of 50 ml. From this solution the ferric chloride is eliminated by extraction with ether as described under alloy No. 1.

The acid solution which is now free of silicon and iron is evaporated to dryness and the residue dissolved in 100 ml of boiling water. The solution is filtered to remove tiny parts originating from the trace amounts. It is warmed to 60°C and enough of 5% KOH is added to effect total precipitation of copper and nickel. The mixture is boiled for 10 minutes and the precipitate allowed to settle. It is filtered through an ashless filter paper by decantation and thoroughly washed with boiling water. The filtrate (No. 1) is reserved for the determination of chromium. The precipitate is ashed and the oxides of copper and nickel are dissolved in 50 ml of boiling 20% HCl. The solution is evaporated to dryness and the residue is now dissolved in 50 ml of boiling water. After this solution has been made slightly acid with HCl an excess of sulfurous acid is added

TABLE 5
PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 4

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Copper	0.087	0.082	0.10
Nickel	0.105	0.114	0.10
Chromium	0.083	0.079	0.08

to be followed by 10 ml of 5% ammonium thiocyanate drop-by-drop with constant stirring. The greenish precipitate changes gradually into pure white cuprous thiocyanate. When it has settled after a few hours it is transferred to a sintered glass filter tube after a thorough washing with cold water and 20% alcohol. The cuprous thiocyanate is dried at 140°C for 20 minutes with gentle suction and, after cooling, weighed. The filtrate is evaporated to a volume of about 50 ml. Ten milliliters of concentrated HNO_3 are added and the solution is boiled for 10 minutes and then again evaporated to dryness. The residue is dissolved in 30 ml of boiling water and in this solution the nickel is determined as described under alloy No. 1. Filtrate No. 1 is evaporated to dryness and the residue dissolved in 30 ml of boiling 10% acetic acid. To the boiling solution a slight excess of 5% barium acetate is added dropwise. The precipitate is filtered through a Neubauer crucible by decantation and washed with hot water and diluted alcohol. The crucible is first gently heated and finally ignited for 10 minutes. The barium chromate is weighed after cooling in a desiccator.

ALLOY No. 5

This alloy which resists extremely high temperatures consists of nickel (60%), chromium (20%), aluminum (10%), molybdenum (5%), micro amounts of titanium, boron, silicon and trace amounts of cobalt, sulfur, and phosphorus. In a 500-ml conical flask 3 g of finely powdered alloy are treated with 1 g of $NaNO_3$, 100 ml of concentrated HNO_3 and 100 ml of concentrated HCl at 60°C for 30 minutes. When the reaction has lessened the mixture is boiled for 30 minutes. The transfer of the

TABLE 6
PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY No. 5

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Titanium	1.88	1.96	2.00
Boron	1.44	1.37	1.50
Silicon	0.94	0.91	1.00

residue after evaporation to a crucible, the fusion with sodium peroxide, treatment of the melt and the repeated process of dissolving in concentrated HCl and evaporating are carried out as described under alloy No. 4. The settled silica is filtered through a Neubauer crucible after it has been thoroughly washed, then it is ignited and weighed. The filtrate is evaporated to dryness and the residue dissolved in 100 ml of boiling 1% HNO_3. Twenty milliliters of 5% ammonium chloride are added and from the boiling solution nickel and aluminum are precipitated with a slight excess of ammonium sulfide. The precipitate is separated from the liquid by decantation and transferred to a folded filter paper after a thorough washing with boiling water. The filtrate is reduced by boiling to a volume of 50 ml, to which 10 ml of 5% HNO_3 are added. After the solution has been cooled in ice water a cold 5% solution of cupferron (the ammonium salt of nitroso-phenyl-hydroxylamine) is added dropwise under stirring until a white precipitate appears on contact of the cupferron with the solution, indicating an excess of precipitant (*1, 4*). It disappears upon stirring while the yellow titanium precipitate settles. After it has been washed clean with cold 3% HCl, containing a little cupferron, the precipitate is transferred to a Neubauer crucible, which is dried on a hot plate and then gradually heated to 1200°C with a Meker burner and ignited for 15 minutes. The organic titanium complex is converted into titanium oxide which

is then weighed. The filtrate which contains sodium chromate, sodium molybdate, sodium borate, and sodium chloride (from the decomposition of the peroxide) is neutralized with NaOH and then evaporated to a volume of 50 ml. Diluted HCl is added drop by drop until the solution turns litmus just red. In this solution, which is made faintly acid with a few drops of acetic acid, the boron is determined according to the method of Rosenbladt and Gooch (*7*). The method is based on the fact that alkali borate when distilled with methyl alcohol gives up the boron in the form of methyl borate, which in the presence of water is completely saponified. The liberated boric acid combines with a known amount of lime to form calcium borate, which is dried and then ignited. The increase in the weight of the lime represents B_2O_3.

ALLOY No. 6

The composition of this alloy is iron (95%), nickel (3%), and micro amounts of vanadium and silicon. (It has great tensile strength and great resistance to stress and torsion.)

Into a 500-ml Erlenmeyer flask 3 g of the alloy in powder form are weighed and boiled with 1 g $KClO_3$ and 100 ml concentrated HNO_3 until solution is complete which takes about 1 hour. The acid lost through evaporation is replaced. The separation, removal and analysis of the silica is carried out in the usual manner. The filtrate from the silica is evaporated to dryness. The residue is dissolved in 100 ml of 10% HCl

TABLE 7
PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 6

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Vanadium	1.43	1.39	1.50
Silicon	0.92	0.98	1.00

from which solution the ferric chloride is extracted with ether as described previously. The acid solution from the extraction is evaporated to dryness and the residue dissolved in 50 ml of boiling water. In this solution, which is made faintly acid with acetic acid, the vanadium present as potassium vanadate is analyzed as follows: (*7*) A slight excess of lead acetate solution 10% strong is added with continuous stirring. The mixture is heated at 50°C for 10 minutes, which effects a color change of the precipitate from orange to white. The latter is washed in the flask several

times with water slightly acidified with acetic acid until the wash water, which is each time siphoned off as completely as possible, leaves no residue. The lead vanadate is then dissolved in 50 ml of hot 5% HNO_3 and enough H_2SO_4 is added for total precipitation of the lead. The mixture is evaporated to a volume of 20 ml, and the heat continued until dense fumes of H_2SO_4 are evolved. After cooling 50 ml of water are cautiously added. The mixture is agitated and after the lead sulfate has settled it is filtered through a folded filter paper by decantation and thoroughly washed with cold water. The filtrate is evaporated to a small volume which is then transferred to a platinum crucible in which it is carefully evaporated to dryness and finally ignited to dull redness. The ignition converts the vanadic acid into V_2O_5 which is weighed.

ALLOY No. 7

This ferrochromium is composed of chromium (70%), iron (25%), micro amounts of nickel, titanium, sulfur and trace amounts of carbon, silicon, and phosphorus.

As with ferrosilicon the most satisfactory method to get ferrochromium into solution is a combined acid and fusion treatment which is carried out on a 3-g sample, as described under alloy No. 4. From the ultimate

TABLE 8

PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 7

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Nickel	1.948	1.974	2.00
Titanium	1.482	1.427	1.50
Sulfur	0.987	0.979	1.00

solution the ferric chloride is removed by ether extraction. The acid solution from the extraction is evaporated to dryness and the residue dissolved in 200 ml of boiling water. To the boiling solution enough of 10% $BaCl_2$ is added to effect total precipitation of the chromate and sulfate. After the precipitate has settled the supernatant liquid which contains the nickel and titanium is carefully siphoned off. The precipitate is boiled with 50 ml water for 5 minutes and again the liquid is siphoned off. This washing process is repeated three times. The precipitate is then boiled in the flask with enough of 20% HNO_3 to dissolve the barium chromate and leave the barium sulfate. The latter is transferred to a

Neubauer crucible after it has received a thorough washing with boiling water. The precipitate is then dried, finally ignited to dull red for 10 minutes, and after cooling weighed as $BaSO_4$. The collected supernatant liquids are evaporated to dryness and the residue, now considerably smaller, is dissolved in 50 ml of boiling water and the solution evaporated to a volume of 20 ml in which the nickel is analyzed as nickel dimethylglyoxime. The filtrate from the precipitate is evaporated to a volume of 30 ml. After cooling 5 ml of concentrated H_2SO_4 are cautiously added. The mixture is boiled for 5 minutes, then cooled in ice water, and the titanium is precipitated with cupferron and analyzed as described under alloy No. 5.

ALLOY No. 8

The composition of this alloy is copper (80%), tin (15%), and micro amounts of lead, iron, aluminum, zinc, and phosphorus.

To 5 g of small chips in a 500-ml conical flask, 1 g of $KClO_3$ and 150 ml of aqua regia are added. The mixture is gently heated, and when the reaction is abating it is boiled for 30 minutes or more until complete solution has been obtained. The solution is evaporated to a volume of about 30 ml, to which 20 ml of concentrated HCl are added and again boiled for 10 minutes. The solution is now cooled in ice water and 30 ml of alcohol are added with constant stirring. The mixture is vigorously agitated and kept in ice water for 20 minutes. The settled lead chloride is transferred to a filter paper after it has been washed clean with an ice cold mixture of alcohol and concentrated HCl (4:1); three washings each time with 30 ml of the mixture are sufficient (filtrate No. 1). The lead chloride on the filter is rinsed into a beaker first with hot water and then with a hot acid ammonium acetate solution (a mixture of ammonium hydroxide and enough acetic acid to render it 2% acid). The mixture is boiled until all lead chloride is dissolved. To the hot solution 10 ml of 5% potassium bichromate are added and the mixture boiled until the yellow precipitate turns to a shade of orange. When it has settled the precipitate is transferred to a sintered glass filter tube after a thorough washing with water, alcohol and ether, then dried at 100°C with gentle suction for 15 minutes and weighed as $PbCrO_4$. Filtrate No. 1 is evaporated to dryness and the residue is rinsed into a flask with a total of 200 ml of hot 3% HCl and boiled until solution is complete. Into the boiling liquid, after the heat has been removed, hydrogen sulfide is passed until the mixture becomes cold. The precipitate consisting of the sulfides

of copper and tin is allowed to settle. It is transferred to a folded filter paper after it has been washed six times with boiling water saturated with H_2S, each time using 50 ml to be followed by careful decantation. The filtrate which contains the iron, aluminum and zinc is evaporated to a volume of about 20 ml. To the boiling solution in a small beaker 5 ml of 3% NH_4Cl are added and then enough of 10% NH_4OH under constant stirring to effect total precipitation of ferric hydroxide and aluminum hydroxide. The precipitate is transferred to a filter paper after repeated washings with boiling water (filtrate No. 2). It is then dissolved on the filter with 20 ml or more of hot 10% HCl. The solution is evaporated to dryness and the residue dissolved in 30 ml of boiling water.

TABLE 9

PERCENTAGES OF MICRO AMOUNTS OF ELEMENTS IN ALLOY NO. 8

	Percentages		
Element	Wet chemical analysis	Physiochemical method	Expected
Lead	0.96	0.93	1.00
Aluminum	0.48	0.54	0.50
Iron	0.25	0.28	0.25
Zinc	0.058	0.064	0.050
Phosphorus	0.016	0.014	0.010

To the boiling solution enough of 10% NaOH is added with constant stirring to convert the initially formed aluminum hydroxide into the soluble sodium aluminate. The formed iron hydroxide is transferred to a filter paper after it has been washed clean with boiling water (filtrate No. 3), and again dissolved on the filter with 20 ml of hot 10% HCl. After the solution has been evaporated to dryness the residue is brought into solution with 30 ml of boiling water. From this solution the iron is reprecipitated with NH_4OH. The ferric hydroxide is transferred to a Neubauer crucible after a thorough washing with boiling water, then ignited over a Tirrell burner for 10 minutes and after cooling weighed as Fe_2O_3. Filtrate No. 3 is evaporated to a volume of 10 ml, to which 5 ml of 3% NH_4Cl are added. To the boiling solution a slight excess of 10% NH_4OH is added for total precipitation of the aluminum. The precipitate is transferred to a Neubauer crucible after repeated washings with 2% ammonium nitrate, then ignited and finally weighed as Al_2O_3. Filtrate No. 2 is evaporated to a volume of 20 ml and then slightly acidified with acetic acid. Ten milliliters of 5% sodium acetate are added and into the boiling solution

hydrogen sulfide is passed for 5 minutes. The zinc sulfide is filtered through a Neubauer crucible by decantation and washed repeatedly with 1% acetic acid and hot water. It is then dried, gently ignited, and held for 5 minutes in the full flame of a Bunsen burner. After cooling in a desiccator it is weighed as ZnO. The filtrate is evaporated to dryness and the residue is quantitatively washed with a total of 50 ml of 10% HNO_3 into a 150-ml flask with ground-in stopper. The solution is boiled for 10 minutes, and the phosphorus is then analyzed with ammonium molybdate solution as described under alloy No. 1.

SUMMARY

The wet chemical analysis of micro amounts of elements in various alloys is described. Although this selection of alloys is for obvious reasons a limited one, it still provides a good representation of those elements most often encountered in alloys as micro amounts. By choosing a course of analysis, which includes the removal of one or more of the main constituents prior to the analysis of the micro amounts, it is shown that the entire analysis can be carried out on a single sample weighing. The percentages obtained by wet chemical analysis are in good agreement with those obtained by physicochemical methods, and both match the expected figures.

REFERENCES

1. BAUDISCH, O., Quantitative Trennung mit Cupferron. *Chem. Ztg.* **33**, 1298 (1909).
2. BRUNCK, O., Zur Bestimmung des Nickels mit Dimethylglyoxim. *Z. Angew. Chem.* **27**, 315 (1914).
3. LORENZ, N., Eine Methode zur Phosphorsaeure Bestimmung. *Z. Anal. Chem.* **46**, 193 (1907).
4. LUNDELL, G. E. F., AND KNOWLES, H. B., The use of cupferron in quantitative analysis. *J. Ind. Chem.* **12**, 344 (1920).
5. LUNDELL, G. E. F., HOFFMAN, J. I., AND BRIGHT, H. A., "Chemical Analysis of Iron and Steel." Wiley, New York, 1932.
6. NEUBAUER, H., UND LUCKER, F., Die von Lorenz'sche Methode der Phosphorsaeure Bestimmung. *Z. Anal. Chem.* **51**, 161 (1912).
7. TREADWELL-HALL, "Analytical Chemistry," Vol. II, 8th ed. Wiley, New York, 1935.
8. TSCHUGAEFF, L., Ueber komplexe Verbindungen der Dioxime. *Z. Anorg. Chem.* **46**, 144 (1905).
9. YAGODA, H., AND FALES, H. A., The separation and determination of tungsten and molybdenum. *J. Am. Chem. Soc.* **58**, 1494 (1936).

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Micro Measurement of Milk Fat

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There are three standard methods for the measurement of milk fat: that of Babcock (*1*, p. 373), that of Gerber (*1*, p. 294), and that of Roese-Gottlieb (*1*, p. 372). They involve the use of either a strong acid or else violatile solvents to separate the fat, which is then measured either volumetrically in special glassware or gravimetrically. The procedures are time-consuming and duplication is sometimes difficult.

Recently Fleet and Linzell (*2*) reported a very simple method for determining fat in fresh milk. They found that the fat concentration obtained by a method similar to that of Roese-Gottlieb was 0.740 $\times$ percent cream obtained by centrifugation of samples in microhematocrit tubes. Thus the procedure for fat determination can be reduced to the simple routine of centrifugation of samples in microhematocrit tubes, measurement of percent cream with the microhematocrit reader, and multiplication of the percent cream by the correlation factor.

Fleet and Linzell obtained their correlation factor with fresh goat milk. The fat globules in fresh milk are 2-10 μ in diameter and they are reduced to 1/10 to 1/100 of this size by homogenization (*3*). Hence the correlation factor of 0.74 obtained by Fleet and Linzell cannot be used for determinations of fat in homogenized milk. The results obtained with homogenized milk are presented in this report.

MATERIALS AND METHODS

Commercial homogenized cow's milk was used. Duplicate samples in microhematocrit tubes,[1] flame sealed at one end to have a flat bottom, were spun for 30 minutes in a centrifuge[2] at 14,500 $\times$ *g*. The percent

[1] Heparinized capillary tube, O.D. 1.3-1.5 mm, length 75 mm, Scientific Products Division, American Hospital Supply Corporation, Evanston, Illinois.

