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devoted to the application of microtechniques in all branches of science

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Microchemical Journal, Vol. 15, No. 2

Briefs

Synthesis of 2-Imidazolidinone-^{14}C. W. B. BURTON, *Shell Development Company, Biological Sciences Research Center, Modesto, California 95352.*

Radioactive 2-imidazolidinone labled with carbon 14 in the 4 position was synthesized on a microscale. Ethylenediamine-^{14}C and urea are the reactants.

Microchem. J. **15,** 161 (1970).

Use of Microcosmic Salt as a New Titrant for the Microdetermination of Acetic and Glycolic Acids. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

This is the third article by the author on the use of the reagent for the determination of organic acids.

Microchem. J. **15,** 171 (1970).

The Analysis of Binary and Ternary Thin Alloy Films of Tantalum, Titanium, Niobium, Zirconium, Molybdenum, and Beryllium. K. F. SUGAWARA AND D. E. CAMPBELL, *Corning Glass Works, Sullivan Park, Corning, New York 14830.*

An accurate and rapid analytical scheme to cope with films involving refractory-type metals is described. The sensitivities of the methods are such that even minute samples can be adequately handled.

Microchem. J. **15,** 173 (1970).

Differential Estimation of Low Energy Beta-Emitters, ^{3}H, ^{14}C, and ^{35}S in Multi-labeled Organic Samples. M. F. BARAKAT AND MAGDA ABDEL-GHANY, *Nuclear Chemistry Department, Atomic Energy Establishment, Cairo, U. A. R.*

Samples were ignited using different ignition systems and, through chemical discrimination between different radioactive oxidation products, the differential estimation of these low energy beta-emitters was achieved.

Microchem. J. **15,** 184 (1970).

Microdetermination of Fluorine in Organic Compounds by Direct Measurement with a Fluoride Electrode. J. PAVEL, R. KUEBLER, AND H. WAGNER, *Central Research, J. R. Geigy SA, Basle, Switzerland.*

A method for routine microdetermination of fluorine in organic compounds is described, using hot-flask combustion and a fluoride-specific electrode for direct measurement of fluoride concentration. The accuracy of the method meets the requirements of microelemental analysis. The method is rapid and simple.

Microchem. J. **15,** 192 (1970).

Microdetermination of Fluorine in Organic Compounds with a Fluoride Ion Electrode Following an Oxygen Flask Combustion. D. A. SHEARER AND G. F. MORRIS, *Analytical Chemistry Research Service, Canada Department of Agriculture, Ottawa, Ontario, Canada.*

Fluorine-containing organic compounds were analyzed by the oxygen flask combustion in a polyethylene or polypropylene flask and using a fluoride specific ion electrode for the titration. Benzoic acid was added to the samples containing a high percentage of fluorine in order to obtain complete combustion.

Microchem. J. **15,** 199 (1970).

Oxidation of Ferrous Sulfate with Ag(III). P. K. JAISWAL, *Department of Chemistry, M.M.M.V. Bhat Par Rani, Deoria, India.*

Ag(III) was used as an oxidant in the oxidation of ferrous sulfate to ferric sulfate.

Microchem. J. **15,** 205 (1970).

Thin-Layer-Chromatographic Separation of Some Polynuclear Hydrocarbons. R. E. SCHAAD, *Department of Hygiene and Work Physiology of the Swiss Federal Institute of Technology, Zurich, Switzerland.*

Polycyclic aromatic hydrocarbons, which disturb the quantitative fluorimetric determination of benzo(*a*)pyrene at 405 nm, may be separated on acetylated cellulose with ethanol/dichloromethane/water (20:10:1). The interfering polycyclics are also separated from each other, except benzo(*b*)fluoranthene and benzo(*k*)fluoranthene.

Microchem. J. **15,** 208 (1970).

Indirect Polarographic Method for the Microdetermination of Sulfur in Organic Compounds. SAFWAT W. BISHARA, *National Research Centre, Dokki, Cairo, U. A. R.*

An indirect polarographic method is described for the microdetermination of organically-bound sulfur using barium as pilot ion. Results are within the prescribed limits of accuracy, namely, $\pm 0.3\%$. Nitrogen, chlorine, bromine, and sodium do not interfere. Interference due to phosphorus is simply eliminated. by using an acidic medium prior to precipitation. Iodine, if present, should not interfere.

Microchem. J. **15,** 211 (1970).

Direct Microdetermination of Oxygen by Static Flash Combustion Pyrolysis. Z. ŠTEFANAC, Z. SLIEPČEVIĆ, AND Z. RAKOVIĆ-TRESIĆ, *Institute of Organic Chemistry and Biochemistry, University of Zagreb, Zagreb, Yugoslavia.*

The pyrolysis and the conversion to carbon monoxide of the oxygen contained in organic samples are simultaneously effected by static flash combustion at 1120°C in an empty quartz tube of the sample covered with carbon. Consistent

and minimized blank values, prolonged life of the pyrolysis tube, and the elimination of an extensive conditioning whenever the combustion tube is repacked justified further investigations of this preliminary procedure particularly in consideration of performance on the ultramicro scale.

Microchem. J. **15,** 218 (1970).

Applications Involving the Iodide Ion. V. Determination of Small Amounts of Platinum and Analysis of Its Mixtures with Some Metal Ions. H. KHALIFA AND B. N. BARSOUM, *Faculty of Science, Cairo University, Giza, U. A. R.*

The stoichiometric reaction between mercuric and iodide ions is basis of a rapid and reliable potentiometric method. Potential breaks were large enough so that the end points are clear. Platinum was determined in the presence of other metals.

Microchem. J. **15,** 224 (1970).

An Ultramicromethod for the Determination of Conjugated and Total Bilirubin in Serum or Plasma. MARTHA I. WALTERS AND H. W. GERARDE, *Department of Pathology, Ohio State University Hospital, Columbus, Ohio 43210; and Fairleigh Dickinson University, Teaneck, New Jersey 07666.*

An ultramicro chemical procedure for bilirubin using the Unopette System has been described that requires only 50 μl of sample for the measure of total bilirubin and an additional 25 μl for the measure of conjugated bilirubin. The test can be set up and completed in less than 12 minutes. Because of the intense color of azobilirubin, the reaction is highly sensitive; the reaction is also linear from 2 to 50 mg of bilirubin per 100 ml of serum.

Microchem. J. **15,** 231 (1970).

Relationship Between Sensitivity and Precision in Atomic-Absorption Flame Photometry. J. RAMÍREZ-MUÑOZ AND W. F. ULRICH, *Beckman Instruments Inc., Fullerton, California 92634.*

Precision results, standard deviation or relative standard deviation, can be expressed in terms of either readings or concentrations. This paper is the continuation of previous studies on sensitivity.

Microchem. J. **15,** 244 (1970).

Chemico-analytical Selectivity and Sensitivity in Atomic-Absorption Flame Photometry. J. RAMÍREZ-MUÑOZ, *Beckman Instruments, Inc., Fullerton, California 92634.*

This is another paper in the series on the subject of sensitivity by the author. This paper states that the main causes of loss of sensitivity are improper choosing of instrumental parameters and matrix composition.

Microchem. J. **15,** 253 (1970).

The Dilution Process in Sample Preparation for Atomic-Absorption Flame Photometry. J. RAMÍREZ-MUÑOZ, *Beckman Instruments, Inc., Fullerton, California 92634.*

A graph is presented and discussed to assist in choosing the appropriate dilutions for original samples in atomic-absorption flame photometry.

Microchem. J. **15,** 271 (1970).

The Concentration Process in Sample Preparation for Atomic-Absorption Flame Photometry. J. RAMÍREZ-MUÑOZ, *Beckman Instruments, Inc., Fullerton, California 92634.*

A graphical representation can be used for choosing the appropriate preliminary concentration factors in preparing samples for atomic-absorption flame photometric determination. The graph has been constructed on the basis of the sensitivity obtainable with a given instrumental system. The use of the graph can be extended to multielement concentrating extractions.

Microchem. J. **15,** 277 (1970).

Microdetermination of Vitamin B_6: Gold Chloride as Oxidizing Agent. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad-2, India.*

Vitamin B_6 was determined in micro amounts by oxidation with gold chloride in alkaline medium. Oxidation was done by adding a known excess of gold chloride and alkali solutions to vitamin B_6 solution. The reaction mixture was heated and then the remaining excess of gold chloride was titrated back. The amount of gold chloride used corresponds to the vitamin B_6 oxidized, 18.5 atoms of oxygen are required for the complete rupture of the molecule. Traces of organic compounds did not interfere, but excess alkali inhibited the reaction.

Microchem. J. **15,** 281 (1970).

Analytical Reactions in Trifluoroacetic Acid. II. Organic Spot Test Parameters. HARVEY W. YUROW AND SAMUEL SASS, *Chemical Research Laboratory, Research Laboratories, Edgewood Arsenal, Maryland 21010.*

Color formation observed in oxidation of aromatic compounds in trifluoroacetic acid will depend essentially upon three parameters. These are the oxidation potential of the organic substrate and electrophilicity of its oxidation intermediates, oxidizing power of the reagent, and Lewis basicity of the cosolvent.

Microchem. J. **15,** 285 (1970).

Solvent Effects in Photometric Analysis. E. SAWICKI, T. W. WINFIELD AND C. R. SAWICKI, *Consumer Protection and Environmental Health Service, National Air Pollution Control Administration, U.S. Department of Health, Education, and Welfare, Public Health Service, Cincinnati, Ohio 45226.*

This paper considers the role of solvents in organic trace analysis. It includes new research and new concepts. Stress is placed on solvent-effected changes of

definite or potential use in organic trace analysis of environmental pollutants. Many techniques and factors affecting the electronic spectra are discussed.

Microchem. J. **15,** 294 (1970).

Simultaneous Determination of Hydrogen and Nitrogen in Organic Compounds by a Gas-Volumetric Method Using a Newly Designed Pt–P_2O_5 Electrolytic Cell. KAN-ICHI NAKAMURA, KIKUSHIGE ONO, AND KATSURO KAWADA, *Sankyo Central Research Laboratories, Shinagawa, Tokyo, Japan.*

Combustion is done in a vertical tube and the gaseous products are passed through copper oxide, cobalt oxide, silver, and copper using pure carbon dioxide as the carrier gas. The water is absorbed in the Pt–P_2O_5 cell and the nitrogen is determined with a dispersion type azotometer. The absorbed water is then electrolyzed and the gases are measured in the same azotometer.

Microchem. J. **15,** 364 (1970).

MICROCHEMICAL JOURNAL 15, 161–170 (1970)

Synthesis of 2-Imidazolidinone-^{14}C

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Received August 15, 1969

INTRODUCTION

Studies have shown that 2-imidazolidinone has interesting biological activity which inhibits the normal development of insects. Its chemosterilant properties *(2)* have prompted others to investigate the mode of action and its metabolic fate. As a tracer compound was needed to aid in these studies, 2-imidazolidinone was synthesized labeled with radioactive carbon in the molecule.

There are several known methods for the preparation of 2-imidazolidinone; the reaction of ethylene glycol or ethanolamine with urea, ethylenediamine with carbon dioxide, and ethylenediamine with urea or phosgene. The method decided upon was patterned after that reported by Schweitzer *(1)*, which consists of the reaction between ethylenediamine and urea in the presence of a small amount of water.

In this method, urea is heated slowly with ethylenediamine–water azeotrope yielding 2-imidazolidinone as the major product. As was discussed by Schweitzer, water is necessary to moderate the reaction; without it, yields of only 10% were obtained and sometimes an uncontrollable reaction occurred. Using water to moderate the reaction, yields of 60% have been obtained on a macro scale in our laboratories, and yields to 100% have been reported in the literature. The reaction sequence is illustrated in Fig. 1.

As the reactions in Fig. 1 show, this method lends itself to labeling with carbon 14 in the 2 or 4 or 4,5 positions of the molecule. To label the 2 position, urea carbon 14 could be used as the isotopic starting material, to label the 4 or 4,5 positions the appropriately labeled ethylenediamine-^{14}C may be employed. It was decided that the radioactivity in the 4 position would be more valuable in the metabolic studies planned.