[2] International Micro-Capillary Centrifuge, Model MB, International Equipment Company, Needham Heights, Massachusetts.

cream was immediately obtained with a microhematocrit reader.[3] The range of fat concentrations was extended by mixing commercial homogenized skim milk and dehydrated (under refrigeration) commercial homogenized whole milk in varied proportions to have a range of 0.6-5.7 g% of fat.

The absolute fat values were obtained by both the Babcock and the Roese-Gottlieb methods. Their accuracy was first checked out by measurements of known amounts of olive oil added to skim milk. Since the charring reaction often made duplication difficult with the Babcock method, it was modified as follows:

1. From a milk sampling pipette (17.6 ml) 18.0 g of milk were introduced into 18.0 g of 95% of concentrated sulfuric acid in a Babcock bottle. Charring did not occur in this concentration of the acid.
2. As in the Gerber method, 1.6 ml of iso-amyl alcohol were added to facilitate the rise of the fat in the capillary neck.
3. The bottle was centrifuged for 3 minutes at 800 rpm (radius = 20.8 cm).
4. Boiling distilled water was added to bring the top of the fat column up to the 4.0% mark.
5. The bottle was recentrifuged for 3 minutes.
6. It was then placed into a bath at 56°C for 3 minutes to control expansion of column.
7. The fat column was read off in grams percent.

RESULTS AND DISCUSSION

The correlation of determinations by the Roese-Gottlieb and modified Babcock methods with known values of fat in skim milk is shown in Fig. 1. The standard error of estimate was 0.02 with the Roese-Gottlieb method and 0.09 with the modified Babcock method. Hence the former method was used to get the absolute values of fat in the bovine milk samples for correlation with percent cream.

The relation of percent cream to percent fat in homogenized milk is shown in Fig. 2. The least squares equation for the inverse relationship, namely, percent fat (Y) to percent cream (X) is

$$Y = 0.008 + 0.610\,X.$$

[3] Adams Micro-Hematocrit Reader, A-2970, Clay-Adams, Inc., New York 10, New York.

The standard error of estimate was 0.05. The Y-intercept is negligibly small and can be omitted.

With this equation and microhematocrit measurements of percent cream, the percent fat in 12 replicate samples of a milk specimen was 3.25 g% with a standard deviation of $\pm$ 0.027. This compared favorably with the results obtained by the Roese-Gottlieb method, 3.18 g% $\pm$ 0.011, and by the modified Babcock method, 3.34 mg% $\pm$ 0.050.

The determination of milk fat by means of its correlation with cream is simple, inexpensive, accurate, and requires only about one-tenth of the

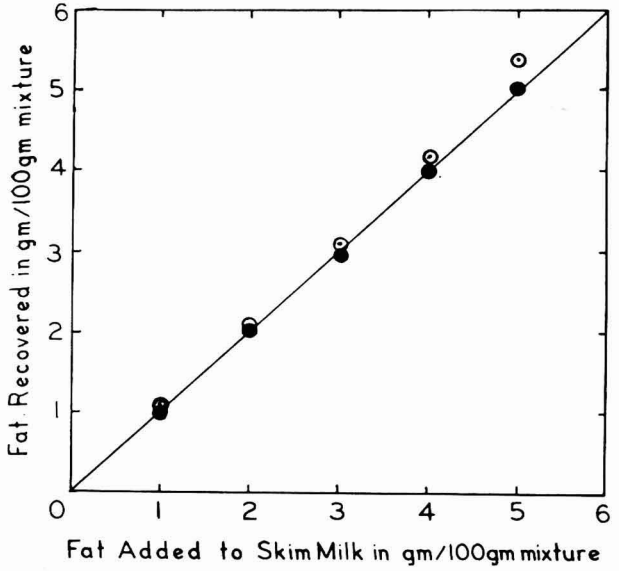

FIG. 1. Correlation of recovered fat values by the Roese-Gottlieb (●) and modified Babcock methods (⊙) (Y-axis) with known values of fat in skim milk (X-axis). Each point is the mean of duplicate analyses. The curve is the line of equality.

time for execution required by the standard methods. In addition, as Fleet and Linzell pointed out, since only a very small amount of milk is required to fill a microhematocrit tube, fat determination in a droplet of milk, as from a mouse or a rabbit, can be easily and accurately made.

The factor correlating cream to fat varies with the size of the fat globules, the duration of centrifugation, and the centrifugal force. Although Fleet and Linzell centrifuged for a shorter length of time (15 minutes vs. our 30 minutes) and with a smaller centrifugal force (10,000 $\times$ g vs. our 14,500 $\times$ g), yet they achieved more compact packing of whole milk

cream than we did of homogenized milk cream, because the fat globules are larger in the former than in the latter. It follows that the factor correlating cream to fat must be independently determined for each different combination of globule dimension, centrifugation time, and centrifugal force.

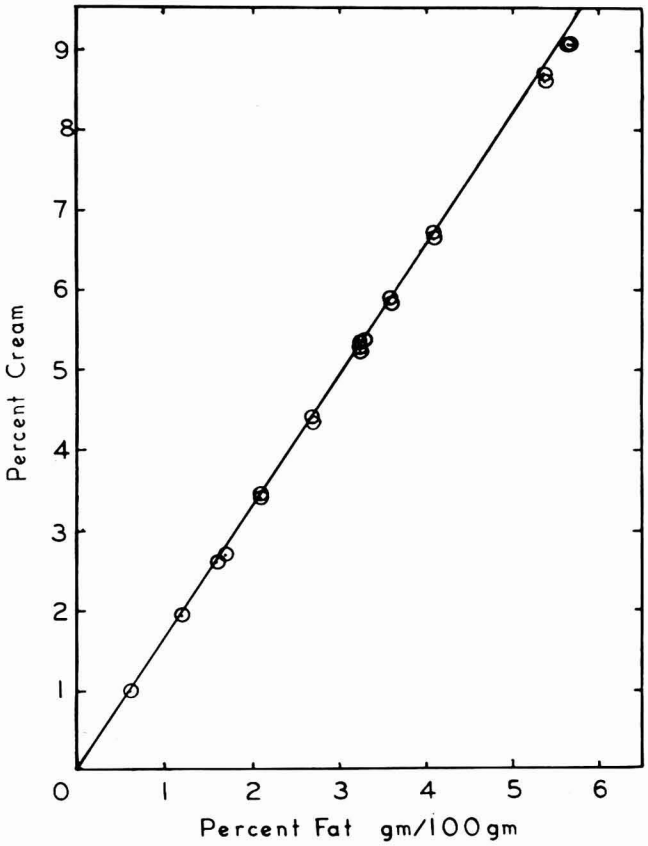

FIG. 2. Correlation of percent cream with percent fat in homogenized milk. Each point is the mean of duplicate analyses. The curve was calculated by the method of least squares.

SUMMARY

Fleet and Linzell have reported that total fat is 0.74 × percent cream (measured with a microhematocrit tube) in fresh milk. In homogenized milk the correlation factor has been found to be 0.61 because the fat globules are smaller in homogenized than in fresh milk. A modification of the Babcock method for the determination of fat in homogenized milk is presented. This includes the use of diluted sulfuric acid to

prevent charring, and of iso-amyl alcohol to facilitate the rise of the fat in the neck of the Babcock bottle.

REFERENCES

1. American Public Health Association. "Standard Methods for the Examination of Dairy Products," 11th ed. American Public Health Association, New York, 1960.
2. Fleet, I. R., and Linzell, J. L., A rapid method of estimating fat in very small quantities of milk. *J. Physiol* **175**, 15P-17P (1964).
3. Weiser, H. B., *In* "Colloid Chemistry," 2nd ed., p. 342. Wiley, New York, 1949.

MICROCHEMICAL JOURNAL 12, 99-116 (1967)

Electrochemical Oxidation of Some Aromatic Amines in Acetonitrile Medium. I. *N,N*-dimethylaniline, Triphenylamine, Diphenylamine and Di-4-tolylamine

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The possibility of electrochemical, especially of polarographic oxidation of aromatic amines, and the possibilities of the analytical application of these findings have begun to be studied on a wider scale only in the recent 15 years, when the application of nonaqueous solvents and mainly of solid electrodes had overcome some of the limitations of polarography with the dropping mercury electrode for oxidation processes in aqueous media. The detailed literature review given by Dvořák in his study (*1*) shows, that the electrochemical oxidation of amines is a very complicated process, which may be briefly characterized as follows:

Substances derived from phenylenediamines are, in principle, capable of being oxidized, forming substances of quinoide structure; but under certain conditions they may also form stable radicals. One-electron oxidation products are frequently substances of the type of Würster salts. Substances derived from primary amines often oxidize to form products, which originate by the chemical reaction of primary products of the electrode reaction. These primary products are most frequently radicals, which are however unstable, mainly in the case of simple molecules, reacting either mutually or with the original nonoxidized amine. In the case of more complicated molecules, which are able to stabilize the radical structure, these radicals may be isolated. Very often these reactions are complicated by side reactions and subsequent reactions. For many amines investigated up to now, these oxidation products are not known.

In our study, we have attempted to make a contribution to the study of the electrochemical oxidation of some structurally similar derivatives

of diphenylamine and *p*-phenylenediamine or benzidine in acetonitrile medium on a rotating platinum electrode. The object of this work was to determine the possibility of the polarographic determination of these substances, to find the influence of the number of amino groups and their substitution by the phenyl and methyl group on the $E_{\frac{1}{2}}$ value and possibly on the formation of reaction products, and to compare the measured $E_{\frac{1}{2}}$ values with the HOMO energy values calculated by means of the simple MO-LCAO method.

For the individual substances we have each time investigated the concentration dependence of the magnitude of the limiting current and $E_{\frac{1}{2}}$ value, influence of water, temperature, acids and rate of polarization on the magnitude of the limiting current and $E_{\frac{1}{2}}$ value. We have attempted to solve the problem of the number of electrons exchanged by coulometric generation at constant potential. We tried to assess the products of the electrode reaction formed from the reduction curves, recorded after the generation process.

In this first communication we are stating results obtained in studying *N,N*-dimethylamine, triphenylamine, diphenylamine and di-4-tolylamine.

EXPERIMENTAL

Reagents

Acetonitrile. Reagent-grade acetonitrile, made by the BDH and Lachema Companies, was used to prepare stock solutions of the amines studied and to prepare the electrolyte. The modified procedure A-1 according to Coetzee *et al.* (*2*) was found best for the purification of the solvent. CH_3CN, thus obtained (fraction 81.5°C), contained $3 \cdot 10^{-3}\%$ water (determined by titration according to Fischer). The solvent was kept in a stock bottle in the dark, under a seal with P_2O_5.

$NaClO_4$. Neither the commercial preparation nor a salt obtained by neutralizing reagent grade NaOH with the reagent-grade acid were found to satisfy the conditions given. The supporting electrolyte alone was found to have a relatively great anodic current, starting already at potentials of + 0.8 v (SCE). Therefore, the procedure according to Biedermann (*3*) was used to prepare $NaClO_4$; by this method, sodium perchlorate is obtained from reagent-grade sodium carbonate and reagent-grade perchloric acid. The perchlorate thus prepared was best suited to the work in question. To convert the dihydrate to the anhydrous salt, and to store this, conventional processes were employed: drying at 150°C for 24 hours, and storage in vacuo above P_2O_5. Stock solution of 0.1*M* $NaClO_4$ in acetonitrile (sup-

porting electrolyte) was kept in a stock bottle fitted with a burette under a seal with P_2O_5, in the dark. The burette was filled by means of the pressure of dry nitrogen.

Stock solutions of the individual amines were prepared by directly weighing the pure substance so as to make a $4 \cdot 10^{-3}M$ solution. Acetonitrile, from which oxygen had been removed by a stream of nitrogen, was used to dissolve the amines in an atmosphere of nitrogen. The stock solutions were kept in the dark in a refrigerator. All the amines were very kindly placed at our disposal by Dr. P. Smejtek, CSc., and Dr. J. Honzl, CSc. of the Macromolecular Chemistry Research Institute of the Czechoslovak Academy of Sciences. The purity of the amine samples was checked by means of their melting points.

A $0.1M$ perchloric acid solution in acetonitrile was prepared by diluting $10M$ $HClO_4$ reagent grade, with acetonitrile. The solution also contained 0.3% water.

A $5 \cdot 10^{-3}M$ perchloric acid solution in acetonitrile was prepared by precise dilution of the $0.1M$ solution: it also contained $1.5 \cdot 10^{-2}$% water. Both $HClO_4$ solutions were prepared each time just before use, as they are relatively unstable (*4*).

Apparatus

All polarographic measurements were carried out with the Type PO 4 Polariter, made by the Radiometer Co.

All measurements were carried out in a vessel illustrated in Fig. 1, which is in fact a modification of the Pecsok and Juvet vessel (*5*). The vessel consists of three main parts: the thermostated space proper, space for the reference electrode, and an intermediate space.

The comparison electrode used was a calomel electrode with a saturated NaCl solution, whose potential was 6 mv more negative than that of the SCE. (A saturated KCl solution was not used, since on contact of the aqueous KCl solution with the $NaClO_4$ acetonitrile solution, $KClO_4$ precipitated, increasing excessively the resistance of the vessel.) The NaCl solution was connected to the intermediate space by a medium-density sinter plate (*6*), covered with a layer of 5% agar in saturated NaCl solution (*8*); the intermediate space was filled with $0.5M$ $NaClO_4$ CH_3CN solution, connecting with the measurement space proper by a dense sinter plate (*7*).

The solution in the intermediate space was replaced at frequent inter-

vals (at least once a day). Under these conditions, no observable chloride traces penetrated into the working space, not even in the course of several hours. In this arrangement the resistance of the vessel was 1 to 1.5 kΩ (measured between the calomel electrode and RPE).

A two-way capillary valve, sealed into the bottom of the working space, served to remove the electrolyte from the working space and to pass a stream of nitrogen through the electrolyte.

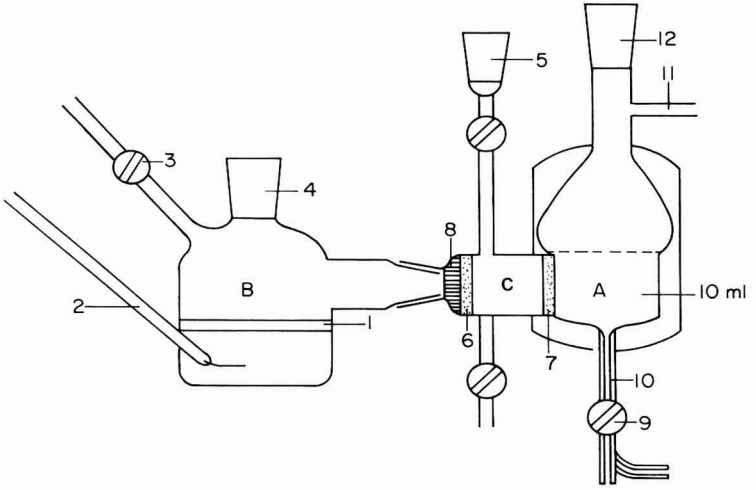


FIG. 1. Polarographic vessel: A, working space; B, space for reference electrode; C, intermediate space; 1, mercury surface ($S = 15.2$ cm^2) with a layer of calomel and NaCl crystals; 2, tube with platinum contact; 3, deaeration tap; 4, filling opening with ground joint and opening for the calomel electrode for checking purposes; 5, ground joint; 6, medium-density sinter plate; 7, dense sinter plate; 8, agar; 9, capillary two-way tap for passing nitrogen through the solution and emptying the working space; 10, capillary; 11, inlet of nitrogen above the working space; 12, opening for the rotating platinum electrode (ground joint).

The rotating platinum electrode (RPE) has already been described earlier (*6*). The wire diameter of the platinum electrode was 0.5 mm, its length immersed in the solution was 2.5 mm. Penetration of oxygen and moisture from the air were avoided by a stream of nitrogen, introduced through tube 11 (Fig. 1).

The conductivity of solutions was measured with a Type CDM-2 Conductoscope by Radiometer.

Coulometry and constant potential electrolysis. The PO-4 Polariter, with voltage variation device switched off, served to generate the products

of electrode processes and for the coulometric determination of the number of electrons transferred in the course of the electrode reaction. The Polariter was used as a voltage source and at the same time for recording the dependence of the current on time. The voltage applied between the operating calomel and generating platinum electrode was adjusted manually to make the potential of the platinum-generating electrode correspond to the potential at which the limiting current is attained. The charge transferred was determined by recording the time dependence of the current and weighing the area thus obtained (the reproducibility of the individual generations was about 3%).

The generating platinum electrode was realized by a glass tube with 22 turns of platinum wire, 0.5 mm in diameter, of a total length of 42 cm, wound around it. The wire was sealed in as in the RPE; the electrode rotated at 1400 rpm. The electrode surface was about 6.6 cm^2.

The operating calomel electrode used was the calomel electrode already described; no polarization of this electrode was to be found even at maximum currents of 100-200 μA (*7*).

Since most of the substances studied were sensitive to atmospheric oxidation, and with respect to work in a nonaqueous medium, oxygen traces were removed and nitrogen was dried in an absorption column with a catalyst, according to Meyer and Ronge (*8*), and heated to 200°C, followed by a column with a molecular sieve to remove moisture. With respect to the relatively high vapor tension of acetonitrile, the purified nitrogen was saturated with acetonitrile vapor (*9*) in a washing bottle placed in the opening of a Höppler thermostat.

The amine stock solutions were measured by means of an all-glass microburette with micrometric screw, of the Agla type, total volume 0.5 ml. When a volume of 0.01 ml is being measured this microburette guarantees a precision of $\pm 5 \cdot 10^{-5}$ ml.

Procedure of Recording

Electrode preparation. When the electrode was out of operation for some time, it was found suitable first to polarize it in a supporting electrolyte solution for several minutes at 2 v against an SCE. In other cases the RPE was wiped carefully each time before a curve was recorded.

The intermediate space was washed, and the sinter plate 7 (Fig. 1) flooded with water and acetone and dried with stream of nitrogen each day before measurement was started. To suck the washing liquid through the sinter plate, tap 9 and ground joint 12 were closed (Fig. 1), and the

vacuum was connected to tube 11. After filling the intermediate space with a 0.5*M* $NaClO_4$ solution in CH_3CN the solution was sucked through the sinter plate 7 (Fig. 1), and the intermediate space was closed by taps. (It is essential to suck the solution through the sinter plate 7 to decrease the otherwise large resistance of the plate.)

The working space proper was always washed with acetonitrile and dried with a stream of nitrogen. After filling the space with the supporting electrolyte and removal of oxygen by means of a stream of nitrogen, the curve of the electrolyte was recorded. Then the required amount of the amine stock solution was added by means of the microburette to give a solution of the required concentration. All current values given were measured with the same electrode, and the data are corrected for the residual current of the supporting electrolyte.

The half-wave potentials were assessed graphically and corrected for the potential gradient within the solution. The values given are calculated for the saturated calomel electrode. Unless otherwise stated, curves were recorded at a polarization rate of 200 mv/min or 400 mv/min at a temperature of 20°C from negative to positive potentials in a volume of 10 ml 0.1*M* $NaClO_4$ acetonitrile solution.

N,N-*dimethylaniline*

N,N-dimethylaniline (DMA) gives one well-developed wave in acetonitrile (Fig. 2), whose height is proportional to the concentration and whose

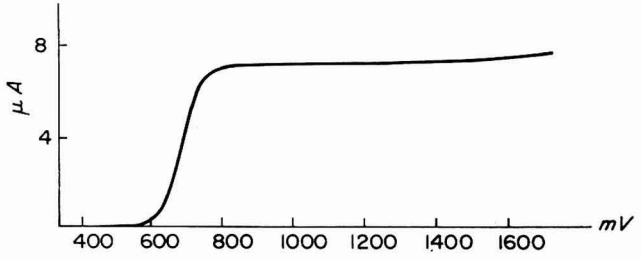


FIG. 2. Oxidation of *N,N*-dimethylaniline ($c = 4.94 \cdot 10^{-5} M$) on the RPE.

$E_{\frac{1}{2}}$ value is practically independent of the concentration. For low concentrations, values of $E_{\frac{1}{2}}$ and i/c rise somewhat, at high concentrations the reverse case pertains (Table 1).

The half-wave potential practically does not vary with the temperature in the 20°-40°C range, and the temperature coefficient of the limiting current is roughly 1.1% per 1°C.

The presence of water in the solution is not manifested up to a concentration of 1%. At higher water concentrations the oxidation of water begins to become effective at potentials higher than 1 v, causing the plateau to deform; the waves become more elongated in shape and the half-wave potential rises (for 5% water the rise is roughly 20 mv).

TABLE 1

DEPENDENCE OF THE LIMITING CURRENT AND HALF-WAVE POTENTIAL OF *N,N*-DIMETHYLANILINE ON CONCENTRATION

c (moles/liter)	i (μA)	$E_{\frac{1}{2}}$ (mv)	i/c (μA liters/mmole)
$0.5 \cdot 10^{-5}$	0.82	705	164.0
$1 \cdot 10^{-5}$	1.61	700	161.0
$1.99 \cdot 10^{-5}$	3.08	685	154.8
$2.98 \cdot 10^{-5}$	4.42	685	148.3
$4.94 \cdot 10^{-5}$	7.18	680	145.3
$5.91 \cdot 10^{-5}$	8.58	675	145.2
$1.90 \cdot 10^{-4}$	28.0	680	147.4
$2.79 \cdot 10^{-4}$	40.3	670	144.4
$4.44 \cdot 10^{-4}$	61.4	680	138.3
$5.96 \cdot 10^{-4}$	77.8	680	130.5

The presence of perchloric acid in the solution causes the wave-height to decrease, approximately in proportion to the acid concentration, and at a molar ratio of $[HClO_4]/[DMA] = 1.25$ the wave dissappears totally. The logarithmic analysis is linear, with a reciprocal slope of 1/63 mv ($2 \cdot 10^{-5}$-$6 \cdot 10^{-4} M$). When 10 ml of a $2 \cdot 10^{-5} M$ DMA solution were electrolyzed at a potential of 900 mv, corresponding to the limiting current, a charge was consumed corresponding to the transfer of 2.3 electrons from each DMA molecule. The oxidation product formed was yellow in color and gave two reduction waves and two oxidation waves that were elongated and badly developed (Table 2). With time, the color of the solution weakened rather quickly, and the reduction waves shifted to the region of oxidation currents. Reverse reduction at a potential of 250 mv, corresponding to the limiting current of the second reduction wave caused a colorless solution to form, which gave two well-developed and two elongated oxidation waves. Further oxidation at 430 mv, i.e., between the first two waves, gave a yellow-green solution that gave one reduction wave and three oxidation waves, which rapidly shifted into the range of oxidation currents until a state of equilibrium

was reached, where about one-half of the wave with $E_{\frac{1}{2}} = 330$ mv lay above the zero current line and one-half below.

According to literature data (*10-12*) the main product of the electrochemical oxidation of DMA in aqueous solutions is the oxidized form of tetramethylbenzidine (TMB). Assuming the same course of the reaction in acetonitrile medium also, the oxidation product of DMA should give two reduction waves corresponding to the reduction of the oxidized form

TABLE 2

GENERATION OF THE OXIDATION PRODUCTS OF *N,N,*-DIMETHYLANILINE

Solution	color	ν^a	$E_{\frac{1}{2}}$ (mv)	i (μA)	Wave
$2 \cdot 10^{-5} M$ DMA	colorless		690	3.02	Ox[b]
Product of oxidation at 900 mv	yellow		480	0.65	1.Red[b]
		2.3	320	0.33	2.Red
			cca 880	cca 0.4	1.Ox[c]
			cca 1050	cca 0.4	2.Ox[c]
Product of reduction at 250 mv	colorless		330	0.4	1.Ox
			500	0.59	2.Ox
			cca 860	cca 0.4	3.Ox[c]
			cca 1050	cca 0.4	4.Ox[c]
Product of oxidation at 430 mv	yellow-green		330	0.53	Red
			495	0.65	1.Ox
			cca 860	cca 0.4	2.Ox[c]
			cca 1050	cca 0.4	3.Ox[c]

[a] Number of electrons per one molecule.
[b] Ox = oxidation wave; Red = reduction wave.
[c] Elongated waves.

of TMB. A comparison of the $E_{\frac{1}{2}}$ values and wave heights of the oxidation products of DMA and TMB shows that actually two reduction waves form at potentials close to the reduction of the oxidized TMB form, but their height is lower than assumed, and moreover the DMA oxidation product has two elongated oxidation waves at more positive potentials.

Considering the reaction course according to literature data (*10-12*), one molecule of the oxidized TMB form should originate from two DMA molecules, two protons being set free. The overall concentration of the oxidized TMB form should, therefore, be one-half of the DMA concentration. The somewhat more positive values, and decreased waves compared to the values expected may be caused by the influence of the hydrogen

ion liberated in the formation of TMB from DMA. The presence of the acid actually causes such a shift and lowering of the wave-height in the case of TMB, as our experiments have shown. A similar comparison may be made also in the case of products corresponding to the reduced TMB form (or of the radical intermediate product). The color of the oxidation and reduction products of TMB and DMA at the corresponding potentials also is the same. The formation of two more oxidation waves, which do not occur in the oxidation of TMB, however, shows that beside the oxidized TMB form another side product is formed in the oxidation of DMA, which may be similar to the product formed when two DMA molecules are joined in the ortho- and para-positions. This product is mentioned in the study by Adams *et al.* (*10-12*), but no details are given. Due to the instability of the reaction products, which causes a shift of the reduction waves to the region of oxidation currents, it is impossible to determine precisely the number of electrons transferred in the process of oxidation of DMA, but the value of 2.3 obtained coulometrically, and the formation of the oxidized TMB form as the main reaction product show that the wave in question is a two-electron wave.

Triphenylamine

Triphenylamine (TPA) gives two oxidation waves in acetonitrile (Fig. 3). The first one is well-developed, proportional to the concentration, and

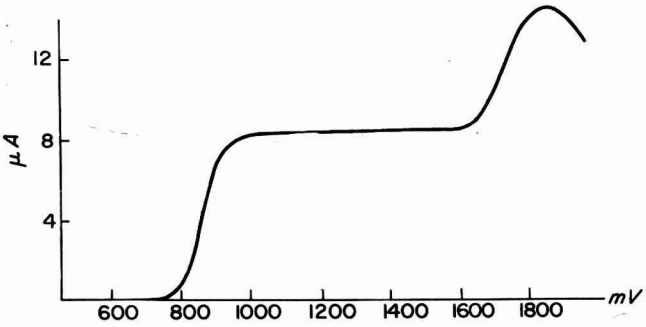


FIG. 3. Oxidation of triphenylamine ($c = 7.84 \cdot 10^{-5} M$) on the RPE.

the second has the shape of a peak ($E_{\frac{1}{4}}$ about 1700 mv) whose relative height decreases with the increasing concentration (electrode passivation).

With the increasing temperature the height of the first wave rises in the range of 20°-50°C, with a temperature coefficient of 1% per 1°C

and its half-wave potential rises by about 0.5 mv per 1°C. The second wave increases in an irregular manner with the temperature (Table 3).

The presence of water is not manifested up to a concentration of 0.1%. At higher water concentrations the $E_{\frac{1}{2}}$ value of the first wave does not vary, but its plateau and the second wave are influenced by the accompanying oxidation of water due to the relatively high potentials, so that the influence of water cannot be evaluated.

TABLE 3

DEPENDENCE OF THE LIMITING CURRENT AND HALF-WAVE POTENTIAL OF THE FIRST WAVE OF TRIPHENYLAMINE ON ITS CONCENTRATION

c (moles/liter)	i (μA)	$E_{\frac{1}{2}}$ (mv)	i/c (μA liters/mmole)
$1 \cdot 10^{-5}$	1.09	850	109.0
$1.99 \cdot 10^{-5}$	2.13	855	107.0
$2.98 \cdot 10^{-5}$	3.20	860	107.4
$3.96 \cdot 10^{-5}$	4.24	855	107.1
$4.94 \cdot 10^{-5}$	5.36	860	108.5
$9.76 \cdot 10^{-5}$	10.4	860	106.6
$1.45 \cdot 10^{-4}$	15.5	860	106.9
$1.90 \cdot 10^{-4}$	20.7	860	108.9
$2.35 \cdot 10^{-4}$	25.3	860	107.7
$2.79 \cdot 10^{-4}$	30.2	860	108.2
$3.64 \cdot 10^{-4}$	39.0	865	107.1
$4.44 \cdot 10^{-4}$	49.1	870	110.6
$5.22 \cdot 10^{-4}$	58.2	875	111.5

The presence of perchloric acid influences neither the $E_{\frac{1}{2}}$ value nor the wave-height of the wave, but it does increase the second wave. When perchloric acid is present in a 1000-fold excess, the height of the first wave decreases somewhat. Its plateau is shorter, as the second wave is growing; it continues to grow until it has increased to the fourfold (compared to the height in the absence of the acid), but it retains the shape of a peak.

Logarithmic analysis of the first wave gives a value of 1/60 mv ($2.3 \cdot 10^{-4} M$ solution).

When 10 ml of a $2 \cdot 10^{-5} M$ TPA solution in anhydrous acetonitrile are electrolyzed at a potential of 1 v, corresponding to the limiting current of the first wave, a charge is consumed corresponding to the transfer of 1.92 electrons from one TPA molecule. The intensively blue solution obtained gives two well-developed reduction waves (Table 4). Reduction at a

potential of 450 mv, corresponding to the limiting current of the second reduction wave consumed a charge, corresponding to 0.97 electrons (related to one TPA molecule), and the solution formed gave two well-developed oxidation waves. During the reduction process, the color of the solution changed from blue through olive-green, yellow-green, yellow-brown, and light yellow to colorless.

TABLE 4
GENERATION OF THE OXIDATION PRODUCTS OF TRIPHENYLAMINE

Solution	Color	ν	$E_{\frac{1}{2}}$ (mv)	i [μA]	Wave
$2 \cdot 10^{-5} M$ TPA	colorless	1.92	860	1.65	Ox
Oxidation product at 1000 mv	blue	0.97	770	0.58	1.Red
			635	0.56	2.Red
Reduction product at 450 mv	colorless		645	0.60	1.Ox
			785	0.64	2.Ox
Oxidation product at 690 mv	brown-yellow	0.45	635	0.58	Red
			780	0.64	Ox

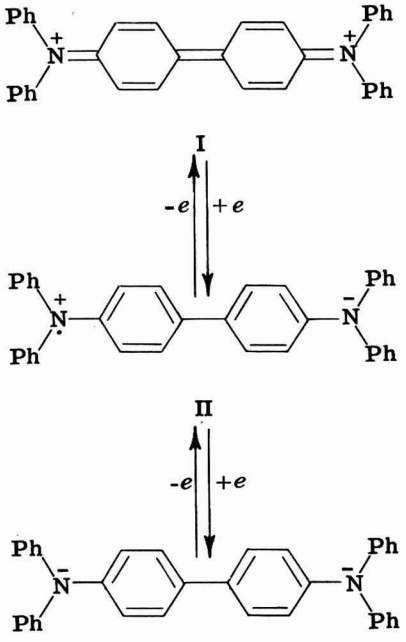
When oxidation was continued at a potential of 690 mv, i.e., between the two waves, a charge of 0.45 electrons was consumed (related to one TPA molecule) and the solution formed, which gave one reduction and one oxidation wave, was colored brown-yellow. All the solutions obtained in the course of the generation processes were stable. Agreeing results were obtained by generating the same amount of TPA in a solution which was also 0.1N in $HClO_4$.

It is known from the literature, that TPA is oxidized to N,N,N',N'-tetraphenylbenzidine (TPB) or its blue oxidized form. The intermediate product is a compound of the type of the Weitz radical (*17*), which, with respect to the free reactive para-position, reacts under formation of a benzidine derivative.

Assuming that on electrochemical oxidation in acetonitrile medium the oxidized TPB form will also be formed, two electrons would have to be transferred from one TPA molecule. The value of 1.92 electrons actually found agrees well with this assumption. The blue color of the solution also supports this view. The oxidation product of TPB formed (I) would

consume, when reduced to the fully reduced form (III), two electrons per molecule, and the resulting product ought to be colorless.