Considerable alteration was necessary to reduce the scale several thousandfold from that reported in the literature to a microscale of 1

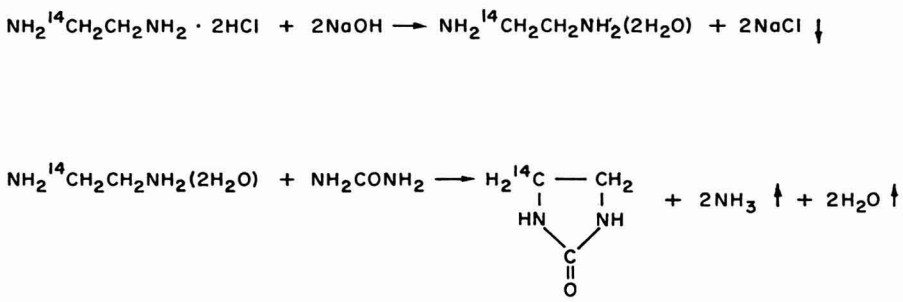


FIG. 1. Reaction scheme for 2-imidazolidinone-^{14}C.

mmole or less. Because ethylenediamine-^{14}C was available only as the hydrochloride salt, the first step in the synthesis was the ready conversion of the hydrochloride salt to the free amine. Because of the neutralization, the ethylenediamine solution contained 37% water, which was a higher proportion than that used in the previously reported preparations. Ethylenediamine water azeotrope which contains 31% water was employed in the earlier synthesis. The diamine carbon-14 solution was reacted with urea for about 4.5 hours. The temperature was increased gradually to a terminal temperature of 210°C. In most trial experiments, the diamine azeotrope was used and in some instances the terminal temperature was as high as 240°C.

Several methods of isolation of 2-imidazolidinone from the reaction mixture were explored. Sublimation was satisfactory if the sample was fairly pure, however, if the sample was rather impure, the yield of sublimate was low, although the purity of the sublimate was satisfactory. Solvent extraction of the reaction mixture, using chloroform, worked the best. This method was used in the radioactive preparation. Although no attempt was made to chromatograph the reaction mixture directly, it would appear that such a method could be successfully employed without a prior cleanup procedure.

In the trial experiments, the yield varied generally between 36–65%. Some of the better preparations were higher than 65% in yield with 80% being the maximum obtained on a 1 mmole scale. The average yield of the trials of 2-imidazolidinone on this scale was 54%. The reaction conditions and the results of the trials are tabulated in Table 1.

METHODS

Partition Chromatography

Purification by solvent extraction increased the purity of the technical product several percent but not sufficiently for metabolic studies. Various liquid–liquid and adsorption chromatographic systems were investigated.

TABLE 1

TRIAL PREPARATIONS OF 2-IMIDAZOLIDINONE

Reference no.	Reactants (mg)			Reaction		Isolation		Product		Purity		
	Urea	Water	Ethylene diamine	Time (hr)	Temp (°C) 110→to	Procedure	Polymer [a]	(mg)	% Yield [b]	% by IR	mp (°C)	Yield 2-ID [c]
1	60	29	61	4.75	220	—[d]	—	39	45.7	95	133–135	43.4
2	60	58	61	4	210	—[d]	—	42	48.8	>95	132–134	46.4
3	60	58	121	4	220	—[d]	60	66	76.7	<50	<110	<38.4
4	55	29	61	2.5	215	—[d]	—	57	72.2	98	129–132	70.8
5	55	29	61	2.25	208	—[d]	—	46	58.2	98	126–130	57.0
6	55	29	61	4.25	210	—[d]	27	57	72.0	95	132–134	68.4
7	55	29	61	4.25	210	—[d]	38	59	75.0	95	132–135	71.4
8	55	29	61	4.5	210	—[e]	24	69	85.9	92	132–134	79.1
9	55	29	61	3.75	210	—[f]	21	50	62.8	>95	128–130	59.8
10	60	29	61	3.25	180	—[g]	21	35	40.9	>95	126–128	38.9
11	55	29	61	5	210	—[d]	24	55	68.7	>95	133–135	65.3
12	55	29	61	4.25	195	—[d]	28	39	48.8	>95	133–135	46.4
13	55	29	61	4.5	200	—[d]	24	36	45.5	98	132–134	44.6
14	44	—[h]	—[i]	5	210	—[d]	17	24	38.0	95	132–134	36.1
15	44	—[h]	—[i]	4.5	200	—[d]	22	29	46.0	90	129–131	41.4

[a] Polymeric material insoluble in extracting solvent.

[b] Determined on a weight basis.

[c] Yield, weight of 2-imidazolidinone (2-ID) times purity based on starting urea.

[d] Chloroform extraction.

[e] Methanol extraction.

[f] A MeOH extraction gave 25 mg of polymer and 71 mg of product followed by chloroform extraction.

[g] A MeOH extraction gave 34 mg of polymer and 55 mg of product followed by chloroform extraction.

[h] Dihydrate from neutralization, 28 mg.

[i] Ethylenediamine dihydrochloride, 102.5 mg.

The most effective method of purification of those tried was paper chromatography. In this system, of ethyl acetate, acetic acid, and water in the ratio 3:1:1 on Whatman No. 3 paper, the difference between the R_f value of 2-imidazolidinone and the impurity located nearest on the paper gram was 0.15 R_f units, sufficient for a complete separation.

Trials were made to determine if 20–30 mg of impure 2-imidazolidinone could be purified in high yields. It was found by weight and carbon-14 measurements the 2-imidazolidinone could be quantitatively extracted from Whatman No. 3 paper with water. To determine if any change occurred, in the compound during chromatography, samples were analyzed by infrared methods, melting point measurements, co-chromatography and bioassay after being chromatographed and compared to authentic material. As evidenced by a slight lowering of melting point the 2-imidazolidinone eluted from paper contained trace impurities. These contaminants, however had no effect on the insect toxicity, infrared spectra, or R_f value on paper in two different systems.

Elution of a paper chromatogram of 2-imidazolidinone made using a few micrograms of 2-imidazolidinone-^{14}C and 30 mg of nonradiomaterial resulted in a recovery of 83% by carbon-14 measurement. The melting point of the recovered material was 132–134°C. A portion was chromatographed and an autoradiogram was prepared. Only one area of darkening was visible on the film, and the radiochemical purity was determined to be >97%.

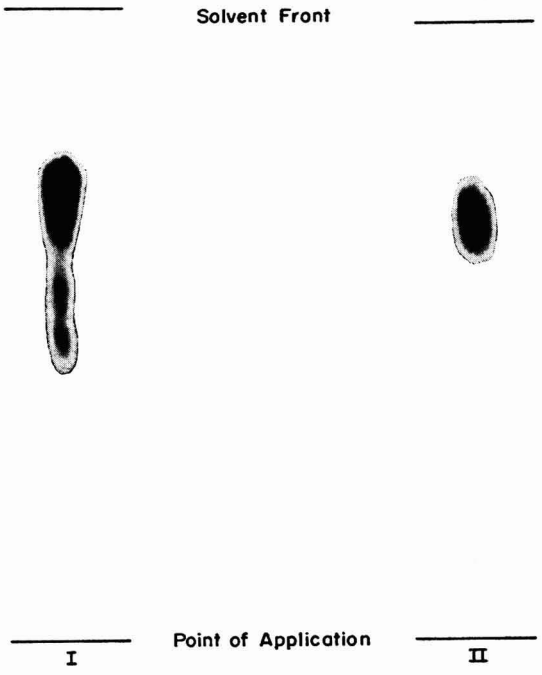
Based on this information the major portion of the radioactive 2-imidazolidinone was purified in this manner. An autoradiogram of a paperchromatogram of the labeled material before and after purification is shown in Fig. 2. Radioassay by strip or zonal scanning showed the final product had a minimum purity of over 98%.

Characterization

The chemical purity of the 2-imidazolidinone from the trial experiments was determined by infrared analysis and melting point measurements. Because 2-imidazolidinone is not sufficiently soluble in suitable solvents the usual solution method of obtaining an infrared spectrum was not applicable. Accordingly, the potassium bromide disc method was used. Solid KBr spectra of 2-imidazolidinone shows:

3.02 μ assigned to –NH absorption band
3.39 μ, 3.45 μ assigned to –CH absorption band
5.9 μ assigned to $-C=O$ absorption band.

Most trials, which were carried out in a manner similar to the radio-



I – 2-Imidazolidinone ^{14}C as isolated from reaction mixture

II – 2-Imidazolidinone ^{14}C after purification by chromatography

FIG. 2. Autoradiograph of paper chromatogram of 2-imazolidinone-^{14}C.

active preparation, had purities as determined from their infrared spectra to be greater than 95%. Others where the reaction variants were changed to a considerable degree had purities of 90–95%. In the latter samples there were small impurity bands at 8.3 and 10.7 μ.

By a comparison of melting points, the purity could be estimated within a few percent. Melting points were compared with the purity obtained by infrared analyses and it was observed that all trials having a purity of over 95% as determined by infrared analysis had a melting point of 132 to 135, and those with a purity of 90–95% by infrared determination had a mp of 128 to 131.

Bioassay

The toxicity to *Drosophila* from several of the trial experiments was compared with authentic 2-imidazolidinone. The toxicity at 100 ppm was essentially identical in all instances as the inhibition of adult emergence was 95–100%

The toxicity to the large milkweed bug, *Oncopellus faciatus*, of certain 2-imidazolidinone samples was evaluated. The samples were given continuously in the adult feed at 100 ppm following known procedures. All

samples from 2-imidazolidinone trials, the carbon-14 labeled material and the authentic standard caused failure of the milkweed bug egg to hatch.

Paper Chromatography

Paper chromatograms of 2-imidazolidinone-^{14}C were made using two different solvent systems. One consisted of *n*-butanol, acetic acid, and water in a ratio of 70:11:19; the other was ethyl acetate, acetic acid, and water in ratio of 3:1:1. The papergrams were prepared by spotting 40 μg or less of nonlabeled 2-imidazolidinone and 1 or 2 μl of radioactive 2-imidazolidinone-^{14}C or mixtures of the two in methanolic or aqueous solutions. The paper, Whatman No. 1, was formed into cylinders and developed using an ascending solvent front with the solvents indicated above. The ethyl acetate mixture was found more suitable, as more distinct spots were found than with the butanol mixture, and was used in identification and separation work. The papergram was allowed to develop until the solvent front had traveled 15 $\pm$ 1 cm. It was then hung in the hood until the solvent had evaporated, and then it was suitably identified with radioactive ink. The papergrams were fastened to a sheet of 8 $\times$ 10-inch paper covered with metallic foil and placed in contact with a sheet of Kodak no screen X-ray film. The film was exposed 24 to 72 hours depending on the amount of radioactivity present. After exposure, the film was developed, fixed, and washed according to the manufacturer's directions.

The film was brought into register by aligning the markings. The areas containing carbon 14 were sectioned, isolated, and counted using a Packard Tri-Carb liquid scintillation spectrometer. Where samples were co-chromatographed with nonlabeled 2-imidazolidinone or nonlabeled samples were analyzed, colormetric methods were used for visualization. The papergrams were sprayed with a 2% solution of dimethylamine benzaldehyde and placed in hydrogen chloride vapors for color development. A bright yellow color was observed for 2-imidazolidinone. Typical R_f values on paper developed using ethyl acetate system are:

	Co-chromatographed		
Carbon 14	Carbon 14	Nonlabeled	Nonlabeled
.72	.72	.73	.73

Counting Procedures

Either aliquots of solutions or isolated sections of papergrams were counted in a Packard Tri-Carb liquid scintillation spectrometer using normal instrument settings, standard scintillation solutions and counting techniques.

Experimental Procedure

Into a semimicro glass reaction tube (10 × 150 mm), was transferred 0.77 mmoles (102.5 mg) of ethylenediamine-^{14}C dihydrochloride. The carbon-14 diamine (sp act 2.6 mCi/mmole) was obtained from the Volk Radiochemical Company, Chicago, in the form of the dihydrochloride salt, and was transferred to the reaction tube within a glove box. To this was added 1.68 mmoles (68 mg), of finely ground sodium hydroxide.

The tube was equipped with an external copper cooling coil, and connected at the top to a glass coiled condenser with an exit to a cold trap cooled with liquid nitrogen. The mixture was placed on a heating stage and heated to 100° for 30 minutes. The neutralization was rapid as noted by the evolution of heat and the formation of water. To improve mixing of the contents, the tube was cooled to condense the reactants to the bottom of the tube. The solution was again heated for 15 minutes at 110°.

Into another reaction tube (10 × 130 mm), was placed 0.74 mmoles (44 mg) of urea. This was connected to the vacuum manifold and evacuated. The tube containing the ethylenediamine-^{14}C hydrate was likewise connected to the manifold, frozen, evacuated, and the contents were distilled into the reaction tube. The reaction tube, after removal from the manifold, was fitted with a baffled exit tube fitted to a cold trap. The tube was placed on a heat exchanger controlled by a calibrated powerstat. The temperature of the mixture was gradually raised during a period of 4.5 hours to 210°C. The heat was increased at such a rate that the temperature was maintained between the limits of the time temperature curve displayed in Fig. 3. The product was extracted with four portions of 1.5 ml of hot chloroform. These were combined and after cooling, the precipitate was separated by filtration using a Skau apparatus. The solvent was distilled from the filtrate using the vacuum manifold. This operation was repeated twice, collecting crops two and three. Weights of the three crops were 13.5, 11.0, and 6.2 mg. A sample of each was chromatographed on paper using the system described in a following section, and an autoradiogram was prepared (see Fig. 2). All crops appeared to be more or less identical, each having a purity of about 80%. The crops were combined at this point yielding 31 mg. Radioautograms were prepared of the insoluble material, and of the reaction mixture. It was found that the chloroform precipitate (16 mg) was 50% 2-imidazolidinone-^{14}C and the reaction mixture was 20%. Considering the 2-imidazolidinone from the latter as not recoverable, this gives a chemical yield of 48% based on ethylenediamine hydrochloride.