Since one TPB molecule forms from two TPA molecules, its concentration must be one-half that of TPA, and the two-electron reduction of TPB must correspond to the transfer of one electron (related to one TPA molecule). The value of 0.97 found agrees very well. Since a radical intermediate (II) may form in the oxidation of TPB (III), and the



III

polarograms of the redox system assumed show two waves, an oxidation product has also been generated at an inflection potential between the two waves, which should correspond to the oxidation of TPB to the radical cation (II) and the charge consumed should correspond to 0.5 electron (related to one TPA molecule). Although the half-wave potentials of the two waves are rather close to each other, a yellow-brown intermediate has actually been generated, as both waves are well developed and steep. The value of 0.45 electrons found agrees well with the assumed value.

Differing from the results obtained for DMA, the results of TPA generation are well reproducible, as the solutions formed are very stable

and the waves well developed; and no reaction side products are formed on TPA oxidation, capable of being detected polarographically. The Weitz radical type intermediate could not be proved (even in acid medium) even in orientative experiments with cyclic polarography.

Diphenylamine

In anhydrous acetonitrile medium, diphenylamine (DPA) gives one well developed oxidation wave whose height is proportional to the concentration (Table 5), which is followed at potentials of around 1600 mv

TABLE 5
DEPENDENCE OF THE LIMITING CURRENT AND HALF-WAVE POTENTIAL OF DIPHENYLAMINE ON CONCENTRATION

c (moles/liter)	i (μA)	$E_{\frac{1}{2}}$ (mv)	i/c (μA liters/mmole)
$0.50 \cdot 10^{-5}$	0.8	835	160.0
$1.99 \cdot 10^{-5}$	3.14	835	157.8
$3.96 \cdot 10^{-5}$	6.48	830	163.6
$7.84 \cdot 10^{-5}$	13.0	835	165.8
$1.54 \cdot 10^{-4}$	25.2	845	163.6
$3.30 \cdot 10^{-4}$	55.6	845	168.5
$4.91 \cdot 10^{-4}$	82.4	835	167.8
$6.39 \cdot 10^{-4}$	108	830	163.0

by a low peak (Fig. 4), after which the current starts to decrease (electrode passivation).

With the increasing temperature the first wave rises in the range of 20°-50°C, with a temperature coefficient of 1.2% per 1°C and the $E_{\frac{1}{2}}$ value does not vary.

The presence of water in the solution is not manifested up to a concentration of about 0.1%. At water concentrations above 1% the limiting current achieved continues to rise, as the oxidation of water begins to participate.

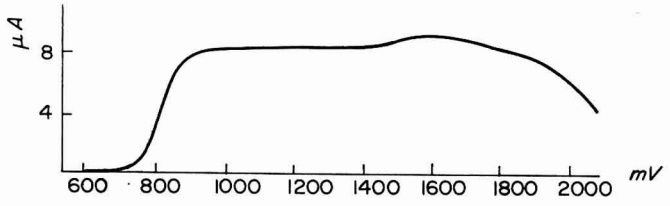


FIG. 4. Oxidation of diphenylamine ($c = 4.94 \cdot 10^{-5} M$) on the RPE.

The presence of perchloric acid decreases the height of the first wave and increases its $E_{\frac{1}{2}}$ value. This first wave gradually converts into the second one, which at perchloric acid concentrations of over $10^{-2}M$ (i.e., an approximately 200-fold excess) loses the character of a peak, becoming very elongated and badly developed.

Logarithmic analysis of the first wave, obtained in a $2 \cdot 10^{-5}M$ solution gives a straight line with a slope of 1/62 mv.

On electrolysis of 10 ml of a $2 \cdot 10^{-5}M$ DPA solution (Table 6), at a potential corresponding to the limiting current of the first wave (1000 mv),

TABLE 6
GENERATION OF THE OXIDATION PRODUCTS OF DIPHENYLAMINE

Solution	Color	v	$E_{\frac{1}{2}}$ (mv)	i [μA]	Wave
10 ml $2 \cdot 10^{-5}M$ DPA	colorless		885	2.56	Ox
Product of	violet		680	0.74	1.Red
oxidation		2.1	560	0.6	2.Red
at 1000 mv			cca 250	cca 0.3	[a]
Product of	colorless		575	0.66	1.Ox
reduction		0.95	700	0.74	2.Ox
at 500 mv			cca 280	cca 0.2	[a]
Product of	yellow		705	0.8	Ox
oxidation		0.4	575	0.64	Red
at 640 mv			cca 300	cca 0.3	[a]

[a] Elongated reduction current rise.

a charge was consumed, corresponding to the transfer of 2.1 electrons from one DPA molecule. The solution obtained was colored intensively violet, and gave two reduction waves, followed by a small, elongated current rise around 300 mv. In the reduction of this solution at a potential of 500 mv, corresponding to the limiting current of the second reduction wave, a charge was consumed corresponding to 0.95 electrons (related to one DPA molecule) and the colorless solution formed gave two oxidation waves and an elongated current rise (in the region of reduction currents) at potentials around 300 mv.

The continued oxidation at a potential of 640 mv, i.e., between the two oxidation waves, consumed a charge corresponding approximately to 0.4 electrons (related to one DPA molecule), but due to the very close half-wave potentials of the two waves this is only an approximate value.

The solution formed gave one oxidation and one reduction wave (and an elongated reduction current rise around 300 mv). The mutually corresponding waves were always found at practically equal potentials, and the solutions were stable.

According to literature data, DPA is oxidized in aqueous medium to diphenylbenzidine (DPB) or its oxidized form (*13-14*). The results of the generation of DPA oxidation products show (Table 6) that the oxidized form of DPB is the main product of electrochemical DPA oxidation in acetonitrile medium also. Differing from TPA, however, a small amount of an intermediate is also formed (the slight increase of the reduction current at potentials around 300 mv corresponds to this intermediate). We have used the following experiment to convince ourselves that the waves of generated products at 680 and 560 mv actually correspond to DPB; DPB has been prepared by the oxidation of DPA with bichromate in a medium of sulfuric acid and reverse reduction of the diphenylbenzidine violet by a solution of ferrous ions (*13*). The colorless product was then made alkaline and DPB extracted with toluene. After evaporating the toluene, the residue was dissolved in acetonitrile. The DPB solution thus obtained gave two oxidation waves of approximately equal height, with half-wave potentials of 570 and 700 mv, which are identical with the oxidation waves obtained by polarographic oxidation of a DPA solution that had been oxidized at 1 v and then reduced at 0.5 v. The color of the solution, corresponding to a semiquinoid intermediate of oxidation in acetonitrile, was yellow, while according to literature data the solid, water-insoluble semiquinoid is green. However, the green semiquinoid, prepared by oxidation of DPB to the first oxidation stage (*13*), dissolves in acetonitrile to form a yellow solution, which explains this apparent discrepancy.

Differing from the oxidation of DPA with bichromate in a weakly acid medium, where DPA is first oxidized to DPB (which can be isolated) and is then oxidized in the following stages to the semiquinoid and benzidine violet (*13*), benzidine violet forms immediately in electrochemical oxidation on the platinum electrode in acetonitrile medium (the mechanism of the direct oxidation of DPA to DPB violet has already been proposed by Wieland (*15*) and Thiele (*16*)). The half-wave potential of DPB oxidation in acetonitrile medium is more negative than the $E_{\frac{1}{2}}$ value of DPA, so that even if DPB is formed as intermediate, it is immediately oxidized (at DPA oxidation potentials) up to diphenylbenzidine violet.

Di-4-tolylamine

In a medium of acetoniltrile, di-4-tolylamine gives two oxidation waves: the first one is well developed, its half-wave potential does not vary with the concentration and its height is proportional to the concentration (Table 7); the second has the shape of a peak (see Fig. 5) which becomes sharper at higher concentrations.

TABLE 7

DEPENDENCE ON THE LIMITING CURRENT AND HALF-WAVE POTENTIAL OF DI-4-TOLYLAMINE ON CONCENTRATION

c (moles/liter)	i_1 (μA)	$E_{\frac{1}{4}}$ (mv)	i_2 (μA)	$E_{p/2}$ (mv)	i_1/c (μA liters/mmole)
$1 \cdot 10^{-5}$	1.16	700	1.3	1500	116.0
$1.99 \cdot 10^{-5}$	2.29	705	2.5	1530	115.1
$2.98 \cdot 10^{-5}$	3.44	710	3.8	1520	115.4
$3.96 \cdot 10^{-5}$	4.60	705	5.1	1540	116.2
$4.94 \cdot 10^{-5}$	5.37	710	7.4	1530	116.0
$1.17 \cdot 10^{-4}$	14.0	700	12.4	1550	119.7
$2.27 \cdot 10^{-4}$	26.9	705	22.6	1540	118.5
$3.64 \cdot 10^{-4}$	43.7	710	24.0	1550	120.0
$5.22 \cdot 10^{-4}$	61.1	710	—	—	117.0
$6.64 \cdot 10^{-4}$	77.2	710	—	—	116.3

With the increasing temperature the height of the first wave rises in the range of 20°-50°C, with a temperature coefficient of approximately 1% per 1°C, and its $E_{\frac{1}{4}}$ values does not vary. The second wave increases with a temperature rise from 20° to 30°C by about 60%, and then decreases with the continuing temperature rise; as electrode passivation is the more rapid at higher temperatures, the sharper the maximum.

The presence of water in the solution is not manifested up to a concentration of 0.1%. At higher water concentrations the height of the first wave decreases somewhat, as also does its $E_{\frac{1}{4}}$ value (decreasing at 5% water content by 20 mv). Due to the high potential values the plateau of the first wave and the second wave are influenced by the accompanying oxidation of water, so that the influence of water on the second wave cannot be evaluated.

The presence of perchloric acid decreases the limiting current of the first wave and shifts the half-wave potential to positive values. The height of the second wave increases somewhat in the presence of perchloric acid, and another low wave appears between the first and the second wave. At

perchloric acid concentrations greater than about $3 \cdot 10^{-2} M$, all the waves are suppressed.

The logarithmic analysis gives straight lines with slopes of 1/56 ($5 \cdot 10^{-5} M$ solution) and 1/64 mv ($5 \cdot 10^{-4} M$ solution).

Attempts to generate oxidation products of di-4-tolylamine were unsuccessful. Values of the charge passed through the solution in the oxidation of di-4-tolylamine at the potential of the limiting current of the first wave were nonreproducible (around 2.5 electrons related to one molecule). The solution after the generation gave several elongated, low

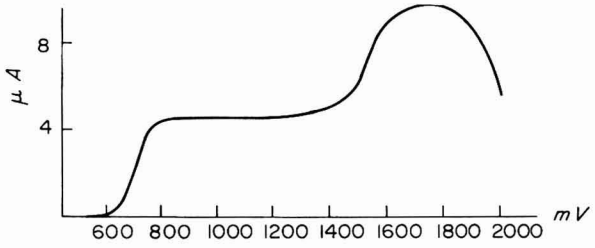


FIG. 5. Oxidation of di-4-tolylamine ($c = 3.96 \cdot 10^{-5} M$) on the RPE.

reduction waves, which shifted into the region of the oxidation currents, and one elongated oxidation wave whose foot lay at potentials somewhat more negative than the potential needed for generation, so that the generated product was slowly oxidized to a higher stage. It was therefore not possible to determine the number of electrons involved in the exchange corresponding to the first wave.

The first oxidation product may well be a compound of the type of the Weitz radical (*17, 18*); however, the product is unstable and converts to more stable oxidation products (phenazine derivatives).

SUMMARY

The polarographic behavior of *N,N*-dimethylaniline, triphenylamine, diphenylamine, and di-4-tolylamine has been studied in acetonitrile medium on a rotating platinum electrode. It has been found possible to apply the oxidation waves of these substances to their polarographic determination, considering the water content of the solvent, temperature and acidity of the medium. Constant current coulometric generation has been used to study the number of electrons exchanged in the reactions. The products formed in the electrode reaction are discussed.

REFERENCES

1. DVORÁK, V., Electrochemical oxidation of aromatic amines. PhD Thesis, Charles University, Prague, Czechoslovakia, 1965.

2. COETZEE, J. F., CUNNINGHAM, G. P., MCGUIRE, D. K., AND PADMANABHAN, G. R., Purification of acetonitrile as a solvent for exact measurements. *Anal. Chem.* **34**, 1139-1143 (1962).
3. BIEDERMANN, G., The hydrolysis of metal ions. XIV. The hydrolysis of the indium(III) ion. *Arkiv Kemi* **9**, 227-293 (1956).
4. KOLTHOFF, I. M., AND IKEDA, S., Polarographic and acid properties of thorium perchlorate in acetonitrile. *J. Phys. Chem.* **65**, 1020-1026 (1961).
5. PECSOK, R. L., AND JUVET, R. S., JR., Versatile polarographic cell. *Anal. Chem.* **27**, 165-166 (1955).
6. DVORÁK, V., NĚMEC, I., AND ZÝKA, J., Study of the properties of lead tetraacetate as oxidizing and oxidimetric reagent in analytical chemistry. II. Polarographic behavior of tetravalent lead. *Microchem. J.* **11**, 172-182 (1966).
7. SHAMS EL DIN, A. M., NILSSON, O., AND WRANGLÉN, G., The polarizability of the saturated calomel electrode. *J. Electroanal. Chem.* **2**, 497-500 (1961).
8. MEYER, F. R., AND RONGE, G., Entfernen von Sauerstoff aus Gasen mittels aktiven Kupfers. *Angew. Chem.* **52**, 637-638 (1939).
9. WILLEBORDSE, F., Thesis, University of Amsterdam, 1959.
10. MIZOGUCHI, T., AND ADAMS, R. N., Anodic oxidation studies of *N,N*-dimethylaniline. I. Voltammetric and spectroscopic investigations at platinum electrodes. *J. Am. Chem. Soc.* **84**, 2058-2061 (1962).
11. GALUS, Z., AND ADAMS, R. N., Anodic oxidation studies of *N,N*-dimethylaniline. II. Stationary and rotated disk studies at inert electrodes. *J. Am. Chem. Soc.* **84**, 2061-2065 (1962).
12. GALUS, Z., WHITE, R. M., ROWLAND, F. S., AND ADAMS, R. N., Anodic oxidation studies of *N,N*-dimethylaniline. III. Tritium tracer studies of electrolysis products. *J. Am. Chem. Soc.* **84**, 2065-2068 (1962).
13. KOLTHOFF, I. M., AND SARVER, L. A., Properties of diphenylamine and diphenylbenzidine as oxidation-reduction indicators. *J. Am. Chem. Soc.* **52**, 4179-4191 (1930).
14. SARVER, L. A., AND KOLTHOFF, I. M., Electrochemical properties of diphenylbenzidine sulfonic acid. *J. Am. Chem. Soc.* **59**, 23-25 (1937).
15. WIELAND, H., Zur Oxydation der sekundären und tertiären aromatischen Amine. (XX. Über ditertiäre Hydrazine und damit zusammenhängende Gebiete.) *Chem. Ber.* **52**, 886-893 (1919).
16. THIEL, A., Uber Diphenylamin und Diphenylaminblau. *Z. Elektrochem.* **35**, 274-278 (1929).
17. BECKER, F., The stability of ammonium and ammonium salts of the arylamines. *Chem. Ber.* **86**, 1150-1157 (1953).
18. WEITZ, E., AND SCHWECHTEN, H. W., Über den Ammonium Charakter der Tri- und Diarylamine (II). *Chem. Ber.* **60**, 545-551 (1927).

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Improved Determination of Carbon and Fluorine in Highly Fluorinated Substances

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Despite its importance, the combustion tube method for the decomposition of organic fluorine compounds has received limited attention. A previous study reported by this laboratory (*4*) made reference to several procedures in use at that time. More recent combustion techniques have been described by Monand (*6*), Gelman and co-workers (*5*), Dubnikov and Kalyagin (*2*), and by Curry and Mellon (*1*). Of these, only the work of Gelman and his co-workers allows the simultaneous determination of other elements together with fluorine.

In our work described previously (*4*), the sample is burned in moist oxygen at 1100°C in a quartz tube. Carbon is determined gravimetrically as carbon dioxide, and fluorine is determined acidimetrically as hydrofluoric acid. We have used this procedure in our laboratory for 11 years with satisfactory results. Various improvements have been made in reagents, procedure, and apparatus. The present study describes these improvements.