Thirty mg of 2-imidazolidinone-^{14}C (80% pure) sp act 2.6 mCi/mmole were dissolved in 50 μl of water. This was applied on a piece of

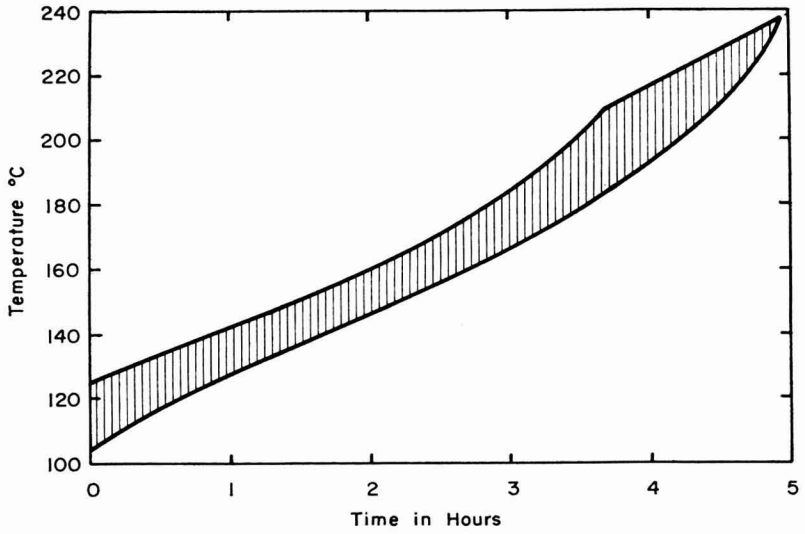


FIG. 3. Heating curve for 2-imidazolidinone.

Whatman No. 3 chromatographic paper (19 × 20 cm) by means of a micropipette, over about 9 cm of the paper 24 mm from the edge. The flask was rinsed with 50 μl of water and this was applied along the starting line extending on the next 8 cm of the paper.

After drying, the paper was placed in a rectangular tank preconditioned for 1 hour with the mobile phase. Using the ascending technique, the paper was developed using ethyl acetate, acetic acid, and water in a ratio of 3:1:1 parallel with the machined direction of the paper. The papergram was allowed to develop until the solvent front had traveled about 15 cm (1.5 hours). After drying, the papergram was mounted on heavy paper covered with foil, and a clear thin plastic sheet was formed into an envelope around the papergram. The papergram was placed on Kodak no screen X-ray film and exposed 10 minutes and developed according to manufacturers instructions. The papergram was brought into register and marked to indicate the area containing the 2-imidazolidinone-^{14}C. A section of the paper ca. 35-mm wide containing the 2-imidazolidinone-^{14}C was removed. This was fashioned to a point at one end suspended by a thread. The sectioned papergram was enclosed in a cylindrical glass tank and eluted with 15 ml of water after being wet. A streaking pipette was used to give a constant flow of water to the top of the papergram. The eluent was collected and the solvent was removed.

The yield was 24 mg. An infrared scan of 2-imidazolidinone-^{14}C was

comparable to reference standard. Analysis in two paper chromatographic systems showed a radiochemical purity of over 98%. The bioassay of the ^{14}C product was comparable to authentic product.

The chloroform precipitate was purified in the same manner, yielding 8 mg of pure 2-imidazolidinone-^{14}C.

RESULTS

Radiotracer 2-imidazolidinone was prepared in a yield, purity, and biological activity comparable to microscale trials. As shown Table 1, certain factors appeared to be quite important considerations for a successful reaction. The dimensions of the reaction flask are very important depending upon the scale of the experiment. The shape of the reaction flask is also vital to insure a steady removal of the small amount of water present. Details of the reaction flask dimensions are reported in the Experimental Procedure section.

It was found that an excess of 5% of the ethylenediamine over the urea enhanced the yield, however when an excess of over 10% was employed, the yield of 2-imidazolidinone-^{14}C was decreased.

The water content of the aqueous ethylenediamine solution in the range of 31–37% had little effect on the yields of 2-imidazolidinone-^{14}C. Other concentrations were not evaluated.

The rate of heating appeared to be critical in order to insure a successful result. A suitable heating curve for temperature vs. time is shown in Fig. 3. Experiments which varied greatly from this curve gave reduced yields. This is, no doubt, a function of the water removal which in macro trials could be controlled by more conventional means. Better yields were obtained if the terminal temperature could be reached in a short time interval.

The effect of an inert atmosphere such as nitrogen did not noticeably alter the reaction. In general, the higher the terminal temperature, the better the yield. Usually the final temperature was determined by the point at which the reaction mixture bumped onto the sides of the tube, thus, going out of the heating zone, and forcing an interruption of the experiment.

The literature reports 2-aminoethyl urea as a side product in the reaction of ethylenediamine and urea in the presence of water. The 2-aminoethyl urea is reported to yield both 2-imidazolidinone and a high melting polymer. In our trials, usually some high melting polymer was formed. Analysis of this material by infrared spectroscopy showed the absence of 2-imidazolidinone and that it contained peaks at 2.97 (OH) and 6.0 (C=O) which suggested a polymeric structure. Some trials showed an impurity which had an R_f value of .49. In the radioactive preparations,

two impurities were in evidence having R_f values of .58 and .49. The impurity having an R_f value of .49 was tentatively identified as $H_2NCH_2CH_2NHCOCH_2CH_2NH–CONH_2$.

SUMMARY

Radioactive 2-imidazolidinone labeled with carbon 14 in the 4 position has been synthesized because it was needed to facilitate the study of the metabolic fate of this interesting chemosterilant. It was prepared from the reaction between ethylenediamine and urea in the presence of water. Thirty-two mg of labeled 2-imidazolidinone was obtained in a yield of 48% based on the starting ethylenediamine-^{14}C. Its specific activity was 2.6 mCi/mmole. It assayed over 98% after purification by paper chromatography.

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MICROCHEMICAL JOURNAL 15, 171–172 (1970)

Use of Microcosmic Salt as a New Titrant for the Microdetermination of Acetic and Glycolic Acids

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In earlier publications from this laboratory, methods were suggested for the microdetermination of some organic acids such as sulfanilic*(3)*, iodoacetic, and oxalic*(4)* acids, etc., by titration with a microcosmic salt solution. In the present paper a similar procedure is described for the microdetemination of acetic and glycolic acids. It is observed that the results are concordant and precise, and compare well with other methods *(1, 2, 5)* proposed earlier.

EXPERIMENTAL METHODS

Reagents used. Acetic acid (A.R.B.D.H.); glycolic acid (Riedel 57%), microcosmic salt (E Merck), and bromcresol purple (B.D.H.).

Procedure. To a given solution of acid, add some distilled water to raise its volume to about 15 ml, followed by 1 or 2 drops of 0.1% solution of bromcresol purple indicator. The solution is yellow at this point. Now titrate it with a standardized microcosmic salt solution until the yellow color is completely discharged and the solution is a faint purple.

TABLE 1

MICRODETERMINATION OF ACETIC ACID

Sample no.	Vol of soln (ml)		Acetic acid (mg)		Error (mg)
	0.001 *M* Acetic acid taken	Microcosmic salt 0.001 *M*	Found	Theoretical value	
1	5.00	5.08	0.305	0.300	0.005
2	2.50	2.48	0.149	0.150	0.001
3	1.00	0.94	0.056	0.060	0.004
4	0.50	0.56	0.034	0.030	0.004

TABLE 2
MICRODETERMINATION OF GLYCOLIC ACID

Sample no.	Vol of soln (ml)		Glycolic acid (mg)		Error (mg)
	0.001 *M* Glycolic acid taken	Microcosmic salt 0.001 *M*	Found	Theoretical value	
1	5.00	5.04	0.383	0.380	0.003
2	3.00	3.06	0.229	0.228	0.001
3	1.00	0.98	0.074	0.076	0.002
4	0.50	0.52	0.039	0.038	0.001

RESULTS

The results are given in Tables 1 and 2; acids were estimated over a range of 0.380–0.030 mg. The results are concordant and precise.

SUMMARY

The acetic and glycolic acids were determined in micro quantities with a titrant, i.e., microcosmic salt solution, bromcresol purple was used as indicator. Estimations were carried out in the range of 0.380–0.030 mg, with a maximum error of ± 0.005 mg.

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The Analysis of Binary and Ternary Thin Alloy Films of Tantalum, Titanium, Niobium, Zirconium, Molybdenum, and Beryllium

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Reliable analytical results were essential to studies of other parameters critical to the preparation of sputtered alloy films. Generally, these films were of the order of several thousand angstroms thick and weighed less than 2000 μg. The alloys deposited both as binary and ternary systems involved the refractory-type metals, titanium, tantalum, zirconium, niobium, beryllium, and molybdenum. Thus, the methods developed required a high degree of sensitivity, selectivity, and precision. It was anticipated that the chemical data would also aid in the application and calibration of X-ray fluorescence spectrometry to the analysis of these systems.

The literature sources investigated were devoid of reports for the chemical analysis of metallic films involving those particular metals. However, spectrophotometric procedures for individual elements were available and many which appeared to exhibit satisfactory dependability were evaluated in the laboratory. Based upon this investigation, appropriate procedures were selected and incorporated with some modifications into an overall scheme for the analysis of metallic films.

Luke (*10*) and Hill (*6*) reported that tantalum could be determined using phenylfluorone as the chromogenic reagent following a methylisobutylketone extraction step. The two procedures differed somewhat in that Luke destroyed the organic by wet oxidation while Hill re-extracted the tantalum back into the aqueous phase prior to the final color development. Hill's method was simpler and more rapid. Two very selective and sensitive spectrophotometric methods reported for the determination of titanium involved the Tiron (*12*) and tri-*n*-octylphosphine oxide (extraction) procedures (*5, 13*). The most specific method for the determination of zirconium involved extraction with tri-*n*-octylphosphine oxide followed by color development directly in the organic phase using pyrocatechol violet (*5, 11, 14*). The main advantage of this pro-

cedure is its very high tolerance for sulfate. Niobium is commonly determined by extracting the thiocyanate complex and measuring the absorbance directly in the organic phase (*9*). However, since other refractory metals also form thiocyanate complexes, the method did not appear as advantageous as the 4-(2-pyridylazo)-resorcinol (PAR) method (*2*). Molybdenum is usually determined by the thiocyanate (*8*) or the toluene-3,4-dithiol extraction method (*7*). Again, for reasons of specificity, the toluene-3,4-dithiol method seemed to possess a definite advantage. Beryllium has been determined by the acetylacetone extraction method (*1*) as well as by a method relying upon lake formation (*4*). Generally, colors based on metal chelate formation are more stable than those of lakes, and hence are to be preferred.

MATERIALS AND METHODS

Reagents and Standards

1. Acetate buffer solution (Ti procedure), pH 5.0. Mix equal volumes of 1 M sodium acetate and 1 M acetic acid. Adjust the pH to 5.0 with glacial acetic acid or sodium acetate crystals.

2. Acetate buffer solution (Nb procedure), pH 5.8. Dissolve 80 g of ammonium acetate in distilled water. Add 6.5 ml of glacial acetic acid and dilute to 1 liter. Using a pH meter, adjust the pH to 5.8 with glacial acetic acid or 28% ammonium hydroxide (sp gr 0.90).

3. Buffer solution (Ta procedure), pH 5.0. Add 350 ml of glacial acetic acid to 250 ml of distilled water and mix. Dissolve 250 g of ammonium acetate in the resulting solution and then add 10 ml of 10% solution of benzoic acid in methanol. Dilute the solution to 1 liter. Dissolve 0.5 g of gelatin in 500 ml of hot distilled water, cool, and add to the buffer solution. Adjust the pH to 5.0 with either 28% ammonium hydroxide or glacial acetic acid.

4. Phenylfluorone, 0.01% alcoholic. Disssolve 0.010 g of phenylfluorone in 4 to 5 drops of 12 *M* HC1 and 10 ml of ethanol. Dilute the solution to 100 ml with ethanol. Prepare this solution just prior to use.

5. Pyrocatechol violet solution, 0.05% alcoholic. Dissolve 10 mg of pyrocatechol violet in 20 ml of reagent grade denatured ethanol. Prepare this solution just prior to use.

6. 4-(2-Pyridylazo)-resorcinol (PAR), 10^{-3} M. Dissolve 0.30 g by triturating with the minimum volume of 5% sodium hydroxide solution and diluting to 1 liter. The disodium salt is water soluble.

7. Toluene-3,4-dithiol. Break a 5-g vial of toluene-3,4-dithiol beneath the surface of 1 liter of 2% sodium hydroxide solution in a plastic container. Warm the solution slightly to about 30–35° C and magnetically stir until dissolution is complete. Add 10 ml of thioglycollic acid, mix, and decant the solution into 4-oz polyethylene bottles. When

stored under refrigeration, the solution is stable for at least 3 months.

8. Tri-n-octylphosphine oxide (TOPO), 0.1 M. Disolve 1.93 g in 50 ml of reagent grade cyclohexane.

9. Standard solutions (Be, Zr, Mo, Ta, Ti, Nb). Standard solutions were prepared from the pure metals (purity $\geqslant$ 99.95%). Beryllium was dissolved with dilute sulfuric acid. The remaining metals were dissolved with dilute hydrofluoric and nitric acids. The concentrations and diluent for each of the standards are as follows:

Be	1 μg/ml	2% H_2SO_4
Zr	5 μg/ml	2% HF
Mo	5 μg/ml	2% HF
Ta	10 μg/ml	2% HF
Ti	10 μg/ml	2% HF
Nb	20 μg/ml	2% HF

Procedure for Dissolution

Place the slide (1 × 1 inch is a convenient size) film-side down directly on top of 4 g of fused sodium bisulfate containing several drops of sulfuric acid. A 75-ml platinum lid dish of the concave type works well as a container. Invert a similar platinum dish lid over the first lid as the cover. After fusing very gently for about 5 minutes with a Bunsen burner, cool the fusion melt. Add distilled water to the fusion vessel, then place on a steam bath or warm hot plate until the glass substrate loosens from the platinum, enabling it to be transferred with distilled water to a beaker. Heat the beaker until the salt dissolves completely from the substrate. Cool the solution and transfer it to a volumetric flask.

If the alloy film is tantalum–titanium, sufficient hydrofluoric acid is added to the sample solution contained in a plastic beaker so that the resulting stock solution upon final dilution is approximately 2% in hydrofluoric acid. If the alloy film is niobium–zirconium–molybdenum, 2 drops of 49% hydrofluoric acid per 100 ml are added to prevent hydrolysis. The final volume depends, of course, upon the amount of each element in the stock solution. To minimize etching of the glass, the dilute hydrofluoric acid solution should be diluted almost to volume in the plastic beaker prior to transferral to a volumetric flask and final dilution. After dilution to the exact volume, store the stock solution in a plastic bottle.

Analysis of Tantalum–Titanium Films

Tantalum procedure. Transfer a 5.00-ml aliquot (25–50 μg) from the stock solution to a 125-ml separatory funnel and add, with mixing, 5.0 ml of 12 *N* hydrochloric acid. Extract the aqueous phase for 5 minutes

with 5.0 ml of methyl isobutylketone (MIBK). After draining and discarding the aqueous phase, scrub the organic phase once with 5 ml of a solution that is 0.4 *N* hydrofluoric acid and 6 *N* hydrochloric acid (5 ml of 4 *N* HF + 25 ml of 12 *N* HCl + 20 ml of H_2O). After draining and discarding the aqueous phase, rinse the excess acid from the organic phase by rocking the separatory funnel gently with 2–3 ml of distilled water for 5–10 seconds, and discard the aqueous phase. Repeat. Add 5.0 ml of 10% EDTA solution and 5 ml of buffer solution (pH 5.0) to the separatory funnel with mixing. Extract the tantalum from the MIBK by shaking it on a mechanical shaker for 5 minutes. Add 1 ml of ethanol and mix briefly to break up the emulsion. Allow the phases to separate and collect the aqueous phase in a 25-ml volumetric flask. Wash the MIBK with small portions of buffer solution and add the washings to the volumetric flask. After adding 3.0 ml of 7% aluminum chloride solution (7 g of $AlCl_3 \cdot 6H_2O$/100 ml solution) and 5.0 ml of phenylfluorone solution with mixing, dilute the solution to volume with buffer (pH 5.0) and allow the color to develop for 45 minutes. Measure the absorbance in 1-cm cells at 550 mμ using distilled water as the reference solution. A blank is carried through the complete procedure.

Titanium procedure. Transfer an aliquot containing about 50–100 μg of titanium to a platinum crucible, add 3-ml of sulfuric acid and heat to strong SO_3 fumes. Cool the crucible, rinse the wall with distilled water, and fume again to completely volatilize the hydrofluoric acid. Cool and transfer the solution to a 100-ml beaker with about 5 ml of distilled water. While the beaker is being cooled in a cold water bath, add 5 ml of 4% Tiron solution and a small piece of congo red indicator paper. Slowly add 1:1 ammonium hydroxide solution with stirring until the congo red indicator paper just commences to change color from red to blue. After cooling the solution, make the final pH adjustment to 4.5 with 1:5 ammonium hydroxide solution, using a pH meter. Add 5 ml of acetate buffer solution (pH 5.0). Transfer the solution to a 50-ml volumetric flask, dilute to approximately 40 ml, and then add 2 drops of thioglycolic acid to reduce any traces of iron. After diluting the solution to volume, mix and allow the color to develop for 5 minutes. Measure the absorbance at 410 mμ versus distilled water using 1-cm cells. Carry a blank through the complete procedure.

Analysis of Niobium–Zirconium–Molybdenum Films

Niobium procedure. Transfer an aliquot from the stock solution containing about 50–100 μg of niobium to a platinum crucible. Add 10 drops of sulfuric acid and evaporate just to dryness. The residue is dissolved with 10 ml of 10% tartaric acid solution by warming. When

completely dissolved, the solution is transferred to a 100-ml beaker and the pH is adjusted to 5.8($\pm$ 0.05) with 1:2 ammonium hydroxide. Add 5.0 ml of 0.02 *M* EDTA solution and readjust the pH to 5.8. Add 5.0 ml of PAR solution plus 5.0 ml of buffer (pH 5.8) with mixing and then transfer the solution to a 50-ml volumetric flask with distilled water. After diluting to volume, allow the color to develop for 1 hour. Measure the absorbance at 550 mμ versus water using 1-cm cell. Carry a blank through the complete procedure.

Zirconium procedure. Transfer an aliquot containing approximately 20–50 μg of zirconium to a platinum crucible, add 10 drops of sulfuric acid, and evaporate the mixture to dryness. After cooling, add 10 ml of 7 *N* nitric acid and warm it to dissolve the residue. Cool and then transfer the solution to a 125-ml separatory flask with an additional 10 ml of 7 *N* nitric acid. Extract the solution on a mechanical shaker for 15 minutes with 5.00 ml of 0.1 *M* TOPO solution. After discarding the aqueous phase, add 20 ml of 7 *N* nitric acid and scrub the organic phase for an additional 10 minutes. Discard the aqueous phase and drain the organic phase into a 10-ml beaker. Droplets of the aqueous phase will cling to the wall and also to the bottom of the beaker. Avoiding the droplets of aqueous phase, carefully pipet a 2.00-ml aliquot from the organic phase and transfer it to a 25-ml volumetric flask. Add successively 10 ml of ethanol, 1.50 ml of pyrocatechol violet solution, and 5 ml of pyridine to the flask with mixing in an exhaust hood. Dilute the flask to volume and allow the color to develop for 10 minutes The absorbance is measured at 655 mμ versus ethanol using 1-cm cells. A blank is carried through the complete procedure.

Molybdenum procedure. Transfer an aliquot containing approximately 20–50 μg of molybdenum to a platinum crucible, and, following the addition of 4 ml of sulfuric acid, evaporate the solution down to light fumes. After cooling, rinse the walls of the crucible with distilled water, and fume again. Cool, and carefully add 8 ml of 1:2 HC1 slowly with mixing, using a cold water bath for cooling the crucible. Transfer the cooled solution to a 125-ml separatory funnel with two 4-ml portions of 1:2 HC1. Add 10 drops each of 49% hydrofluoric acid add 20% hydroxylamine hydrochloride solution with mixing. Add 10 ml of toluene-3,4-dithiol solution and agitate it on a shaker for 15 minutes. Extract the molybdenum–toluene-3,4-dithiolate complex twice with 7-ml portions of carbon tetrachloride by shaking for several minutes. Transfer the organic extract to a 25-ml volumetric flask and dilute to volume with carbon tetrachloride. Measure the absorbance at 680 mμ versus carbon tetrachloride using 1-cm cells. Rinse and clean the separatory funnels and cells immediately after use to minimize corrosive attack

on the glass by dilute hydrofluoric acid solution. A blank is carried through the complete procedure.

Analysis of Niobium–Zirconium–Beryllium Films

Beryllium procedure. Transfer an aliquot containing approximately 5 to 15 μg of beryllium to a 100-ml beaker and evaporate to SO_3 fumes twice with several drops of sulfuric acid. Dilute by adding 2.0 ml of 10% EDTA solution and 25 ml of distilled water. Using 10% sodium hydroxide, adjust the pH of the solution to 7.5. After adding 5 ml of 5% aqueous acetylacetone solution, readjust the pH to 7.5. Allow the solution to stand for 5 minutes and then extract successively with 10-; 5-; and 5-ml portions of chloroform. Transfer the chloroform phases to a separatory funnel containing 50 ml of 0.1 *N* sodium hydroxide solution. After rocking each flask very gently back and forth 20 times, allow the layers to separate and discard the aqueous layer. Repeat the scrubbing step two more times. The chloroform layer is drained into a clean dry 50-ml beaker and then decanted into a 50-ml volumetric flask. The beaker is rinsed once with chloroform which is also decanted into the flask. After diluting to volume and mixing, measure the absorbance at 295 mμ versus chloroform using 1-cm cells. The absorbance of the solution is stable for at least 12 hours. A blank is carried through the complete procedure.

RESULTS AND DISCUSSION

Several complex alloy film systems were analyzed, including those consisting of tantalum–titanium, niobium–zirconium, niobium–zirconium–molybdenum, and niobium–zirconium–beryllium. The method of dissolution not only had to be drastic enough to dissolve the refractory-type alloy metals completely, yet also selective enough to avoid dissolving the 96% silica substrate which, of course, precluded the use of hydrofluoric with other common acids. Of the dissolution methods investigated, only a sodium bisulfate fusion satisfied the above requirements. Several drops of sulfuric acid added to the sodium bisulfate increased the effectiveness with which the metals were attacked and, in addition, decreased the tendency of certain metals such as tantalum and niobium to hydrolyze. In two systems, tantalum–titanium and niobium–zirconium–molybdenum, a small volume of hydrofluoric acid was added to the stock sample solution to prevent hydrolysis.

Two different types of container materials were evaluated for the sodium bisulfate fusions. One was fabricated from fused silica with optimized dimensions to accommodate the substrate. The second consisted of a standard type platinum lid made for a 75-ml platinum dish.

second lid inverted over the first, serving as the cover during the fusion. Fusions were completed on the concave side of the platinum lid with a Due primarily to better heat conduction, fusions were completed much more efficiently in platinum than in fused silica.

Each procedure for the analysis of these films was completely free from sulfate interference. Hence, the fusion flux presented no special problems. Hydrofluoric acid, however, did adversely affect the procedures for zirconium, niobium, beryllium, and titanium, and thus had to be completely volatilized prior to the determination of these elements. With molybdenum and tantalum, the proper amount of hydrofluoric acid had to be present to ensure complete extraction.

The methods for the preparation of the individual calibration curves were the same as that employed for the determination of the respective elements in the analysis of the films. Where a fuming step with sulfuric acid or an extraction step was included, the same stepwise procedure was followed for the preparation of the calibration curve.

An interference study was completed for each determinative method used in the analysis of a particular alloy film system (Table 1). Interference from an accompanying element in the film was considered significant or positive if it caused a change in absorbance of 0.010 or more. As noted, the analytical procedures employed were very selective in all cases.

To adapt to film analysis some of the procedures reported in the literature, a few modifications were made. Both Hill and Luke recommended that the absorbance measurements for the tantalum phenyl-

TABLE 1

INTERFERENCE STUDY

Element determined	Element added	Amount added (μg)	Interference
Ti	Ta	200	None
Ta	Ti	100	None
Mo	Nb	100	None
Mo	Zr	100	None
Nb	Zr	300	None
Nb	Mo	100	None
Nb	Be	100	None
Zr	Nb	15	Positive [a]
Zr	Mo	100	None
Zr	Be	100	None
Be	Nb	100	None
Be	Zr	100	None

[a] At least, up to 100 μg of niobium interference can be compensated for; see Fig. 1 and discussion.

fluorone complex be made at 530 mμ for maximum sensitivity. In this work, 550 mμ was selected as the optimal wavelength for the absorbance measurement, because at this wavelength the absorbance of the blank was minimized without seriously sacrificing sensitivity.

Contrary to indications by previous workers (*11*), a positive interference is observed when zirconium is determined in the presence of niobium. In this work it was further observed that the degree of effect of niobium on the zirconium calibration was no more serious when 100 μg of niobium was present than in the presence of 15 μg of niobium, which amounts to about a 10% positive relative error, is shown in Fig. 1. Consequently, the effect could readily be compensated for. A new calibration curve was simply established for the determination of zirconium in the presence of niobium by adding 15 μg of niobium to the blank and each standard used in establishing the calibration.