MATERIALS AND METHODS

Apparatus

The modified apparatus is shown in Fig. 1. It consists of a conventional Mariotte bottle (A) for measuring the volume of oxygen used for combustion, a guard tube containing Anhydrone (B), micro absorption tube (D) and tare tube (C), each filled with Ascarite and Anhydrone. The U-tube (E) is filled with Anhydrone, and the bubble trap (F) contains concentrated sulfuric acid. The Grote-type absorber (G) has been modified by the addition of a 10/30 joint to facilitate removal of the acidic material. It is made of quartz and has a medium frit. The spray trap is borosilicate glass. Electric furnace (H), 11 cm long, is kept at 415°C. The Lindberg Micro Combustion Furnace (I) is 20.5 cm long and normally

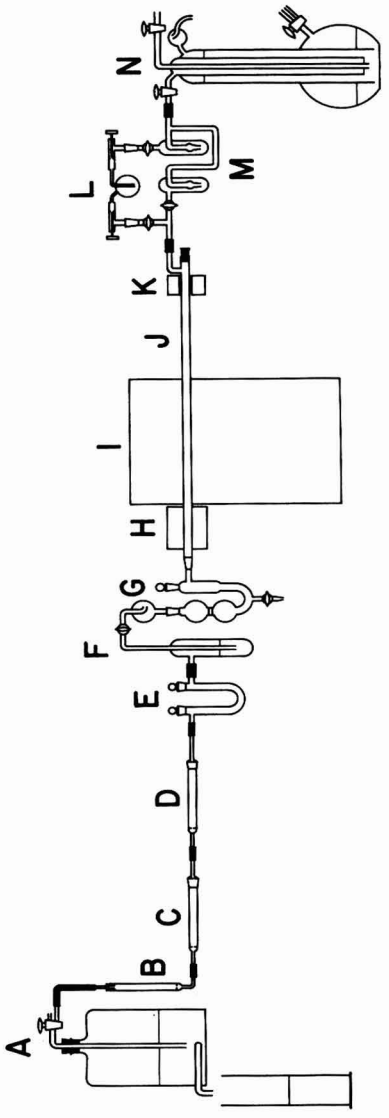

FIG. 1. Combustion apparatus.

operates at 1100°C. For operation above 1100°C, a furnace is used which is constructed by winding platinum wire on an Alundum core and covering it with Norton R.A. 1139 refractory cement (Norton Co., Worcester, Mass.). The quartz combustion tube (J) is approximately 65 cm long and 11 mm O.D. The side arm is 4 mm O.D., and the exit end has a 10/30 male joint. It is packed, beginning at the exit end, with 11 cm of silver gauze, 2.5 cm of silver vanadate granules (Coleman Instruments, Inc., Maywood, Illinois), 4.7 cm of quartz chips, 4.7 cm of platinum screen, 4.7 cm of quartz chips again, and finally another 4.7-cm section of platinum screen. This final platinum screen protrudes out of the entrance end of the furnace about 1 cm. The movable electric furnace (K) is 5 cm long and is operated at 800° to 900°C. The gas-handling bulb (L) is about 17 cm long over-all, and is fitted with valves having Teflon needles (Fischer and Porter Co., Warminster, Pa.). The gas-splitting device (M) allows changing the ratio of oxygen gas going through the sample bulb (L) to that which goes by it. The bubblers saturate the oxygen with moisture. The all-glass pressure regulator (N) has been described separately (*7*).

When compounds containing nitrogen are analyzed, the Kjeldahl micro distillation apparatus, specified by the Committee for the Standardization of Microchemical Apparatus (*8*), is also used.

Reagents

Sodium hydroxide (0.01 *N*), carbonate-free, which is stored in a polyethylene bottle equipped with an automatic buret. It should not be allowed to stand in contact with the glass buret any longer than necessary.

Phenolphthalein solution, 1%, in ethanol.

Hydrochloric acid, 0.01 *N*.

Boric acid solution, 2% w/v, containing 0.1 g of Duponol per liter of solution.

Potassium hydroxide solution, 40% w/v.

Devarda's alloy.

Antifoam A silicone spray (Dow Corning Corp., Midland, Mich.).

Methyl red indicator.

PROCEDURE

Samples are about 5 to 10 mg in size. The solids are weighed in platinum boats, liquids in quartz capillaries 0.5 to 1 mm I.D., and gases in the gas bulb described. Liquids can usually be weighed with one end

of the capillary open, especially if smaller capillaries are used. Prepurified oxygen enters the combustion apparatus at the pressure regulator (N).

The apparatus is assembled with the preweighed absorption tubes (C and D) in place and about 10 ml of water in the Grote absorber (8 to 9 ml below and 1 to 2 ml above the frit). The joint between the combustion tube and the Grote absorber is lightly greased with Celvacene heavy vacuum grease. This joint is air cooled during combustion. The liquid or solid sample is slowly vaporized into the main furnace by the movable furnace over a period of about 30 minutes with the oxygen gas flow set at 15 ml per minute. When gas samples are being analyzed, the weighed gas bulb is first filled with oxygen. The ratio of oxygen going through the gas bulb to that going by it is adjusted to be very low at first. It is then increased until the oxygen is finally all passing through the sample bulb.

When the sample has been burned, an additional 20 minutes is allowed to sweep out the combustion products. The oxygen flow rate may be increased to 25 ml per minute during this period. The absorption tubes are then removed and weighed. The air which cooled the joint on the combustion tube is turned off, and the joint is allowed to warm up to dry it out. The oxygen flow is continued until the joint is dry.

The Grote absorber is removed, and its contents are transferred to an Erlenmeyer flask. To remove all the acid from the absorber requires scrubbing with a swab made of cotton on platinum wire. Some 0.01*N* NaOH is added directly to the absorber, and final quantitative transfer is assured by putting a drop of indicator in the absorber. The spray trap must be rinsed into the same flask. By using phenolphthalein the combined washings and absorber contents are titrated nearly to the end point with 0.01*N* NaOH. The solution is boiled 3 minutes to expel carbon dioxide, cooled, and the titration completed to the first permanent pink color. A total of about five drops of phenolphthalein solution is used.

If nitrogen is present in the sample, the titration must be corrected for the presence of acidic nitrogen oxides. This is accomplished as follows (*9*):

The neutralized acids are transferred to the Kjeldahl micro distillation apparatus which contains 8 ml of a 40% potassium hydroxide solution and about 1 g of Devarda's alloy. A small amount of Antifoam is added to the still. The nitrogen oxides are reduced to ammonia which is distilled into 5 ml of the 2% boric acid solution containing Duponol. When 25 ml of distillate have been collected, the receiver is replaced with a second receiver, also containing 5 ml of the boric acid solution. A third 25-ml

portion is usually sufficient to complete the distillation. Methyl red indicator is added and the ammonia is titrated with 0.01*N* HCl.

CALCULATIONS

$$\% \text{ C} = \frac{\text{mg } CO_2 \times 0.2729 \times 100}{\text{mg Sample}}$$

$$\% \text{ F} = \frac{\text{ml NaOH} \times N \times 19 \times 100}{\text{mg Sample}}$$

$$\% \text{ F} = \frac{\text{meq HF} \times 19 \times 100}{\text{mg Sample}},$$

(when nitrogen is present)

where meq HF = ml NaOH $\times$ N — ml HCl $\times$ N
= total acidity — acidity of nitrogen compounds.

RESULTS AND DISCUSSION

Sulfur-Containing Compounds

The original work discussed certain sulfur compounds in which the sulfur was present in relatively low valence states (—1, 0), which yielded high values both for carbon and fluorine. To correct for this, vanadium pentoxide was added to a section of the combustion tube packing and an acid permanganate bubbler was inserted into the combustion train following the Grote absorber. Without these reagents, acidic sulfur-containing combustion products were absorbed in the hydrofluoric acid solution and in the Ascarite. The vanadium pentoxide and permanganate complete the oxidation to sulfate and retain the sulfate, thus preventing high results.

By the addition of the silver vanadate section to the combustion tube, as described above, the vanadium pentoxide and permanganate can be eliminated. The silver vanadate, the preparation and use of which have been described by Ebeling and Malter (*3*), is more convenient to use than is the vanadium pentoxide. The vanadium pentoxide migrated down the tube and coated the silver and platinum, which soon caused them to be ineffective. The silver vanadate-packed tubes, on the other hand, have a normal tube life. It is necessary to pack only one type of combustion tube, and it may be used for all types of compounds, whether they contain sulfur or not. The silver vanadate is in pellet form, coated on zirconium oxide, but the zirconium oxide does not interfere, probably because the zirconium fluoride is thermally unstable while the oxide is a very

high-melting compound. At the position specified in the combustion tube, the silver vanadate granules are at about 875°C. Some compounds which contain lower valence sulfur and which have been analyzed with the silver vanadate-containing combustion tube, are shown in Table 1.

TABLE 1
SULFUR COMPOUNDS ANALYZED WITH SILVER VANADATE PACKING

Compound	Carbon %		Fluorine (%)	
	Calculated	Found	Calculated	Found
$(C_3F_7)_2S_2$	17.91	18.1	66.14	66.6
$(C_7F_{15})_2S_2$	20.95	21.0	71.04	71.4

Nitrogen-Containing Compounds

When nitrogen is present in the compound being analyzed, nitrogen oxides are formed which dissolve in the Grote absorber causing high fluorine results. Formerly such compounds were analyzed only for carbon by the combustion procedure. Fluorine was then determined by a separate method. It is now possible to also determine fluorine in the presence of nitrogen by making the correction as described. Not all the nitrogen in the samples is converted to nitrogen oxides during the combustion, but all the excess acidity in the Grote absorber is accounted for by this correction. Several examples are given in Table 2. Halogens and sulfur do not interfere since they are retained in the combustion tube. In one case a sample was analyzed which contained eight elements (C, H, O, N, P, F, Cl, an —S—S— linkage) and an SO_2 group, by using the silver vanadate in the tube packing and by applying the correction for nitrogen.

Other Improvements

Several other changes will be noted in the procedure from the previous work. The use of the quartz Grote absorber eliminates the interference of boron which is experienced when borosilicate glass absorbers are used. Since boron causes the formation of hydroxyfluoroboric acid which is too weak to be titrated, one must allow for the presence of the weaker acid by assigning a titer to the sodium hydroxide. Such a titer is obtained by analyzing several pure fluorochemicals. The use of the quartz absorber eliminates this lengthy process, and the sodium hydroxide may simply be standardized against a primary standard such as potassium acid phthalate.

TABLE 2

COMPOUNDS ANALYZED USING THE CORRECTION FOR NITROGEN

Compound		Carbon		Acidity	Acidity, nitrogen	Acidity	Fluorine	
Empirical formula	mg Taken	Calculated (%)	Found (%)	Total (meq)	compounds (meq)	HF (meq)	Calculated (%)	Found (%)
$C_9H_8F_3NO_4S$	11.254	38.16	38.1	0.1257	0.0055	0.1202	20.13	20.3
$C_{14}H_{10}F_9NO_3$	9.053	40.88	40.8	0.2018	0.0057	0.1961	41.58	41.2
$C_{11}H_{10}F_6N_2O_3$	10.856	39.76	40.0	0.1991	0.0032	0.1959	34.31	34.3

By extending the combustion and sweeping periods and slowing down the oxygen flow rate, a more controllable decomposition is obtained. While previously a sample would occasionally distill into the main furnace too rapidly, this seldom happens now. Extension of the sweeping time also assures complete removal of the carbon dioxide from the water in the absorber. Quartz capillaries avoid the problem of sticking to the combustion tube which is characteristic of glass capillaries. The Fischer and Porter valves on the gas sampling bulb are less subject to leakage than are the small glass stopcocks formerly used.

The procedure can be recommended for any laboratory which is engaged in the determination of two or more elements in highly fluorinated materials. It is especially valuable for gases and particularly when there is a limited amount of material available.

REFERENCES

1. CURRY, R. P., AND MELLON, M. G., Spectrophotometric determination of inorganic fluoride and of fluorine in organic compounds. *Anal. Chem.* **29**, 1632-1637 (1957).
2. DUBNIKOV, L. M., AND KALYAGIN, I. A., Colorimetric determination of fluorine in fluorine-containing organic compounds. *Zavodsk. Lab.* **29**, 1298-1301 (1963).
3. EBELING, M., AND MALTER, L., Carbon-hydrogen analysis of sulfur- and halogen-containing compounds using a silver vanadate packing. *Microchem. J.* **7**, 179-184 (1963).
4. FREIER, H. E., NIPPOLDT, B. W., OLSON, P. B., AND WEIBLEN, D. G., Determination of carbon and fluorine in highly fluorinated substances. *Anal. Chem.* **27**, 146-149 (1955).
5. GELMAN, N. E., KORSHUN, M. O., AND NOVOZHILOVA, K. I., Simultaneous microdetermination of fluorine, carbon, and hydrogen. *Zh. Anal. Khim.* **15**, 342-346 (1960).
6. MONAND, P., Microdosage du fluor dans les composes organiques a l'aide du titrage haute-frequence. *Bull. Soc. Chim. France, 1956,* 704-707.
7. NIPPOLDT, B. W., AND POLLMAN, G. J., All-glass pressure regulator. *Anal. Chem.* **26**, 1526-1527 (1954).
8. STEYERMARK, A., ALBER, H. K., ALUISE, V. A., HUFFMAN, E. W. D., KUCK, J. A., MORAN, J. J., AND WILLITS, C. O., Recommended specifications for microchemical apparatus. *Anal. Chem.* **23**, 523-528 (1951).
9. WHITE, L. M., AND MARION, C. L., Kjeldahl microdigestions in sealed tubes at 470°C. *Anal. Chem.* **23**, 363-365 (1951).

MICROCHEMICAL JOURNAL **12**, 125-132 (1967)

Ion Specific Electrochemical Behavior of Macrotetrolides in Membranes[1]

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Received November 10, 1966

In connection with the use of antibiotics as tools for metabolic studies an ion specific behavior of nonactin homologs (*3, 4*), gramicidins (*1, 6*) and valinomycin (*5*) has been observed in rat liver mitochondria. It was proposed that these compounds act *via* a mechanism related to cation movements with the specific cation requirements depending in some manner on the molecular configuration of the antibiotic (*3-5*). The obvious question arises whether these compounds show an analogous behavior *in vitro*. This report presents data on the ion specific effects of nonactin homologs in electrochemical cells.

MATERIALS AND METHODS

EMF measurements have been performed on the following electrochemical cell:

Ag; AgCl, inner solution//membrane//sample/0.1*M* NH_4NO_3/KCl std., Hg_2Cl_2; Hg

The aqueous inner solution was 0.1 *M* in the chlorides of all cations tested in one set of experiments and was buffered to pH 8 with acetic acid (ca. 0.5*M*) and triethanolamine (1.0*M*).

At room temperature supersaturated solutions of nonactin homologs in carbon tetrachloride were transferred onto sintered glass discs (Porosity G2 to G5, Jena, Germany) to form the membrane. Membranes may also be prepared by treating filter paper (Millipore TH filters for Infrared Spectroscopy, Millipore Filter Corp., Bedford, Mass.), polyethylene film (0.1 mm and 25 μ), nylon mesh (0.0010 in. aperture, threads: 254 in.

[1] Partly presented at the Sommerversammlung der Schweizerischen Chemischen Gesellschaft in Solothurn, October 1, 1966. (See *8*.)

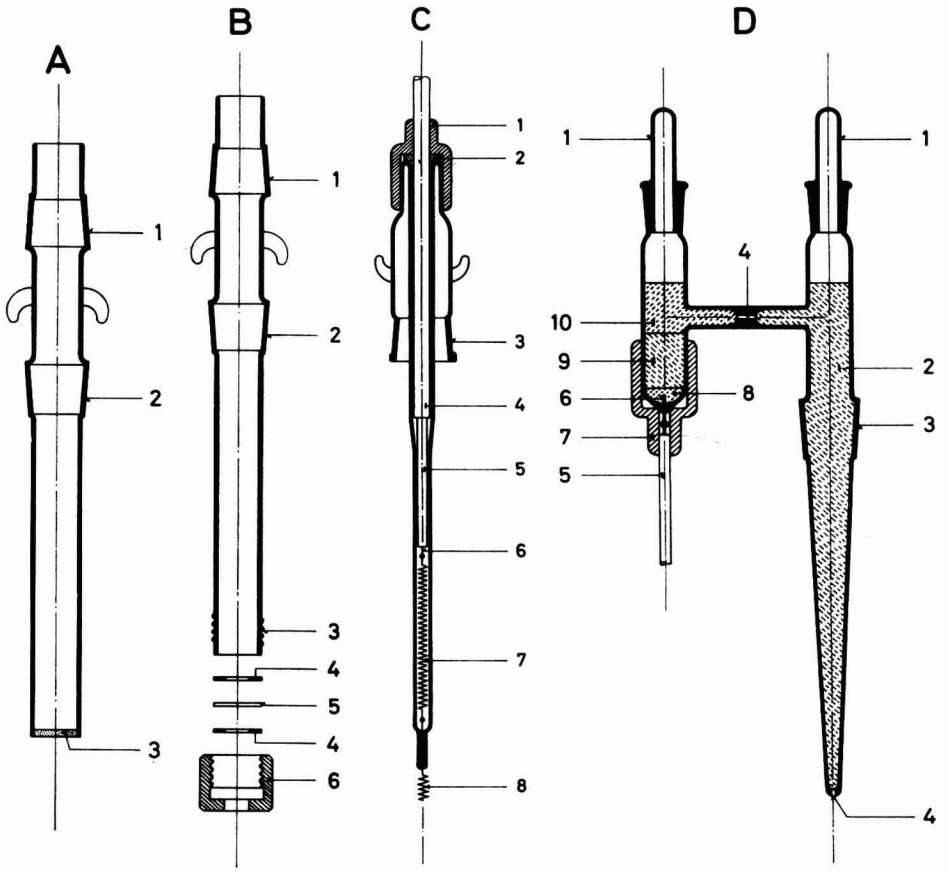

FIG. 1. Electrode assembly.

A: Electrode body with sintered glass disc. (1)—Ground glass joint for C (3); (2)—ground glass joint to fit into measuring cell; (3)—sintered glass disc.

B: Electrode body for using foils as membranes. (1)—Ground glass joint for C (3); (2)—ground glass joint to fit into measuring cell; (3)—glass thread; (4)—rubber rings; (5)—membrane; (6)—Teflon counterpart.

C: Inner reference electrode for A and B. (1)—Rubber sleeve; (2)—metal ring; (3)—ground glass joint for A or B (1); (4)—electrical shield and insulation; (5)—polyethylene insulation; (6)—copper lead welded to spiral; (7)—silver spiral welded to platinum wire; (8)—platinum wire (silver is electrodeposited and chloridized).

D: Reference electrode. (1)—Stoppers for filling electrode with electrolyte; (2)—0.1*M* NH_4NO_3 in water; (3)—ground glass joint to fit into measuring cell; (4)—porous sintered oxide plugs; (5)—electrical connection; (6)—platinum wire; (7)—rubber sleeve; (8)—mercury/calomel; (9)—cotton wool; (10)—KCl (satd.) in water.

warp, 409 in. woof; Nybolt 25 T II, Schweiz. Seidengazefabrik, Zürich) or gel formers (Thixcin B 968/1238, Nuclear Enterprises, Edinburgh; Thixotropic Gel Powder CAB-O-SIL, Packhard Instrument GmbH, Frankfurt) with supersaturated solutions of nonactin homologs in benzene or carbon tetrachloride. Details of the cell used are shown in Fig. 1. The EMF values of the cell were measured relative to an aqueous test solution as sample being 0.001*M* in the chlorides of all cations tested in one set of experiments. The solution was buffered to pH 8 with acetic acid and triethanolamine. To measure the signal due to a specific cation the concentration of the cation in question was raised by two orders of magnitude to 0.1*M* in the test solution. The EMF was measured at 25.0° ± 0.1°C with a standard deviation of 0.1 mV as described earlier (*7*).

Doubly distilled water and chemicals of highest purity available were used in the work described.

The nonactin homologs studied were in a mixture of 20% nonactin, 35% monactin, 35% dinactin and 10% trinactin (*2*). We thank Dr. W. Keller-Schierlein and Dr. H. Gerlach for supplying this material.

RESULTS

Preliminary experiments with various supports to prepare the nonactin homolog membranes showed (Table 1) that sintered glass discs led to working systems without any exception. The change of EMF with time for a typical membrane of the sintered glass type is given in Fig. 2. A steady reading is obtained about two hours after changing the sample solution. To obtain such constant values, EMF measurements were carried out during about 2-3 hours for each solution. Before and after testing the

TABLE 1
MEMBRANE SUPPORTS

Membrane support	No. of membranes prepared	No. of membranes showing significant potassium response
Millipore	12	6
Nylon mesh	4	3
Polyethylene 0.1 mm	3	2
25 μ	3	—
Thixcin	17	5
Thixotropic gel powder (CAB-O-SIL)	7	4
Sintered glass discs	18	18

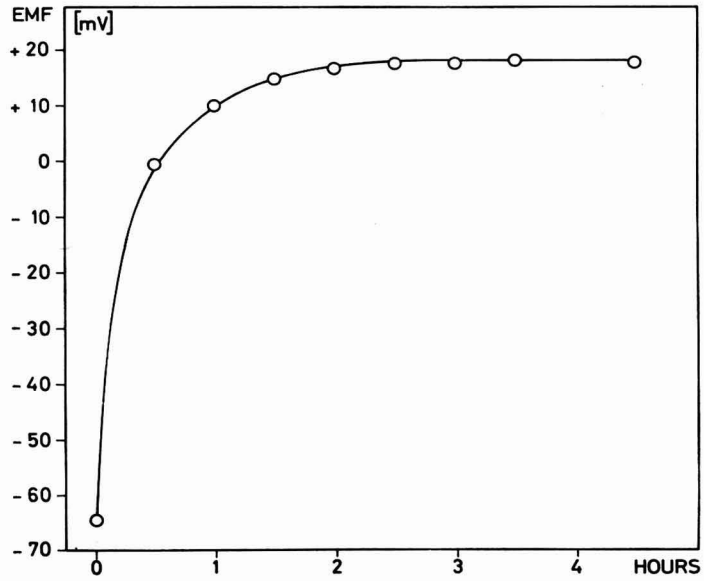

FIG. 2. Potassium response of a cell of the sintered glass type (G4) after transfer from a sample solution being 0.001*M* in NaCl, KCl and $CaCl_2$ (pH 8) to a sample solution being 0.001*M* in NaCl and $CaCl_2$ and 0.1*M* in KCl (pH 8).

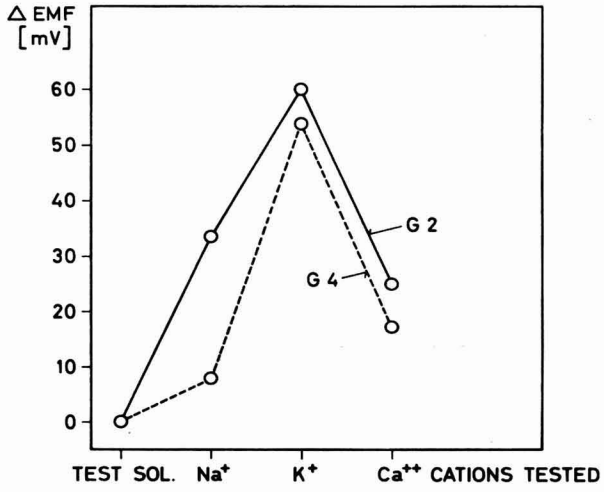

FIG. 3. Influence of porosity of sintered glass on the selectivity for different cations using membranes consisting of a supersaturated solution of nonactin homologs in CCl_4 of low viscosity.

response for a given cation, test solutions were measured as reference. All Δ EMF values are presented relative to the mean values for the two test solutions.

The viscosity of the supersaturated solution of the nonactin homologs as well as the porosity of the sintered glass discs have a significant influence on the specificity of the cell. Low viscosity causes low specificity. The influence of the porosity for a given viscosity is shown in Fig. 3 for

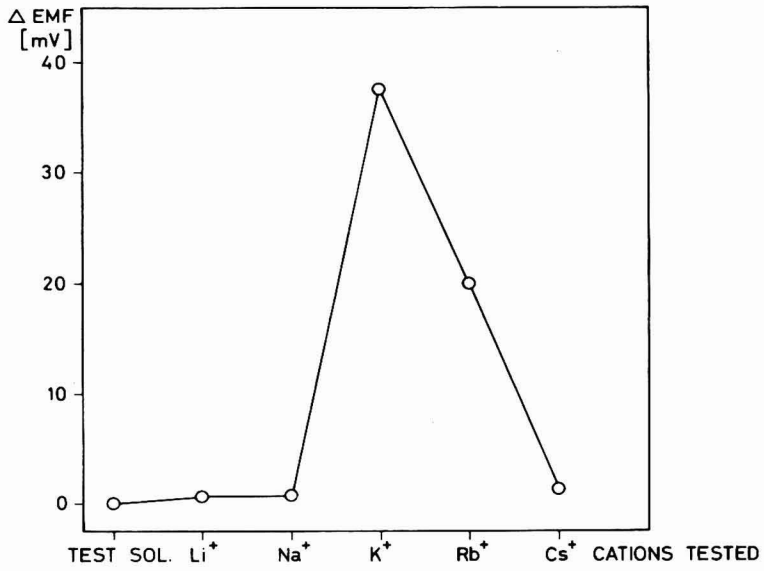

FIG. 4. Response of nonactin homolog membranes to monovalent cations. The radius of the circles corresponds to the standard deviation of a single determination (0.5 mV). The arithmetic mean of the ΔEMF values obtained on three membranes is presented (two G5, one G4). Viscosity of the nonactin homolog solution: 12 poise.

the response to Na^+, K^+, and Ca^{++}. It is obvious that low porosity leads to high specificity. The response to Li^+, Na^+, K^+, Rb^+, and Cs^+ of similar membranes which have been prepared using a solution of the nonactin homologs at a viscosity of 12 poise (Epprecht Rheomat 15, Contraves AG., Zürich) is given in Fig. 4. According to these results, membranes may be prepared having a remarkable selectivity for potassium over sodium. Some selectivity constants K determined in buffered and unbuffered solutions are given in Table 2. These K values have been calculated by using the experimental slope of 32 mV for a change in activity of the cation of one order of magnitude (Table 3).

TABLE 2

SELECTIVITY CONSTANTS DETERMINED

Porosity of sintered glass	Selectivity of potassium over sodium: K_{K^+/Na^+} [a]	
	Unbuffered	pH 8
G2	3	—
G4/G5	499	105

[a] $\log K_{K^+/Na^+} = \dfrac{^{EMF}0.1M\ KCl;\ 0M\ NaCl \quad -\,^{EMF}0.1M\ NaCl;\ 0M\ KCl}{32}$

Viscosity of the supersaturated nonactin homolog solution: 12 poise.

TABLE 3

CHANGE IN EMF FOR A TENFOLD CHANGE IN CATION CONCENTRATION (slope)[a]

Porosity of the sintered glass	Slope [mV]	
	0.1/0.01*M* KCl	0.01/0.001*M* KCl
G5	35.0	30.3
G4	33.3	28.9

[a] The measurements were performed using solutions of KCl in water (pH8), acetic acid ca. 0.5*M*, triethanolamine (1.0*M*). Similar slopes are obtained in unbuffered solutions.

DISCUSSION

In Fig. 5 the ion specificity obtained *in vitro* by using the electrochemical cell described is compared to the ion specific effect of monactin and nonactin at $2 \cdot 10^{-7}M$ on the ATPase induction (*3*, *4*). For the monovalent cations studied there is a close correlation between the behavior of the two systems. The EMF response of the electrochemical cell studied is controlled by the ion transport phenomena in the membrane area. Preliminary measurements by vapor pressure osmometry in methanol at 30°C have shown that the complex formation constant of potassium with the nonaction homologs is about 30 times higher than the one with sodium (*8*). Because of the cation requirements for the activity of compounds such as actin homologs, gramicidins and valinomycin in rat liver mitochondria it has been suggested (*3-5*) that their mechanism of action involves the transport of monovalent cations. The behavior *in vitro* of the compounds mentioned may therefore give additional information about the mechanism and site of the ion transport in subcellular membranes.

The electrochemical cells studied have a far superior specificity for potassium over sodium than the ion specific glass electrodes available so

far. The highest selectivity constants reported for glass electrodes are around 30, whereas values up to 750 have been measured on the cells studied. Membrane electrodes, in which compounds of the type mentioned were used, seem to give an attractive approach for an ion specific transducers. By using adequate supports the speed of response (Fig. 2) may be increased by orders of magnitude and the slope of the electrode function

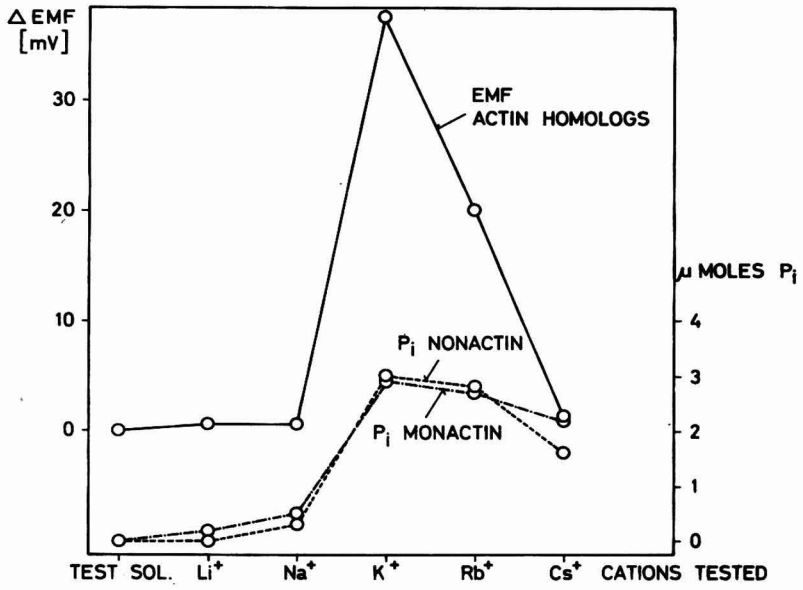

FIG. 5. Comparison of the ion specific effects of nonactin homologs on the ATPase induction (*3*, *4*) with the EMF response of electrochemical cells (see Fig. 4) for monovalent cations.

may become close to theoretical. Corresponding studies as well as work in the field of ion transport phenomena are in progress.

SUMMARY

Electrochemical cells using macrotetrolides (nonactin homologs) on inert supports as membranes show a specificity for cations comparable to the ion specific behavior observed in the metabolism of rat liver mitochondria.

REFERENCES

1. CHAPPELL, J. B., AND CROFTS, A. R., Gramicidin and ion transport in isolated liver mitochondria. *Biochem. J.* **95**, 393-402 (1965).
2. GERLACH, H., AND PRELOG, V., Ueber die Konfiguration der Nonactinsäure *Liebigs Ann. Chem.* **669**, 121-135 (1963).

3. GRAVEN, S. N., LARDY, H. A., JOHNSON, D., AND RUTTER, A., Antibiotics as tools for metabolic studies. V. Effect of nonactin, monactin, dinactin, and trinactin on oxidative phosphorylation and adenosine triphosphatase induction. *Biochemistry* **5**, 1729-1735 (1966).
4. GRAVEN, S. N., LARDY, H. A., AND RUTTER, A., Antibiotics as tools for metabolic studies. VI. Damped oscillatory swelling of mitochondria induced by nonactin, monactin, dinactin, and trinactin. *Biochemistry* **5**, 1735-1742 (1966).
5. MOORE, C., AND PRESSMAN, B. C., Mechanism of action of valinomycin on mitochondria. *Biochem. Biophys. Res. Commun.* **15**, 562-567 (1964).
6. NEUBERT, D., AND LEHNINGER, A. L., The effect of oligomycin, gramicidin and other antibiotics on reversal of mitochondrial swelling by adenosine triphosphate. *Biochem. Biophys. Acta* **62**, 556-565 (1962).
7. SIMON, W., AND WEGMANN, D., Ueber das elektromotorische Verhalten von Elektrodengläsern I. Messungen an Elektrodengläsern in wässrigen Systemen. *Helv. Chim. Acta* **41**, 2308-2322 (1958).
8. ŠTEFANAC, Z., AND SIMON, W., In-vitro-Verhalten von Makrotetroliden in Membranen als Grundlage für hochselektive kationenspezifische Elektrodensysteme, *Chimia (Switzerland)* **20**, 436 (1966).