Hibbits *at el.* (*5*) reported that the organic phase should be centrifuged to prevent traces of aqueous 7 *N* nitric acid from accompanying the cyclohexane in the aliquot withdrawn from the organic phase for the

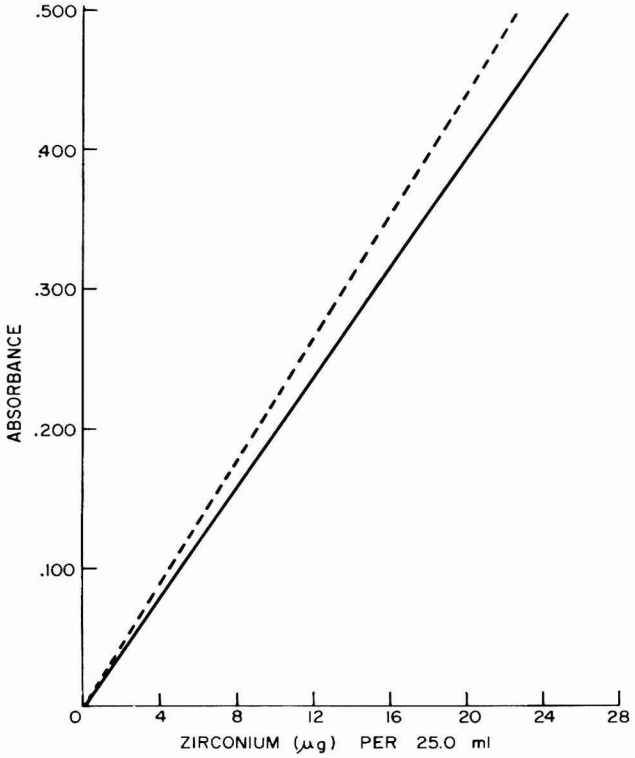

FIG. 1. Effect of niobium upon the calibration curve for zirconium (——), calibration curve for zirconium; (– – –) 15 μg of niobium added to each zirconium standard and the blank.

determination of zirconium. The same results were obtained by merely draining the organic phase into a dry 10-ml beaker and then carefully withdrawing the proper aliquot with a pipet. Small droplets from the aqueous phase adhered to the wall and bottom of the beaker. In each case the calibration curve remained the same.

Adams *et al.* (*1*) made a double extraction of beryllium acetylacetonate; following the first extraction, the organic phase was completely destroyed by wet oxidation and the beryllium was then re-extracted. This took considerable time, so experiments were completed to determine whether a single extraction would provide accurate and reproducible results for the analysis of alloy films. Using a single extraction step, it was determined that neither 100 μg of zirconium nor niobium interfered with the procedure. The scrubbing step was quite critical. Prolonged shaking resulted in low recovery for beryllium, due to partial extraction of the chelate. If the organic phase was not scrubbed sufficiently, the slight excess acetylacetone remaining significantly increased the absorbance. The procedure outlined gave satisfactory results.

Since it would have been extremely difficult to weigh each alloy film accurately, the relative concentration based upon sample weight was not determined. Instead, the total amount of each component in each film sample was reported. Instead of listing analysis results, the data were

TABLE 2

STATISTICAL TREATMENT OF DATA [a]

Film system	Element determined	Range of averaged data pairs (mg)	Pairs of data (k)	SD ($\pm$mg)	95% Confidence limits ($\pm$mg)
Nb–Zr–Be	Zr	0.198–0.240	8	0.006	0.011
Nb–Zr–Mo	Zr	0.950–1.17	11	0.041	0.065
Ta–Ti	Ti	0.387–0.435	10	0.011	0.018
Ta–Ti	Ta	1.23–1.66	9	0.035	0.057
Nb–Zr–Mo	Mo	1.44–2.18	8	0.021	0.035
Nb–Zr–Mo	Mo	0.517–0.551	4	0.004	0.008
Nb–Zr–Mo	Nb	1.63–1.82	12	0.009	0.014
Be–Zr–Nb	Nb	0.540–0.595	8	0.005	0.008
Be–Zr–Nb	Be	0.0371–0.0412	5	0.0005	0.001
Be–Zr–Nb	Be	0.0441–0.0605	7	0.0006	0.001

[a] Standard deviation $= \pm (\Sigma d^2/2k)^{1/2}$, where d = difference between duplicate determinations; k = number of pairs of data. Limits (95% confidence) $= \bar{X} \pm t_{0.5}s_0$ $[s/(N)^{1/2}]$, where X = mean value for duplicate determinations; t = test statistic used to estimate the area under a normal distribution curve for 95% confidence; s = standard deviation; N = number of determinations (2). Duplicate determinations represent two aliquots from the same stock solution.

examined statistically both to summarize the results more concisely and, in particular, to establish the precision of the respective methods. Following a treatment detailed by Bennett and Franklin (*3*) for comparing series of duplicate observations within fairly narrow ranges of absolute values, it was possible to estimate the standard deviations and the 95% confidence limits. The results of this treatment are shown in Table 2.

SUMMARY

Despite the fact that there are numerous current investigations relating to thin alloy films there is a dearth of published analytical procedures concerning their analysis. Thus, this article attempts to provide an accurate and rapid analytical scheme to cope with films involving refractory-type metals. The sensitivities of the methods are such that even smaller size samples can be adequately handled. The methods recommended have been successfully used the past 2 years in this laboratory for the analysis of numerous films. If a film and substrate are subdivided into several sections and the ratio of the concentrations of the elements comprising the alloy is chemically determined to be uniform over the whole film, then one of the sections could logically serve as a standard for X-ray fluorescence analysis. Since the effect of niobium upon the pyrocatechol violet procedure for zirconium had not been previously reported, it appeared to be quite noteworthy, especially in those instances where a maximum degree of accuracy is desired. The data are summarized by a statistical treatment that indicates the relative standard deviation to be between 1 and 4% depending upon the method and the system. The 95% confidence limits are also estimated.

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Differential Estimation of Low Energy Beta-Emitters, ^{3}H, ^{14}C, and ^{35}S in Multilabeled Organic Samples

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INTRODUCTION

Organic compounds labeled with low energy beta-emitters (^{3}H, ^{14}C, and ^{35}S) are extensively applied as tracers in various fields of science, biology, and medicine. It is obvious that the use of multilabeled compounds, either doubly or triply labeled with these isotopes, would be of great value in improving the potentialities of tracer techniques involving the use of these isotopes. This, in turn, implies the necessary application of radioactivity assay techniques that are highly sensitive and, at the same time, capable of affording differential estimation of these low energy beta-emitters. In this respect, most of the already available techniques suffer from one or several limitations. For example, while the solid sample counting technique could not be applied for tritium determination, carbon-14 and sulfur-35 could be collectively determined but with low efficiency due to the well-known losses inherent in that technique (*1*). Alternatively, applying the more efficient liquid scintillation counting technique, the differential estimation of the above-mentioned low energy beta-emitters in cases of their coexistence is only partially possible. Thus, by using the costy pulse hight analyzer coupled with a liquid scintillation detector, it is only possible to differentiate between tritium on the one hand and carbon-14 (*2–5*) and/or sulfur-35 on the other hand. The latter two isotopes emit bata radiations with nearly the same energy. In fact, the differential estimation of carbon-14 and sulfur-35, either in double-labeled compounds or in triple-labeled compounds, when both are present in combination with tritium, could not be acheived by, the nowadays famous, liquid scintillation spectrometry.

In the present work, the highly efficient and sensitive gas flow monitoring counter, erected and successfully applied in our laboratory for carbon-14 (*6*), tritium (*7*) and sulfur-35 (*8*) determination in

labeled organic samples was used for the differential estimation of these isotopes in double- or triple-labeled organic samples.

The technique adopted consists of combustion of the organic multilabeled samples, followed by appropriate chemical discrimination between different oxidation products, then counting using the internal gas flow monitoring counter. Results obtained clearly indicated the successful application of the described technique in the differential estimation of multilabeled organic samples.

EXPERIMENTAL METHODS

Materials

Since no double- or triple-labeled organic compounds were at our disposal, intimate mixtures of organic compounds labeled with carbon-14, sulfur-35 and tritium were used in varying relative amounts. Acetanilide-^{14}C used was obtained from an acetanilide sample irradiated for 48 hours with thermal neutrons in the 2 MW research reactor of the UARAEE and subsequently purified by repeated crystallization. Tritium-labeled *m*-dinitrobenzene was obtained from a sample prepared in the labeled products division, AEE. Commercially available thiourea-^{35}S was diluted through recrystallization after the addition of the appropriate amount of inactive thiourea. The specific activities of the different labeled compounds used were determined by the gas flow monitoring counter using the previously published procedures (*6–8*).

Apparatus

The apparatus used is schematically shown in Fig. 1. It consists of the three ignition systems (A, B, and C), water absorption tubes and the flow monitoring counter. Construction, operation, and counting characteristics of the gas flow monitoring counter, working with argon and butane–butylene mixture, were reported in detail in a previous publication (*6*).

Each of the three ignition systems, when used in combination with the flow monitoring counter, can provide a means for the selective determination of one or two of the radionuclides present in double- or triple-labeled samples. Thus, ignition system A, normally used for sulfur-35 determination (*8*), was also found applicable in carbon-14 determination. On the other hand, tritium could not be determined, since on ignition it is transformed into tritiated water, which is completely absorbed in the water absorption tubes. Moreover, the ignition–reduction system B formerly used for tritium determination (*7*) was also found applicable in the carbon-14 assay with great accuracy. On the other hand, sulphur 35 in labeled organic samples could not be determined

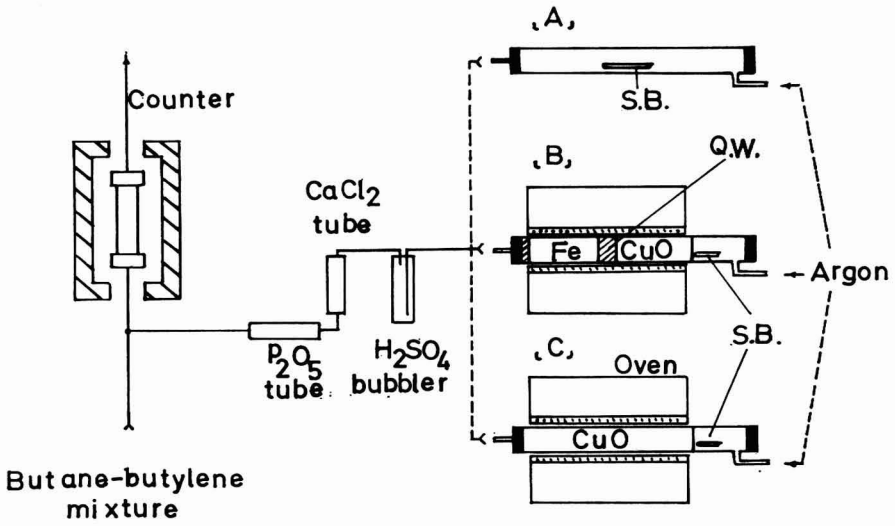

FIG. 1. Schematic representation of the apparatus used in the differential estimation of ^{3}H, ^{14}C, and ^{35}S in multilabeled organic samples: S.B. = sample boat; Q.W. = quartz wool.

since SO_2, primarily formed in the sample boat after ignition, was further oxidized on passing through the ignition tube into SO_3 which condensed readily on the cold parts of the apparatus. Finally, ignition system C was found to be applicable only for carbon-14 determination. Sulfur-35 being transformed into SO_3 readily condensed in the cold parts of the apparatus while tritium being transformed into tritiated water was completely absorbed in the drying tubes indicated in Fig. 1. From experiments using highly radioactive tritiated samples it was found necessary to use water absorption tubes filled with P_2O_5, 15-cm long and 15-mm i.d., in addition to the usually used drying tubes filled with conc H_2SO_4 and granular $CaCl_2$, in order to be sure of the complete absorption of tritiated water. In those ignition systems in which SO_3 was retained in the colder regions it was necessary to use a clean ignition tube and a fresh sulfuric acid bubbler after the assay of 8–10 samples, each of about 3 mg.

Procedures

Using any of the ignition systems mentioned, analysis was started by exactly adjusting the flow rates of argon and butane–butylene mixture, to about 15 ml/minute each, by a series of coarse and fine flow controllers. The high voltage was then applied to the counter tube, the plateau was determined and the proper working voltage was then chosen. Samples were taken by weighing different amounts of *m*-dinitro-

benzene-T, acetanilide-^{14}C and thiourea-^{35}S in a small quartz boat. The sample was then carefully covered with copper oxide and the sample boat, with its load, was then introduced into the ignition tube to be used. After recording the background value the sample was cautiously ignited by a hand blowpipe and at the same time the recording equipment was turned on. Ignition was continued until all the activity was recorded and the background count rate was restored again.