MICROCHEMICAL JOURNAL 12, 133-145 (1967)

Book Reviews

Guide to the Analysis of Pesticide Residues. Vol. 1 and 2. Prepared by H. P. BURCHFIELD and DONALD E. JOHNSON with the assistance of Eleanor E. Storrs for U.S. Department of Health, Education, and Welfare, Public Health Service, Bureau of State Services (Environmental Health), Office of Pesticides, Washington, D.C. under contract with Southwest Research Institute, San Antonio, Texas. Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, 1965. Second Printing, Catalogue number 26/2-P/43/2/ Vol. 1 and 2. $12.75 in set of 2 volumes only.

This set has been prepared in loose-leaf form to make inclusions of new material easy. Procedures are given for detecting chemical pesticides and their metabolic or degradation by-products in human and animal tissues, water, food, soil, and plants. Methods developed abroad as well as in the United States are included. Obviously, the set is an absolute "must" for anyone working in the pesticide field. Analysts in other fields may benefit by the general approach presented. Extensive use is made of sophisticated techniques—all types of chromatography, infrared absorption spectroscopy, etc. Procedures are described for the determination of mg, μg, ng, or even pg quantities of substances.

AL STEYERMARK, *Hoffmann-La Roche Inc.*
Nutley, New Jersey

Advances in Analytical Chemistry and Instrumentation. Vol. 5. Edited by CHARLES N. REILLEY AND FRED W. MCLAFFERTY. Wiley (Interscience), New York, 1966. ix + 398 pp. $14.00.

The growth of analytical chemistry, both in scope and purpose, has been as great as if not greater than that of its sister disciplines. The need for an "advances in" series was obvious several years ago and this is the fifth volume in a continuity which has admirably filled the need.

The fields covered in this latest publication are: Determination of Molecular Weights by Ebulliometry, Automation of the Analytical Process Through Continuous Analysis, Solvent Extraction of Metal Chelates, Interpretation of *K*-Edge X-ray Absorption Spectra of Transition Metal Compounds, Analytical Applications of Microwave Spectroscopy, and Determination of Molecular Structure by Single-Crystal X-ray Diffraction Methods.

The authors are all internationally recognized authorities in their field and the sections are uniformly well-written. Dr. Reilley is to be congratulated on his editorial work and also on the selection of Dr. McLafferty as co-editor.

An outstanding attribute of this series has been the excellence of the indexing. In

works of this sort, a good index is a prime necessity. The index of this volume, like those of its predecessors, is good and complete.

DAVID B. SABINE, *U. S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Crystal Structures. Vol. 3. 2nd Edition. By RALPH W. G. WYCKOFF. Wiley (Interscience), New York, 1965. 981 pp. $27.50.

Well over 2000 compounds are listed in the index of this latest contribution to the permanently bound edition of *Crystal Structures*. More than 400 of these substances are represented by illustrations which include, in most cases, both a projection diagram and a packing drawing of the type that distinguished the earlier volumes. The policy of excluding intermetallic compounds has been continued. The literature coverage extends into 1963.

The present book contains Chapters VIII, IX, and X of the series. Chapter VIII discusses compounds of the type $R_x(MX_4)_y$. Separate sections are devoted to the important cases of $x = 1$, $y = 1$ and $x = 2$, $y = 1$. Chapter IX treats the class $R_x(M_nX_p)_y$ or $R_xM_yX_z$, divided into groups according to the value of z. Hydrates and ammoniates, classified according to the number of solvent molecules, are described in Chapter X. A few compounds which are neither hydrates nor ammoniates, such as $ZnCl_2 \cdot 2N_2H_4$ and $SrO_2 \cdot 2H_2O_2$, are also included.

The standards of accurate reporting established in the earlier volumes have been maintained. One minor error was observed in a drawing, but the results reported here are generally more reliable than the original references. Professor Wyckoff's monumental evaluation of the results of crystal structure analysis will continue to be an indispensable source of structural information.

DONALD E. SANDS, *Dept. of Chemistry, University of Kentucky, Lexington, Kentucky*

Titrimetric Organic Analysis. Part II: Indirect Methods. By M. R. F. ASHWORTH. Wiley (Interscience), New York, 1965. xix + 1023 pp. $32.50.

Part I of this book, on Direct Methods, was reviewed earlier [*Microchemical Journal* **8**, 445 (1964)]. As expected from the nature of organic reactions, the indirect methods yield a much lengthier compendium. Section 1 presents a brief discussion of the general requirements of the procedures, certain features of the titrations, and the types of organic reactions which serve as the bases of the methods. In keeping this section quite general, the author has included some statements that are scarcely meaningful. For example, most good chemists know generally what is meant by an indirect method, but it *is* hard to define; the definition here ("a method of determination involving two or more operations, of which the concluding, measuring operation is a direct titration") is fatuous.

Section 2 comprises the bulk of the book. Here are listed, by the same general

scheme as in Part I, the reagents used in indirect titrations, with many examples and outstanding documentation. This section should be very useful. Section 3 is an index to functional groups and classes of determinable compounds. Finally, there is an index listing all of the compounds mentioned in Section 2 of both Parts I and II.

Only time will tell how much this book will be referred to. This reviewer believes that it will prove useful and that most research groups should have access to it.

A. L. UNDERWOOD, *Department of Chemistry, Emory University, Atlanta, Georgia*

Transformations in Optics. By LAWRENCE MERTZ. Wiley, New York, 1965. viii + 116 pp. $8.95.

Two of the four chapters of this book are devoted to the theory and practice of Fourier transform spectrometry. This is a method in which a spectrum is modulated by the motion of a mirror in a Michelson interferometer. The interference changes from constructive to destructive for a one-quarter wavelength shift of the mirror, and the observed pattern is related to the spectrum by a Fourier integral. In the author's words, this is a disagreeable indirect spectral method, but it has the advantage of being less wasteful of the radiant energy than conventional techniques.

The third chapter, entitled "Fresnel Transformations," is concerned with Fresnel zone plates and related topics. The final chapter describes a wavefront folding double-star interferometer, a device, based on a modification of Lloyd's mirror, which has possible utility in measuring the separation of double stars.

The title of this book is perhaps suggestive of a treatise on the mathematics of transformations. The actual mathematical content, however, is cursory, fragmentary, and nonrigorous. The subject matter is limited, according to the preface, to topics of interest to the author, and the book is intended neither as a textbook nor as a survey. The writing tends to be conversational, but it is frequently obscure, and it is liberally sprinkled with phrases of specialized jargon. Probably every Fourier transform spectroscopist should read this book, and the various experimental techniques presented will interest devotees of more conventional optical systems.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky*

A Laboratory Manual of Analytical Methods of Protein Chemistry. Vol. 4. Edited by P. ALEXANDER AND H. P. LUNDGREN. Pergamon Press, Oxford, England. xii + 233 p. $8.50.

The first three volumes of this series dealt with the field of analytical methods of protein chemistry in three more or less coherent subsections—separation and isolation procedures, analysis and reactivity, and determination of size and shape. This volume, as the editors indicate in their prefatory note, represents a first step in a plan to update the series by the occasional publication of additional volumes containing articles on methods "which have undergone significant improvements."

As a result the collection is somewhat heterogeneous, and the choice of topics in at least two instances strains this reviewer's concept of a manual of analytical methods. The six sections deal with (1) the estimation of thiol and disulfide groups, (2) microtechnics for amino acid analysis and peptide separation based on high voltage electrophoresis, (3) estimation of specific proteins by a gel-precipitation method, (4) thermal polycondensation of α-amino acids, (5) amino acid composition of selected proteins and polypeptides, and (6) dielectric measurements of proteins.

In the first chapter (75 pages), S. J. Leach of Melbourne reviews the principles and procedures for the measurement of —SH and —S—S— groups with and without hydrolysis and evaluates the several technics which have been described by himself and others.

Chapter two (26 pages), by S. Blackburn of Leeds, describes principally the author's methods and apparatus for high voltage electrophoresis.

The third chapter (25 pages), by D. A. Darcy of London, deals with Darcy's work adapting the classical Ouchterlony technic for quantitative measurement and includes details of both a macro- and a micro- version. The role of antiserum specificity in influencing results could profitably have been emphasized more in this report.

S. W. Fox and K. Harada (Florida) review in 22 pages the thermal polymerization of α-amino acids and some of the characterization of the polymers. Both simple (single amino acid) polymers and co-polymers (from amino acid mixtures) are described. The review is more concerned with preparative methods than with analytical methods, and one wonders whether it might not have found a more congenial home in some other compilation.

W. H. Ward (Albany, California) has contributed a 38-page chapter on amino acid compositions of selected proteins and polypeptides. The tabulations reflect the compiler's critical judgments of the analytical data; the author does not discuss methodology or sample purification and preparation technics. Some sequence data are also tabulated. All of these have appeared elsewhere, and the tables do not provide information on analytical methodology.

Under the heading "Dielectric Measurements of Proteins" D. Rosen (London) devotes 30 pages to the problems of measurement rather than the use to which the measurements are put.

The book has author and subject indices and is well printed and bound. No typographic errors were detected. At a cost of just under four cents per (small) page the book seems unnecessarily expensive.

ROBERT A. HARTE, *American Society of Biological Chemists, Washington, D. C.*

Methods of Vitamin Assay. 3rd Edition. Edited by MYER FREED. Wiley (Interscience), New York, 1966. xvii + 424 pp. $14.00.

The second edition of this "bible" has long been unavailable so the Association of Vitamin Chemists has, by popular demand, brought out a third edition—and none too soon. The increasing complexity of the subject and the demands made by new regulations will make its appearance all the more welcome. Like its predecessors, the

book is a manual for the analyst, not a comprehensive review of vitaminology. Literature references are included only where the authors feel that they will be of particular value. The index is skimpy but apparently adequate.

Those who are familiar with the previous editions will appreciate a new and up-to-date handbook; those who are just starting in this intricate field will find it an invaluable laboratory companion.

DAVID B. SABINE, *U. S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Submicro Methods of Organic Analysis. By R. BELCHER. American Elsevier, New York, 1966. vii + 173 pp. $10.00.

The term "submicro" in this context refers to samples of about 30-50 μg, that is, an amount which is just still visible and thus can be handled without lenses and manipulating devises. This scaling-down by a factor of one hundred from conventional microanalysis comes approximately half a century after Pregl's identical scaling-down operation from macroanalysis. The errors are of about the same magnitude as in microanalysis although the amount of "elements and groups which are measured are equivalent to the errors tolerated in microanalysis." This brief description of the situation may serve for the less initiated as a basis for the evaluation of the formidable task Professor Belcher took on when starting a program to develop such techniques. Contaminations, phenomena, and facts that are of no moment when operating on a larger scale suddenly become of utmost importance and are crucial for the success of an analysis. Thus, for example, spectrographically pure ethanol used as solvent in the chloride determination is in part responsible for the blank value since it contains up to 0.3 μg of chloride per milliliter. For these and other reasons it was necessary to subject every method of titration, every reaction employed, etc., although well established and known to function well at higher levels, to a new scrutinizing evaluation under the present circumstances.

The methods have been designed so that a minimum amount of elaborate equipment is required. To eliminate expenses and difficulties with ultramicrobalances all methods are directed towards a titrimetric finish, with the exception of a few (phosphorous, arsenic, fluorine, and certain periodate oxidations) photometric techniques and for carbon and hydrogen which end with a manometric measurement. The ultramicro balance is exclusively employed to weigh the sample.

The introduction to each section contains a listing of all the various investigations and attempts made by Belcher and his group in order to study several possible approaches even if they failed. These sections are of great interest to be read also by persons not immediately interested in the application of the methods eventually developed. The description of the special equipment as well as the procedural details are clearly presented and points of importance are mentioned so that the application of the methods may be possible with the minimum number of failures.

The book exclusively contains methods developed in the author's laboratory and tested by him and his co-workers. Contrary to the situation with other researchers, Professor Belcher is well aware of the limitations of these methods and of their

difficulties and by no means advocates them as the ones designed to replace all other techniques. He gives a clear evaluation of their possibilities and field of application. As to their use in routine work for actual analysis it remains to be seen how much additional and special training technicians will require.

The book is well made although the reproduction of photographs does not readily allow one to distinguish all interesting details. For a mere 170 pages the price of the book is high but persons interested in this and related fields will gladly spend the ten dollars because the wealth of material and suggestions found in the volume are worth the money.

H. FLASCHKA, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia*

The Separation of Biological Materials. Edited by R. A. KEKWICK. Published by the Medical Department, the British Council, London, 1966. 86 pp. $5.00.

This collection of fifteen papers on the separation of biological materials has been published, in magazine form, as a single issue [Vol. **22**, No. 2] of the *British Medical Bulletin*. In a sense, it is a sequel to an earlier issue [Vol. **10**, No. 3, 1954] devoted to the more general subject of "Chromatography."

The papers can be divided into two general groups. The first group deals with newer techniques and techniques which have developed rapidly in recent years. The specific subjects are physical chemistry of porous systems, molecular-sieve chromatography, electrophoretic techniques, density-gradient separations in the ultracentrifuge, liquid-liquid countercurrent distribution, and gas-liquid chromatography. In the second group of papers, the approach is based upon the separation of specific classes of compounds. The classes considered are lipids, subcellular particles, viruses, nucleic acids (isolation of), proteins and protein sub-units, peptides, amino acids, carbohydrates and mucoid substances, and bacterial, cell-wall polymers. Each paper is, of course, highly specific and has been compiled by persons expert in the area.

The most impressive points about this collection are the large number of references cited in each paper and the fact that almost all of them refer to literature published between 1960 and 1966. Thus the papers appear most complete and up to date.

The book is suitable for all persons working in biochemistry. In fact, it is hard to imagine how a modern biochemist could work effectively without the book or the information contained therein. The price is most attractive.

JAMES M. BOBBITT, *Department of Chemistry, University of Connecticut Storrs, Connecticut*

Chemical Data Book. 2nd Edition. Edited by G. H. AYLWARD AND T. J. V. FINDLAY. Wiley, New York, 1966. viii + 88 pp. $2.95.

This limp-bound, little book will be welcomed by those wearied by the increasing weight, bulk, and inconvenience of the modern chemical handbook. Intended as a

teaching aid in high schools, it is designed especially to meet the needs of the Australian school system. It is, of course, incomplete—it has to be—but judicious selections by the authors cover a surprisingly large field. There are 39 tables which will fill a goodly portion of the busy worker's needs. Fundamental constants and conversion factors, properties of the elements and of the most common inorganic and organic compounds, some crystal forms, ionization constants, solubility products, redox equilibria, azeotropic data, and log tables (plus a presentation of the Greek Alphabet in both capital and small letters) are a few of the tables—enough to give a general idea of the coverage.

Those fatigued by the burden of today's complete handbooks will be glad to have this handy pamphlet, packed as it is with an amazing amount of useful information.

DAVID B. SABINE, *U. S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Laboratory First Aid. By K. GUY. Macmillan, London, 1965. 124 pp. 8s.6d. ($1.20).

In this short but comprehensive book, the author discusses the many types of accidents that occur in a modern laboratory. In large industrial organizations, one finds usually a medical staff equipped to take care of such accidents. However, a knowledge of simple and rapid emergency treatment may save a life or minimize the extent of injury. In laboratories when such facilities do not exist, and these include most small universities, a knowledge of first aid practice, as is brought out in this book, can be a great value in protecting the lives of laboratory personnel.

The recommended procedures are quite clear and concise and follow good medical practice. First aid materials and facilities are discussed and the chapters on eye injuries wounds, burns and scalds, shock, asphyxia and poisoning are intelligently handled. Photographs of simulated "accidents" and first aid measures are included.

The importance of safety in other walks of life has been increasingly emphasized and has received particular attention in industry. The low cost of this valuable manual should make it even more welcome on the shelf of every laboratory and particularly where no book of this kind exists.

JOSEPH F. ALICINO, *The Squibb Institute for Medical Research, New Brunswick, New Jersey*

Reflectance Spectroscopy. By WESLEY WM. WENDLANDT AND HARRY G. HECHT. Wiley (Interscience), New York, 1966. viii + 298 pp. $12.00.

This book deals with the theory and practice of reflection techniques and the characterization of substances by the wavelength-dependence of reflected light. It is a welcome book on a subject that has received only limited attention from reviewers. It will be found useful by serious students of quantitative optical phenomena as well as by those who must work semi-empirically with intractable materials for which

the more familiar absorption spectroscopy employing transmitted light is not applicable or not the method of choice. In contrast to the relative simplicity of the Beer-Lambert law for dilute solutions in the case of absorption spectroscopy, the mathematics required for the basic theory of quantitative reflection spectroscopy is both lengthy and advanced. Thus, "Reflectance Spectroscopy" should not be approached by the casual reader except as a source of reference for instrumental methods and examples of what has been studied.

After a short, introductory, first "Chapter," the substance of the book starts with Chapter II, which deals with the fundamental theory of the reflection of light at plane surfaces. A derivation is given of the relationship between the intensity and polarization of light and the angle of incidence and refractive indices of the media. The treatment is given for the reflectivity from both a nonabsorbing and an absorbing second medium and is extended to the case of a second medium of lower refractive index (n_2) than that of the first (n_1), for which reflectivity is total at angles of incidence greater than the critical angle, $\sin^{-1}(n_2/n_1)$, as is commonly well known. It is shown that the phenomenon of attenuated total reflectivity, first put to spectroscopic use by J. Fahrenfort, is a consequence of the penetration of the electromagnetic wave into the second medium, by an amount of the order of magnitude of the wavelength of the radiation, and the fact that the reflectivity is thus attenuated from 100% for a nonabsorbing second medium to lower values for an absorbing one. Experimental methods are reviewed for obtaining the refractive index and absorption coefficient from reflectivity measurements on media in bulk form and thin films.

We have to go to Chapter VI to find the logical continuation of the discussion of internal reflection in its practical aspects. It is important to emphasize the meaning of terms here, as some confusion is possible. As the authors point out in their introduction, they prefer to reserve the term reflectivity for the amount of light reflected at plane surfaces and to use reflectance for the diffuse light reflected from rough surfaces. In the case of the interface between a highly refractive medium in intimate contact with an absorbing solid, liquid, or plastic material of much lower refractive index, for angles of incidence greater than the critical angle we have what can properly be called *attenuated total reflectivity* and even multiply attenuated total reflectivity if multiple reflections are used. This technique yields spectra that are similar to and almost equally well resolved as transmittance spectra and from which the refractive index and absorption coefficient can be calculated accurately through the theoretical equations. There is an error in the last sentence of p. 169: what is meant is that the ATR spectrum of a substance is equivalent to the transmittance spectrum of a sample 0.2 mm thick for an absorption coefficient of 10^{-4}, or 0.004 mm thick for an absorption coefficient of 0.2. These figures are derived from the theoretical equations. ATR spectra of transparent materials are insensitive to sample thickness above 0.005 mm. Results obtained by transmittance and ATR spectroscopy are compared, and it is shown that the Beer-Lambert law is frequently followed in the ATR spectra of both liquid and solid solutions. The above phenomena should be clearly distinguished from *multiple reflectance.* In this case, a common, transparent material of comparitively low refractive index, like glass, is used in contact with the sample merely to increase the number of reflections of the incident light *from the*

surface of the sample; an auxiliary liquid may be used between the flat, glass internal reflector and a rough, solid sample to establish optical contact, in which case the absorption spectrum of the liquid is included in the multiple reflectance spectrum of the solid. Quantitative treatment of such reflectance spectra must be empirical. Chapter VI also gives a good account of the various types of cell employed in these studies.

The material on reflectivity at plane surfaces covers about 60 pages. The remainder of the book, about 240 pages, is devoted to the diffuse reflectance of powdered or rough materials.

As for specular reflectivity, the theoretical sections on diffuse reflectance are sufficient for those who wish to approach quantitative fundamental work in this area. The quantitative relationship between conventional absorption spectra and the diffuse reflectance spectra of layers of partially absorbing, fine powders of at least 2-3 mm thickness are derived. The Kubelka-Munk function, which relates the total diffuse reflectance to the absorption coefficient and scattering coefficient (particle size) is derived, and a table of the function is given in an appendix. The more advanced treatment of N. T. Melamed is also given. Reflectance spectra are compared quantitatively and discussed for several examples. A 35-page chapter is devoted to instrumental accessories for diffuse reflectance. The theory of the reflectance of the integrating sphere, a commonly used accessory, is dangled at the end of the book as the last chapter; this includes a useful practical discussion of the absolute reflectance of substances used for coating integrating spheres and for reflectance standards.

The reflection from solids in states other than beds of fine particles may contain a specular component as well as generally diffuse reflectance; the spectra of such materials can be used more empirically for purposes of comparison and identification. All types of materials from asphalt to zinc salts can be used. Chapters V, VII, and VIII, which together cover 74 pages and include 248 references, give an extensive review of the application of diffuse reflectance spectroscopy to a host of different, industrially important materials, chemical reactions, and surface phenomena. These chapters give an excellent view of the versatility of the reflectance technique and are to be recommended as a source of ideas for those who are seeking to utilize this method.

There is a separate chapter on color measurement. Details of the use of reflectance spectra, in conjunction with tables of energy-distribution coefficients for standard light-sources, are given for the specification of color in terms of the internationally agreed (CIE) system of chromaticity coordinates. Also, for weakly colored systems, the Kubelka-Munk function can be related to a linear combination of the absorption coefficients of mixed colorants.

Generally speaking, each of the sections is quite thorough, and the theory is given from first principles. In this reviewer's opinion, a more readable book would have resulted if the subject matter and chapters had been grouped together in a more logical sequence and some of the lengthy, algebraic derivations placed in appendices. As it is, the relationship between theory and practice is not too well brought out. There would seem to be no necessity to place a large and exhaustive table of conversion factors for energy units in the short, introductory, first chapter. The amount of work that has gone into the presentation of this difficult subject in its detail is

commendable, and the book appears to be free of misprints. Reflection techniques will certainly attract increasing use in the future for both fundamental and semi-empirical studies, and it is hoped that some of the details given above will help those who wish to decide whether they have cause to enter this area.

J. G. PRITCHARD, *IIT Research Institute, Chicago, Illinois*

Biochemistry Laboratory Techniques. By STERLING CHAYKIN. Wiley, New York, 1966. vii + 169 pp. $5.95.

This is a laboratory manual designed for the beginning graduate student in biochemistry. It assumes the basic knowledge such a student would have and places its emphasis on technique. Biochemistry is not neglected but the prime concern is to teach how to carry out laboratory procedures. The first chapter is an excellent review of buffers (a good refresher for experienced workers, too). The other chapters are: Methods for Protein Determination, Properties and Preparation of Yeast Alcohol Dehydrogenase, Isolation of Rabbit Muscle Aldolase, Sephadex and Gel Filtration, Criteria of Protein Purity, Protein Structure (Aldolase), Theoretical Considerations Involved in Isotope Work, Radioisotopes in Biochemical Research, Carbohydrates, Glass Blowing, Oxidative Phosphorylation, and Bacterial Genetics. There are two appendices, one containing conversion tables and tables of constants, the other detailed instructions for setting up a laboratory including equipment and materials needed. Save for an error in the directions for the use of the saturated ammonium sulfate solutions (the directions read "column on the *left*" but should read "column on the *right*"), the book is free of typographical errors and the equations, graphs, tables, and illustrations are all well presented. The book can be highly recommended for the beginning graduated student in biochemistry.

DAVID B. SABINE, *U.S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Dictionary of Optics, Photography and Photogrammetry. By GÜNTER RICHTER. Elsevier, Amsterdam, 1966. xvii + 502 pp. $19.00.

This dictionary contains the English equivalents of about 7800 German optical expressions and the German equivalents of about 8800 English terms. The justification for the compilation is that most of the important literature on optics is in either German or English, and a specialized listing can presumably be more complete and more convenient than a general dictionary. Many of the entries are identified as terms in a certain branch of optics, such as photometry. The gender of each German noun is given.

This volume can be a valuable aid in reading books and articles in optics and related fields. Unfortunately, its price will keep it out of the hands of most of its potential users.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky*

Automatic Methods in Volumetric Analysis. By D. C. M. SQUIRRELL. D. Van Nostrand, Princeton, New Jersey, 1964. 201 pp. $6.75.

Automation of wet chemical analysis is still a young technology. Nevertheless, two directly opposing developments have crystallized in the last few years.

On one hand automated techniques closely following the mechanics of classical titration procedures have been brought to an advanced stage of sophistication. This development and the available technology is well described in Squirrell's book.

He has drawn upon his pioneering, practical experience to describe the latest equipment for automatic spectrophotometric, conductometric, amperometric, and nullpoint detection both in aqueous and nonaqueous systems. In particular, the introductory chapters on titrations to preset end-points and recording potentrometric titrimetric methods should provide a newcomer with considerable and relevant help.

However, only a passing mention of the major force in automatic wet chemistry, that of continuous flow analysis, limits the book for practicing chemists who require a critical survey.

It would have been useful to read of the relative merits of the cyclic and continuous approaches, in particular for on-stream process control. It is in this rugged and uncompromising situation where the differences of the two philosophies become apparent.

JAMES F. MARTEN, *Technicon Controls, Inc. Chauncey, New York*

The Electron Microprobe. Edited by T. D. MCKINLEY, K. F. J. HEINRICH, AND D. B. WITTRY. Wiley, New York, 1966, xvi + 1035 pp. $27.50.

This volume comprises a series of papers delivered at a national symposium sponsored by the Electrochemical Society held in Washington, D.C. on October 12-15, 1964. The theme of the symposium was, The electron microprobe, Instrumentation and Applications.

The volume is divided into four sections; Analysis of light elements; Quantitative analysis; New techniques and instrumentation, and Applications.

The light elements down to beryllium, $\lambda_K = 113$ Å, are now being studied. For this purpose, Franks and Lindsey, and Holliday use ruled gratings. They discuss the principle and methodology in the preparation of these gratings. Holliday recommends the blaze type of grating. Better resolution is obtained with gratings than are obtained at present with lead stearate crystals for the light elements.

Poen Sing Ong reports on the elements fluorine, oxygen, and carbon using a lead stearate-decanoate multilayer crystal on mica. For identification of light elements in metal sections in the range of concentration of 5-10%, the nondispersive pulse analysis method is applied by Wardell. Campbell and Gibbons keep the specimen at liquid nitrogen temperature to minimize the cracking of hydrocarbons and carbonization that normally takes place. The application of the electron probe to thin sections is described by Anderson.

Detailed study of the inherent errors of quantitative analysis with the electron probe, when dealing with mixtures of elements, occupies 300 pages of the book. The basic problems are those of electron backscatter, self-absorption and absorption of the

matrix and fluorescence of the continuum which surrounds the element sought. Colby, Birks *et al.;* Criss and Birks; Shimizu and Shinoda; Cosslett and Thomas; Poole and Thomas; and Heinrich discuss the theoretical approaches to this problem. Extensive tables, especially by Colby (83 pages) and Heinrich (26 pages) yield readily available data such as mass absorption coefficients, correction for X-ray emergence angle and for fluorescence. The problem of the extensive calculations required for this approach to multiple element analysis is discussed by Brown, who proposes a computer program for the solution of this problem.

An interesting study is that of Duncumb and Shields who translate the distribution of electrons with depth into distribution of X-ray emission with depth as being the more important phenomenon for purpose of analysis. Ziebold and Oglivie approach the problem empirically for studying prepared specimens to make atomic number and fluorescence corrections. Hutchins discusses the problems associated with the use of the electron problem for measurement of the thickness of thin films. In order to avoid the nonproportional behavior of flow proportional detectors, Bender and Rapperport suggest that the detector be operated at low detector anode potentials, that low positive ion density in the detector be maintained, and that the longest X-ray wave lengths available and the lowest acceptable counting rates for the standards be used.

Newer instrumentation is discussed in several articles. Openshaw describes the Assoc. Electrical Industries Ltd. scanning microprobe. The ARL microprobe with programmed analysis is presented by Davidson *et al.* Hart and Pilney describe the construction of a microprobe with a compact vacuum path 2θ scanner. The instrument of Japan Electron Optics Lab., discussed by Kimoto and Hashimoto, permits stereoscopic observation in scanning microscopy, using multiple detectors. Duncumb describes the instrument of Tube Investments Res. Lab. of England which combines the electron probe for examination of the morphology of a particle and X-ray microanalyzer for assay of its composition. An application to a similar problem is presented by White *et al.* using the Hitachi instrument. Yakowitz adapts the electron microprobe for accurate measurement of cubic lattice parameters.

The applications section reports the observations on various systems with the electron probe. Phase equilibria and diffusion of one metal into another in solid systems are explored by Shinoda and Kawabe, Rosenbaum and Schadler, Speich *et al.,* and Guy and Leroy. Analysis of iron alloys and inclusions therein are described by Desforges and Charles, Koh, Matsubara, Banerjee, and Bingle.

Analysis of electronic materials such as transistors and semiconductors and these film devices are described by Everhart, Lublin, and Sutkowski; Kyser and Wittry; Ramsey and Weinstein; and Nealey *et al.*

Special problems which are explored are grain boundary migration in hot-pressed tantalum carbide by Klerk and Roeder, analysis of the titanium ore Ilmenite by Temple *et al.,* and the compatibility of selected refractory metals with various ceramic insulation materials by Fornwalt *et al.*

Only two presentations concern themselves with biological problems. Hall *et al.* study calcified arteries and zinc in sperm cells. Mellors *et al.* study the distribution of calcium and phosphorus in bone tissue.

The bibliography compiled by K.F.J. Heinrich covers 189 pages and is comprehen-

sive in its scope. The references are arranged in accordance with the part of the electron probe being explored or specific application desired.

The book is well written and numerous figures and plates illustrate each article. It is encyclopedic in its coverage and for this reason, a must for the chemist or engineer engaged in study in this area. It is of general value to the analytical chemist, especially the microanalyst. For the biochemist and biologist it presents a tool which is becoming of increasing importance.

The editors should be congratulated for the excellent style and method of presentation of so complex a problem.

SAMUEL NATELSON, *Director of Biochemistry, Michael Reese Hospital and Medical Center*

Treatise on Analytical Chemistry. Part II: Analytical Chemistry of Inorganic and Organic Compounds, Vol. 4. Edited by I. M. KOLTHOFF AND PHILIP J. ELVING, with the assistance of Ernest B. Sandell. Wiley (Interscience), New York, 1966. xx + 452 pp. $17.00.

This is Vol. 4 of Section A (Systematic Analytical Chemistry of the Elements) in Part II of the *Treatise*. Part I is on theory and practice and Part III covers analytical chemistry in industry. Eight authors or co-authors have contributed to this volume, each an authority in his respective field. There are six chapters as follows: Silver (69 pp., 238 refs.) by E. P. Przybylowicz and C. W. Zuehlke; Gold (35 pp., 30 refs.) by N. Herz; Calcium (46 pp., 174 refs.) by K. K. Turekian and E. Bolter; Strontium and Barium (65 pp., 170 refs.) by K. K. Turekian and E. Bolter; Radon and Radium (148 pp., 372 refs.) by J. Sedlet; and Aluminum (73 pp., 121 refs.) by G. H. Farrah and M. L. Moss. Each chapter is headed with a detailed outline of its contents. After a brief introduction on the element or elements the chapter covers, there follows sections on the occurrence, properties, and various methods of separation, identification, and determination. Recommended or selected laboratory procedures are given for each of the eight elements treated in the book. There are many figures, graphs, and tables of data which add much to the clarity and usefulness of the book. References are listed at the end of each chapter and total more than eleven hundred. A subject index concludes the book. Printing and paper are good, and the book is attractively bound in cloth.

Volume 4 of Part II maintains the high standard set by preceding volumes in the *Treatise* and is an authoritative and up-to-date reference work on the analytical chemistry of silver, gold, calcium, strontium, barium, radon, radium, and aluminum.

JOHN H. YOE, *Department of Chemistry, University of Virginia, Charlottesville, Virginia*

Announcement

The XXIst International Congress of Pure and Applied Chemistry will be held in Prague, September 4-10, 1967. Three topical subjects have been selected which will be dealt with in three Sections:

Automation in Analytical Chemistry
Toxicological Chemistry
Chemistry of Nucleic Acid Components

Kindly request the Second Circular of the Congress with full information from the

The XXI International Congress IUPAC
Organizing Committee
P. O. BOX 139 PRAHA 6—DEJVICE

The application deadline for those participants who intend to present a paper is March 1, 1967; for others it is May 31, 1967.