Using appropriate combinations of ignition systems, the differential estimation of tritium, carbon-14, and sulphur-35 in multilabeled samples could be achieved as follows:

I—Analysis of double-labeled samples. For double-labeled samples with carbon-14 and sulphur-35, ignition system A could be used to determine the total activity of both nuclides. Then, on using ignition system C, the activity of carbon-14 alone could be determined. The activity of sulfur-35 could be determined by difference.

For samples doubly-labelled with tritium and carbon-14, the total activity could be determined using ignition system B. Then, on using ignition system A or C, the carbon-14 activity alone could be determined and the tritium activity could be evaluated by difference. Alternatively, the tritium activity could be measured using ignition system B to which a carbon dioxide absorption tube was attached and by difference the carbon-14 activity could be determined.

For samples doubly-labeled with tritium and sulphur-35, the tritium activity alone could be determined using ignition system B whereas the activity of sulfur-35 could be determined on using ignition system A.

II—Analysis of triple-labeled samples. The differential estimation procedure for triple-labeled samples consists of three steps. At first, the total activity of carbon-14 and sulfur-35 could be determined using ignition system A. Then on using ignition system C, the carbon-14 activity alone could be determined and by difference the sulfur-35 activity could be evaluated. Tritium activity could be determined by either of two ways. It could be determined together with the carbon-14 activity by using ignition system B and by subtracting the known carbon-14 activity, the activity of tritium could be determined. Alternatively, the tritium activity could be directly determined by using a carbon dioxide absorption tube in series with ignition system B.

RESULTS AND DISCUSSIONS

The results obtained on analyzing three types of double-labeled samples are shown in Table 1. From these results, it is clear that in any of these samples, the activity of any single nuclide or the total activity of both nuclides present were determined, with deviations lying

TABLE 1

RESULTS OF ANALYSIS OF DOUBLE-LABELED SAMPLES

Mixture no.	Amount used (mg)			Activity used (cpm)			Ignition system used	Activity recorded (cpm)	(%)
	Acetanilide-^{14}C	Thiourea-^{35}S	m-Dinitrobenzene-^{3}H	^{14}C	^{35}S	^{3}H			
1	2.10	4.95	—	1703	8479	—	A	10050	98.7
2	4.60	2.50	—	3731	4250	—		7732	96.9
3	6.05	1.25	—	4907	2108	—		6717	95.7
4	1.85	4.40	—	1500	6798	—	C	1446	96.4
5	4.40	2.05	—	3568	3167	—		3645	102.2
6	7.30	1.50	—	5920	2318	—		5895	99.6
7	4.05	—	1.90	3285	—	4180	B	7577	101.5
8	5.20	—	1.00	4217	—	2200		6546	102.0
9	1.30	—	4.00	1054	—	8800		9302	94.4
10	6.30	—	1.30	5109	—	2860	C	5069	99.2
11	3.90	—	1.30	3163	—	2860		3084	97.5
12	1.30	—	4.35	1054	—	9570		1044	99.0
13	—	3.90	3.35	—	13354	11055	A	13844	103.7
14	—	5.10	1.35	—	17325	4455		17596	101.6
15	—	3.05	4.15	—	10196	13695		9987	97.9
16	—	3.70	2.60	—	12269	8580	B	8384	97.7
17	—	3.55	2.35	—	11772	7755		7846	101.2
18	—	1.60	0.85	—	5306	2805		2833	101.0

TABLE 2

RESULTS OF ANALYSIS OF TRIPLE-LABELED SAMPLES

Mixture no.	Amount used (mg)			Activity used (cpm)			Ignition system used	Activity recorded (cpm)	(%)
	Acetanilide-^{14}C	Thiourea-^{35}S	*m*-Dinitrobenzene-^{3}H	^{14}C	^{35}S	^{3}H			
1	1.30	1.00	2.00	1054	3161	6600	A	4381	103.9
2	3.25	1.20	0.95	2636	3764	3135		6337	99.0
3	1.85	2.95	1.85	1500	9254	6105		10378	96.5
4	1.20	2.95	2.20	973	9180	7260		10094	99.4
5	1.95	1.35	1.20	1581	3818	3984	B	5514	99.1
6	1.60	1.15	1.25	1298	3226	4150		5387	98.9
7	1.05	1.45	1.15	852	4067	3818		4610	98.7
8	1.20	1.10	0.95	973	3086	3154		4127	99.5
9	3.25	1.55	1.45	2636	4489	4814		2627	99.7
10	2.80	1.55	1.15	2271	4489	3818	C	2265	99.7
11	2.35	1.25	1.15	1906	3591	3818		1920	100.7
12	2.65	1.60	1.35	2149	4597	4482		2204	102.6
13	1.10	1.25	1.05	892	3061	3486	B	3431	98.4
14	1.25	1.10	1.25	1014	3479	4150	(+NaOH tube)	4131	99.5

within 1–4% only. About 1 hour was necessary for the complete analysis of any of the double-labeled samples, except when sulfur-35 was present in the analyzed sample. In the latter case the analysis time was about 1.5–2 hours.

It could be stated that our technique is much simpler than the lengthy and less efficient technique described by Jeffay and Alvarez (*9*) for the determination of ^{14}C and ^{35}S in double-labeled samples by transforming them into CO_2 and $MgSO_4$, respectively, followed by liquid scintillation counting using different scintillating solutions. Apart from the difficulties in the transformation procedures, use of different scintillating solutions could be a serious source of inaccuracy.

Table 2 shows the results obtained on analyzing triple-labeled samples. From these results, it is obvious that the activity recorded, using ignition system A, represents exactly the combined activity of carbon-14 and sulfur-35 (mixtures 1–4); whereas, the activity recorded, using ignition system B, is equivalent to the activity of carbon-14 and tritium together (mixtures 5–8). Besides, the data recorded, using ignition system C, represents exactly the activity of carbon-14 alone; whereas, those recorded, using ignition system B to which a carbon dioxide absorption tube was attached, represents the tritium activity alone. The time required for the complete analysis of a triple-labeled sample was 2–2.5 hours.

The results presented conclusively show that the described technique could be successfully applied in the differential estimation of the three nuclides in question in double- or triple-labeled samples.

It is important to note that the described technique could also be successfully applied for the direct determination of any given radionuclide in the simultaneous presence of any other one. Thus, ^{14}C, in the presence of either ^{35}S or ^{3}H, could be directly determined using ignition system C. Tritium, in the presence of ^{35}S, could be directly determined using ignition system B; whereas, tritium, in presence of ^{14}C, could be determined by using ignition system B to which was attached a carbon dioxide absorption tube. Finally, sulfur-35, in presence of tritium, could be directly determined using ignition system A whereas sulphur-35 in presence of carbon-14 could be determined only indirectly using the procedure described for the differential estimation of ^{35}S and ^{14}C in double-labeled samples.

SUMMARY

A simple technique is described for the determination of tritium, carbon-14, and sulphur-35 in multilabeled organic samples. Samples were ignited using different ignition systems and, through chemical discrimination between different

radioactive oxidation products, the differential estimation of these low energy beta-emitters was achieved. Radioactivity measurement was carried out using the highly efficient and cheap gas flow monitoring counter. Results obtained showed clearly that the described technique also could be successfully applied, with great accuracy and good reproducibility, for the individual determination of any of the above-mentioned isotopes in multilabeled samples.

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Microdetermination of Fluorine in Organic Compounds by Direct Measurement with a Fluoride Electrode

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INTRODUCTION

The analysis of fluoride by direct measurement with the fluoride-ion-specific electrode is today a widely accepted technique (*1–4*). However, unlike other fields of analytical chemistry in which the use of the fluoride electrode has been recommended (*5–10*), less than full advantage of this sensitive analytical tool has been taken in microelemental analysis (*11*, *12*).

In our preliminary experiments the fluoride electrode was used as an end-point detector in titrations of fluoride with lanthanum nitrate. Some of the difficulties encountered in this procedure were as follows: the equivalence point is not the point of maximal slope but must be determined empirically; the shape of the titration curve is very dependent on the reaction conditions, titration rate, and amount of fluoride to be titrated. Similar limitations have been described by Francis (*11*), Harzdorf (*13*), and Lingane (*1*). Best results were obtained in our laboratory by back-titration of the excess of lanthanum nitrate and by using a slow and constant titration rate. There is no doubt about the accuracy of the titration procedure, if it is performed very slowly and carefully. On the other hand, the need to separate sulfur and phosphorus (which interfere), the use of two standard solutions, and the difficulty in evaluating the titration curves make the procedure time-consuming and necessitate highly skilled personnel. This paper is concerned with the microdetermination of fluorine in organic compounds using the fluoride electrode for the direct measurement of fluoride concentration following a combustion step.

MATERIALS AND METHODS

Apparatus

A hot-flask combustion assembly (*14–17*) was used throughout the experiments. Measurements were made in a 50-ml polyethylene beaker

by means of a model 94–09 fluoride-ion electrode (Orion Research Inc.) and a sleeve-type saturated calomel electrode (Ingold M 303 W) in conjunction with an E 388 Metrohm Herisau Compensator (accuracy ± 0.1 mV or ± 0.001 pH). All weighings were made on a Mettler microbalance M 5.

Reagents

Reagents were p.a. grade Merck. Total ionic strength adjustment buffer (TISAB) was prepared as recommended by Frant and Ross (*3*) and diluted 1:1 with distilled water. Stock solutions were stored in polyethylene bottles.

Procedure

A sample of 1.5–4 mg in weight containing of 0.5–2 mg of fluorine is weighed in a platinum boat on the microbalance. The combustion tube without the sample holder is inserted into the furnace for a preheating period of 3–4 minutes. The tube well is filled with 2 ml of water and the combustion tube flushed thoroughly with oxygen. The sample holder is then quickly inserted into the tube and secured with a steel clamp. After combustion the hot tube is withdrawn and allowed to cool, after which 4 ml of 0.05 *M* KOH is introduced, thoroughly shaken to rinse the entire surface, and washed out with TISAB to make 50 ml. The resulting solution is then stored in a polyethylene bottle for later measurement of its fluoride concentration along with the other samples. Standard solutions are prepared by weighing 1–4 mg of NaF and diluting to 50 ml with 6 ml of water and TISAB. In routine work, all solutions are assayed the same day, together with one or two standard solutions, one after the other. The electrode response is determined after 3 minutes' stirring, using the pH mode of the compensator. The fluoride electrode is conditioned in TISAB at about 5×10^{-6} moles/liter in fluoride.

RESULTS AND DISCUSSION

Determination of Fluoride Ion Concentration

Combustion of various fluorocarbons results in solutions with different relative anionic and cationic compositions from sample to sample. Since the potential developed by the fluoride electrode depends on the fluoride activity of the solution, and since the hydroxyl ion interferes with the electrode response to the fluoride ion (*2*, *4*), variations in both the ionic composition and pH of the solution must be avoided. This can be done by a TISAB procedure as recommended for determination of fluoride in water supplies (*3*). The hot flask containing several milliliters

of solution has to be washed out as thoroughly as possible. Therefore, the 1:1 diluted TISAB was used for this purpose.

A typical calibration curve for the electrode response to sodium fluoride standards in the range 10^{-6}-10^{-2} moles/liter is shown in Fig. 1. The slope of the linear part of the curve proved to be constant as well as close to the theoretical Nernstian response, as reported by other authors (*2*, *3*, *6*) and the manufacturers of the electrode. However, intercepts of the curve varied slightly from day to day (even though no systematic drift was observed), making it impossible to use one reference curve over a long period without considerable loss in precision. This may be due to instability of the reference electrode and variability of the liquid junction potential between the reference electrode and test solution. The observed potential E in a solution of high ionic strength can be expressed in terms of the fluoride concentration c_F as

$$E = E_K - A \log c_F, \tag{1}$$

where A is a constant [ideally 2.303 (RT/F) from the Nernst equation], and the constant E_K is the sum of the potentials at the reference electrode and at the liquid junction between the solution and reference electrode, plus the potential due to the internal filling of the fluoride electrode.

Since the electrode gives a Nernstian response (59.0 mV/pF at 24°C), i.e., the value of A is the same as in pH measurement, the

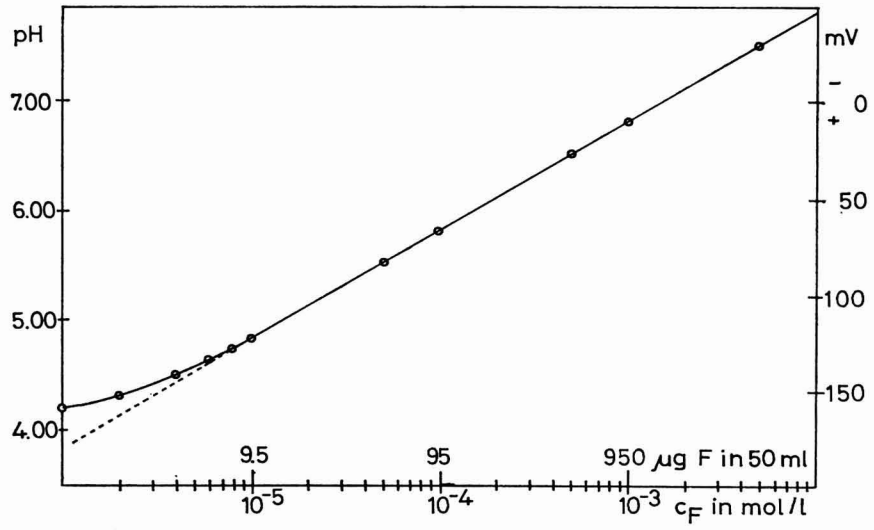

FIG. 1. Calibration curve for the electrode response in pH or mV mode of the instrument vs. fluoride ion concentration moles/liter or µg of F in 50 ml. Measurements were made in a TISAB background at 24°C, sample volume 50 ml.

logarithm of the fluoride concentration can be measured directly using the pH mode of a millivoltmeter with an expanded scale. The values thus obtained differ, however, from the true log c_F scale by an additive constant :

$$\log c_F = S_{pH} + \text{constant}, \tag{2}$$

where S_{pH} is the signal read in the pH mode of the instrument. Like E_K in Eq. (1), this constant is again a function of the particular electrode system used and must be evaluated with a known concentration of fluoride ion. In routine work, daily standardization of the electrode at one concentration allows the fluoride concentration in all unknown solutions of the day to be calculated from Eq. (2). The error involved in this simple calibration method can be read from Table 1. The fluoride electrode was calibrated by means of a sodium fluoride standard containing 1 mg of fluorine in 50 ml of TISAB. A series of 18 sodium fluoride solutions containing 0.4–2 mg of fluorine were assayed. The standard deviation of the absolute error in these 18 analyses (Table 1) was 0.17%.

The percentage of fluorine in the sample is calculated from the formula

$$\% \text{ F} = \frac{c_F \cdot K}{W} \times 100, \tag{3}$$

where c_F is the concentration of fluoride obtained from Eq. (2), K is the adjusting factor (corresponding to the measurement in 50 ml) and W is the sample weight in milligrams.

Analysis of Fluorocarbon Test Substances

Results obtained with three fluorocarbon standards during a 3-month period are shown in Table 2. One or two of these standards were combusted each day along with the unknown samples; none of the results obtained has been omitted here. The standard deviations of absolute error for the 25 analyses of each substance are 0.15, 0.19 and 0.18%, respectively. The slight negative tendency of absolute error was not satisfactory explained. Nevertheless, the accuracy of this method appears to be equal to that of other methods used in microelemental analysis.

Analysis of Fluorocarbons Containing Traces of Fluorine

To ensure that measurement of the fluoride concentration is made in the linear—and preferably always the same—range of the curve shown in Fig. 1, a fluorocarbon sample should contain not less than 0.1 mg of

TABLE 1
DIRECT DETERMINATION OF FLUORIDE CONCENTRATION IN SODIUM FLUORIDE SOLUTIONS
Fluoride electrode calibrated with a single NaF solution; sample volume, 50 ml.

Wt of NaF (mg)	Theoretical wt of F (mg)	F (%)	
		Found	Error
1.002	0.453	45.24	0.00
1.245	0.563	45.43	+0.18
1.508	0.682	45.22	−0.02
1.593	0.721	45.14	−0.10
2.010	0.909	45.46	+0.22
2.022	0.915	45.29	+0.05
2.050	0.927	45.16	−0.08
2.080	0.941	45.50	+0.26
2.566	1.161	45.00	−0.24
2.598	1.175	45.51	+0.27
2.636	1.193	45.48	+0.24
3.015	1.364	45.24	0.00
3.064	1.386	45.19	−0.05
3.107	1.406	45.31	+0.07
3.158	1.429	45.24	0.00
3.534	1.599	45.00	−0.24
3.970	1.796	45.05	−0.19
4.250	1.923	45.09	−0.16

TABLE 2
DETERMINATION OF FLUORINE IN ORGANIC COMPOUNDS

Sample	No. of determinations	Wt (mg)	F found (%; mean)	SD (%)
p-Fluorobenzoic acid [a] (theor., 13.56% F)	25	2.5–3.5	13.35	±0.15
Trifluoracetanilide [a] (theor., 30.14% F)	25	2.0–3.0	30.03	±0.19
N,N'-Bis-4-chloro-3-trifluoromethyl-diphenylurea (theor., 27.33% F)	25	2.0–3.0	27.15	±0.18

[a] BDH microchemical standard.

fluorine. If the fluorine content of the sample is lower, a simple standard addition method enables the usual working region to be reached, so that measurements with the same absolute error are made. This consists of adding a known amount of sodium fluoride to the solution after

combustion. The total concentration of the fluoride in the solution is the sum of the known concentration of fluoride added and the unknown concentration of fluoride in the original sample.

INTERFERENCE

No interference has been observed after combustion of fluorocarbons containing nitrogen, halogens, oxygen, sulfur, or phosphorus.

SUMMARY

A method for routine microdetermination of fluorine in organic compounds is described, using hot-flask combustion and a fluoride-specific electrode for direct measurement of fluoride concentration. The accuracy of the method meets the requirements of microelemental analysis, and it has the great advantage of simplicity and rapidity.

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MICROCHEMICAL JOURNAL 15, 199–204 (1970)

Microdetermination of Fluorine in Organic Compounds with a Fluoride Ion Electrode Following an Oxygen Flask Combustion [1]

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INTRODUCTION

As pointed out by Torma and Ginther (*7*), who recently described a method for the determination of fluorine in feeds using a fluoride ion electrode, the official method (*6*) for fluorine is time consuming and requires much care to obtain good results, the greatest difficulty being the indistinct end point in the titration. They found that the use of the fluoride ion electrode for the final measurement reduced the time required, eliminated the troublesome titration, and resulted in a faster, more precise method. In their method, a relatively large sample (10 g) was digested with perchloric acid and the products were steam distilled prior to measurement of the F^- activity.

In analyzing organic compounds on a microscale, a very convenient technique for the initial decomposition of the sample is the oxygen flask combustion whereby the sample is burned in oxygen and the combustion products are absorbed in an appropriate solution. Fernandopulle and MacDonald (*4*) have recommended the use of quartz or plastic combustion flasks for the decomposition of organic fluoro-compounds to prevent losses of fluoride. Due to the high cost of quartz flasks, we have used a polypropylene combustion flask made from a polypropylene sample bottle.

MATERIALS AND METHODS

Apparatus and Reagents

(a) Fluoride ion activity electrode, model 94–09 (Orion Research Inc., Cambridge, Mass.); internal compartment filled with a 1:1 solution of 0.1 *M* NaF and 0.1 *M* KCl in 4% agar according to Durst and Taylor (*3*).

[1] Contribution No. 120 Analytical Chemistry Research Service.

(b) Reference electrode, saturated KCl, sleeve type (Orion model 90–01.).

(c) Digital pH meter (Orion model 801).

(d) Magnetic stirrer with Teflon-covered stirring bar.

(e) McIlvaine's standard buffer solution (TISAB), pH 7.0. Mix 3.53 ml of 0.1 *M* citric acid solution with 16.47 ml of 0.2 *M* disodium phosphate solution.

(f) Standard sodium fluoride solution: Weigh 2.2101 g of dried recrystallized (*5*) NaF into a volumetric flask and make up to 1 liter. Store in a polyethylene bottle to prevent reaction of fluoride with glass. This solution contains 1000 ppm of F.

(g) Working standards: Suitable aliquots of stock solution are transferred to 100-ml volumetric flasks and made to volume. Aliquots of these solutions to contain 30, 40, 50 μg of F are pipetted into 100-ml polyethylene beakers. They are made basic with 0.1 *N* NaOH solution using 1 drop of phenolphthalein indicator, neutralized with 0.1 *N* HCl, 10 ml of TISAB are added, and then diluted to the 50-ml mark in the beakers.

(h) Combustion flask (Fig. 1). A widemouthed polyethylene or

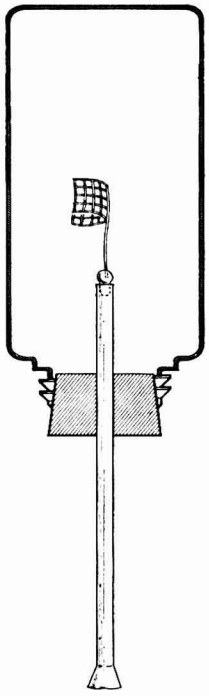

FIG. 1. Polypropylene oxygen combustion flask.

polypropylene bottle of 500-ml capacity is fitted with a one-hole neoprene stopper. A sample support made from standard platinum wire mesh is fused to a 1-cm length of 5-mm Pyrex rod which is sealed with heat and Kronig's cement into an 18-cm length of 6-mm o.d. polyethylene tubing. This is fitted into the neoprene stopper so that the platinum sample support is positioned in the center of the combustion flask when it is inserted.

Determination

Burn a 3–4-mg sample of the organic compound wrapped in filter paper in the usual way. If the sample contains a high percentage of fluorine (e.g., Teflon) mix the sample with 10–15 mg of benzoic acid on the filter paper before combustion. Absorb the combustion products in 10 ml of 0.1 *N* NaOH. After shaking the flask for 10–15 minutes, rinse the sample holder and stopper and transfer the solution to a 100-ml volumetric flask and make up to volume. Pipet an aliquot (enough to contain 40–50 μg of F, usually between 2 and 10 ml) into a 100-ml graduated polyethylene beaker and neutralize it with 0.1 *N* HCl to the phenolphthalein end point; add 10 ml of TISAB buffer and make up to the 50 ml mark. Insert the fluoride ion and standard calomel electrodes and, after exactly 5 minutes, while stirring by means of a magnetic stirrer, take the EMF reading. Similarly, take readings on two working standards which bracket the value found for the sample.

Calculation

To illustrate the method of calculation, an actual example is as follows: A 3.255-mg sample of *p*-fluorobenzoic acid was combusted according to the procedure above and made up to 100 ml from which a 10-ml aliquot was taken for analysis. An EMF reading of 155.0 mV was obtained, while readings of 156.9 and 151.2 were found for standard solutions containing 40 and 50 μg of F, respectively, or 0.57 mV per μg of F.

$$\text{F}(\mu\text{g}) \text{ in aliquot} = 50 - \frac{155.0 - 151.2}{0.57} = 43.33,$$

$$\text{fluorine } (\%) = \frac{43.33}{1000} \times \frac{100}{10} \times \frac{100}{3.255} = 13.31\%.$$

RESULTS AND DISCUSSION

Preliminary experiments with the Orion fluoride ion electrode (Table 1) showed that the stability of the electrode from day to day was not

TABLE 1

DAY-TO-DAY VARIATION OF emf READINGS (mV) WITH A FLUORIDE ION ELECTRODE ON STANDARD FLUORIDE SOLUTIONS

Fluorine (μg in 50 ml)	Mar. 5[a]	Mar. 6[a]	Mar. 7[a]	Mar. 10[b]	Mar. 11[b]	Mar. 19[b]
30	161.1	160.7	159.6	163.0	162.5	161.2
40	157.8	157.1	155.3	156.9	156.5	157.3
50	152.8	152.8	151.0	151.2	151.7	151.5
60	147.4	147.9	146.6	146.8	147.4	148.8

[a] Orion standard calomel electrode used.

[b] Beckmann standard calomel electrode used.

sufficient to allow the use of a calibration curve unless it was prepared at the same time as the determinations were carried out. Since it is simpler to run just two standards which closely bracket the sample in concentration, this procedure was adopted.

When not in use, the electrode was kept soaking in a standard fluoride solution containing 40–50 μg of F. This allowed the use of the electrode after a minimum of equilibration time. The life of the electrode filling was about 1 month.

A number of determinations were carried out on *p*-fluorobenzoic acid using the regular Pyrex combustion flask with nearly acceptable results being obtained. However, using other samples containing relatively high percentages of fluorine, the results were consistently low, even when 10–15 mg of benzoic acid was added before combustion. A possible explanation for these low recoveries of fluorine is the reaction with boron in the Pyrex flask resulting in formation of a fluoroborate complex (*2*) which cannot be measured with the fluoride electrode. Thus an alternative was sought and since the cost of a quartz flask was prohibitively high, a polyethylene combustion flask was improvised. The original flask consisted of a 500-ml narrow-mouthed polyethylene reagent bottle. The results, using this, were encouraging but because of the difficulty of putting the ignited sample through the narrow mouth, a 500-ml widemouthed polypropylene bottle was substituted. Determinations, in these flasks, were carried out on four analytical samples representing a range of fluorine content. A summary of the results is given in Table 2. The second column gives the number of determinations (n), column 3 shows the theoretical percentage of fluorine ($\bar{x}$) and column 4 the

TABLE 2

MICRODETERMINATION OF FLUORINE BY SPECIFIC ION ELECTRODE ANALYSIS FOLLOWING OXYGEN FLASK COMBUSTION USING A POLYPROPYLENE FLASK

Compound	No. of detn (n)	Fluorine (%)		SD (s)	Av abs. dev. (d_m)
		Theory ($\bar{x}$)	Av ($\bar{x}$)		
p-Fluorobenzoic acid [a]	6	13.56	13.29	0.30	0.35
Trifluoroacetanilide (TFA) [a]	6	30.14	30.48	0.38	0.42
TFA + benzoic acid	4		29.91	0.27	0.28
m-Trifluoromethylbenzoic acid (TFMB) [a]	6	29.98	30.25	0.36	0.37
TFMB + benzoic acid	3		30.00	0.80	0.60
Teflon [b]	3	75.98	65.23	7.27	10.75
Teflon + benzoic acid	7		75.86	0.29	0.24

[a] Organic analytical standard, British Drug Houses.
[b] Sample supplied in an IUPAC collaborative study.

average value ($\bar{x}$) of the n determinations. Column 5 shows the standard deviation (s) calculated as follows:

$$S = \left[\frac{\Sigma(\bar{x} - x)^2}{n - 1}\right]^{1/2},$$

and column 6 gives the mean or average deviation (d_m) defined as the average of the absolute deviations from theoretical

$$d_m = \frac{\Sigma|\bar{x} - x|}{n}.$$

In the analysis of trifluoroacetanilide and *m*-trifluoromethylbenzoic acid, some samples were combusted in the presence of added benzoic acid, but, from the results, it appears that this is not necessary. It is necessary, however, in the analysis of Teflon as the results obtained without added benzoic acid were consistenly low. With the addition of the benzoic acid, which supplies hydrogen for the production of HF (1), results are quite acceptable.

CONCLUSIONS

Fluorine-containing organic compounds can be analyzed by oxygen flask combustion in a polyethylene or polypropylene flask and using a fluoride specific ion electrode for the final determination. With samples high in fluorine content (e.g., Teflon) it is necessary to add benzoic acid during the combustion.

ACKNOWLEDGMENTS

We thank Mr. R. B. Carson and Dr. C. S. Shih for helpful suggestions and advice.

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Oxidation of Ferrous Sulfate with Ag(III)

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INTRODUCTION

Reddy and Machin (*2*) oxidized ferrous sulfate to ferric sulfate by means of air oxidation in the presence of pyrolusite as catalyst. Banerji (*3,4*), Elliott (*5*), Mikhelson (*6*), Pound (*7*), Yamamota (*8*), Belopol and Urusov (*9*), Kuzminykh and Babushkina (*10*) oxidized ferrous sulfate to ferric sulfate by air under different conditions and using different catalysts. Damon (*11*) used nitric acid, and Milbauer (*12*) compared the effect of different catalysts in concentrated sulfuric acid at 237°. Reactions with Ag(III) have been described earlier (*1*). The present paper describes the use of Ag(III) as an oxidant for the titrimetric determination of ferrous sulfate. Excess of silver(III) solution was added to the substrate solution. Complete oxidation took place within 10 minutes at room temperature. Substrate solution is oxidized to ferric ion as follows:

$$Fe^{2+} + \tfrac{1}{2}\,O \rightarrow Fe^{3+}$$

so 1 equivalent of Ag(III) is required for 1 mole of ferrous sulfate.

EXPERIMENTAL METHODS

Reagents used. Ferrous sulfate (C.P.B.D.H.) was used. The solution was standardized against standard ceric sulfate solution.

Silver nitrate, potassium hydroxide, sodium bicarbonate, starch, and potassium iodide used were of B.D.H. AnalR grade whereas potassium tellurite, potassium persulfate and iodine used were of C.P.B.D.H. grade.

Ag(III) solution K_5H_4 [$Ag(TeO_6)_2$] was prepared by oxidizing silver nitrate with potassium persulfate in boiling solution, in the presence of tellurate ion as stabilizing agent in alkaline medium ($\sim$0.5 *M*). It is then standardized by addition of known excess of sodium arsenite solution and remaining of it was determined iodometrically in the presence of potassium iodide in bicarbonate medium.

TABLE 1

ESTIMATION OF FERROUS SULFATE

1.30×10^{-2} *M* Ag(III) (ml)	1.00×10^{-2} *M* ferrous sulfate (ml)	Equivalents of Ag(III) consumed
2	1	0.42[a]
2	1	1.02[b]
2	1	1.03[c]
3	2	1.00[c]
4	3	1.04[c]
10	3	0.98[c]

[a] The compound–Ag(III) mixture stood at 30° C for 0 min; [b] 10 min; [c] 30 min.

An aliquot (see Table 1) is added to an excess of Ag(III) solution. The unconsumed Ag(III) is estimated as described above. Five ml of 0.01 *M* sodium hydrogen phosphate is added before addition of sodium arsenite solution to Ag(III) solution to stop the interference of ferric ion formed in the solution mixture with sodium arsenite solution. The equivalence of Ag(III) for 1 g mole of the compound is calculated.

The unused arsenite is determined titrimetrically against iodine solution after allowing the compound–Ag(III) mixture to stand at 30°C for (a) 0 minutes; (b) 10 minutes; (c) 30 minutes before adding the known excess of arsenite solution.

RESULTS AND DISCUSSION

Table 1 clearly shows that ferrous sulfate is oxidized to ferric sulfate at room temperature within 10 minutes. It consumed 1 equivalent of Ag(III) solution. The present work shows the utility of Ag(III) as an oxidant for titrimetric determination of ferrous sulfate.

ACKNOWLEDGMENTS

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Thin-Layer-Chromatographic Separation of Some Polynuclear Hydrocarbons

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INTRODUCTION

The quantitative fluorimetric determination of benzo(*a*)pyrene in an extract of airborne particulates at 405 nm with a fluorescence attachment to the Beckman DU spectrophotometer is always disturbed by similar polycylic aromatic hydrocarbons like benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*e*)pyrene because of the interference of their fluorescence maxima at the wavelength in question. Therefore a separation of these hydrocarbons before quantitative analysis will be useful. The thin-layer-chromatographic methods of Badger et al. (*1*), Kunte (*2*), and Sawicki et al. (*3*) and other authors are fit for complete separation, but unfortunately the mixture has to be developed several times in one or in two directions, and a combination of two or three adsorbents is necessary. In certain cases the adsorbent layers have to be dried for several hours after preparation or development.

The following technique does not have these disadvantages, and, besides, enables the analyst to separate further polynuclear hydrocarbons, e.g., benzo(*a*)anthracene, which are important in cancer research.

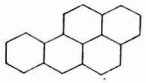
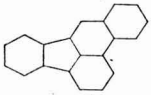
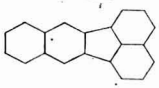
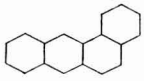
MATERIALS AND METHODS

Reagents and apparatus. Acetylated cellulose (20%) MN 300 AC (Macherey, Nagel & Co., Düren); cyclohexane 99.5% p.a., ethanol 99% p.a., dichloromethane 99.5% p.a. (Merck AG, Darmstadt); benzo(*a*)anthracene, benzo(*a*)pyrene and benzo(*e*)pyrene (Fluka AG, Buchs); benzo(*b*)fluoranthene and benzo(*k*)fluoranthene (Rütgerswerke AG, Castrop-Rauxel). The plates are prepared with an apparatus from Desaga GmbH, Heidelberg, and the chromatograms are analyzed in a Chromato-Vue-Box from Ultra-Violet Products Inc., San Gabriel, Calif.

Preparation of the thin-layer plates. A slurry of 10 g of acetylated

TABLE 1

R_f VALUES OF THE SEPARATED POLYNUCLEAR HYDROCARBONS

Hydrocarbon	Formula	R_f Value
Benzo(*a*)pyrene		0.32
Benzo(*b*)fluoranthene		0.49
Benzo(*k*)fluoranthene		0.51
Benzo(*a*)anthracene		0.62
Benzo(*e*)pyrene		0.70

cellulose—which is enough for preparing 10 plates 10 × 15-cm—in 50 ml of ethanol is poured across the carrier plate to prepare a layer of 250 μ. The coating is allowed to air dry at room temperature. The plates are then stored in a desiccator until ready for use.

Development. After spotting a cyclohexane solution of the test mixture, the plates are developed with a solvent consisting of ethanol/dichloromethane/water (20:10:1) during 30 minutes.

RESULTS

The method was found to be quick and sensitive. The spots of the separated sample were identified under UV light by comparison with chromatograms of the pure substances. The polycyclic aromatic hydrocarbons were separated completely, except the benzofluoranthenes. Table 1 shows the R_f values.

After the separation benzo(*a*)pyrene, which is the most interesting hydrocarbon because of its cancerogenic activity, may be measured as a particular compound.

SUMMARY

Polycyclic aromatic hydrocarbons, which disturb the quantitative fluorimetric determination of benzo(*a*)pyrene at 405 nm, may be separated on acetylated cellulose with ethanol/dichloromethane/water (20:10:1). The interfering polycyclics are also separated from each other, except benzo(*b*)fluoranthene and benzo(*k*)fluoranthene.

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Indirect Polarographic Method for the Microdetermination of Sulfur in Organic Compounds

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INTRODUCTION

From the different methods available for the microdetermination of sulfur, the titrimetric (*1–6*) and gravimetric (*7,8*) finishes predominate. Usually, the organic substance is ignited in an O-filled flask (*1–6, 8–10*).

In the titrimetric determination of sulfur, a general trend can be figured out. This is to absorb the products of combustion in an oxidizing absorbent such as hydrogen peroxide (*1,4,5*), bromine water (*2*), or fuming nitric acid (*2*) and titration of the resuling sulfate with barium perchlorate (*1,4–6*), chloride (*2*), or nitrate (*3*). Some authors (*1,3–5*) recommended addition of sufficient ethanol or isopropanol to give 80% alcoholic reaction medium. Thorin (*1,4,5*), tetrahydroxyquinone (*2*), carboxyarsenazo (*3*), and sulfonazo III (*6*) are suggested as visual indicators. However, in spite of the well-known simplicity of the titrimetric finish, there seems to be some difficulty in detecting the color change of most of the above-mentioned indicators (*6,9*).

Gildenberg (*10*) determined sulfur amperometrically using lead nitrate solution. Both of the amperometric and titrimetric finishes have the disadvantage that phosphorous if present interferes (*7,10*). Ogg (*7*) reported that for compounds containing phosphorous, sulfur should be determined gravimetrically. Lysyj and Zarembo (*8*) applied a gravimetric procedure and eliminated the interference due to phosphorous simply by employing an acidified reaction medium prior to precipitation. Unfortunately, however, the gravimetric finish happens to be somewhat tedious and time-consuming. A method that would be simple and rapid is therefore desired for the microdetermination of sulfur in organic compounds containing phosphorous.

In the proposed procedure, the solution containing the sulfate ion, produced from combustion of the organic sample, is acidified, and

then quantitatively precipitated using a known excess of barium chloride; the unreacted barium ion is measured polarographically. In principle, the method depends on recording the polarogram of a known amount of barium before and after precipitating a certain weight of the organic sulfur compound.

EXPERIMENTAL METHODS

Apparatus

Radiometer polarograph, type P03f with accessories.

Universal U-shaped polarographic cell; the anode compartment is filled with mercury and saturated potassium chloride solution. The capillary used has a drop time of 3–4 seconds under an open head of 60 cm of mercury.

Reagents

All reagents employed are of A.R. grade except M.A.R. *p*-toluenesulfonic acid.

Acetic acid. 0.2 N solution.

Barium chloride. ca. 0.2 N solution was prepared by dissolving 11.7688 g of the dihydrate in 500 ml of water.

Calcium chloride. 0.5 M solution.

Gelatine. 1% solution.

Saturated bromine water.

Recommended procedure

The sample (3–9 mg) is burned in a 300-ml O-filled flask using the Schoniger method. Products of combustion are absorbed in 7 ml of water. Rinse stopper with another 2 ml followed by 1 ml of saturated bromine solution. Excess bromine is removed by heating the solution until almost colorless. While solution is hot, add 0.5 ml of 0.2 N acetic acid, then 1 ml of ca. 0.2 *N* barium chloride solution. Digest under reflux condenser for 20 minutes on hot plate; then allow it to cool for 15 minutes. Transfer quantitatively to 50-ml measuring flask, then add 2.5 ml of 0.5 M calcium chloride, 0.15 ml of 1% gelatine solution, and water up to the mark. Shake mixture well, and transfer it to the polarographic cell. Record the barium wave starting from − 1.80 V applied potential and using a sensitivity of 150 (0.2 μA/mm), damping 3, voltage multiplier 0.8, and compensation of condenser current 2.

Under exactly similar conditions, except for the sample, record the polarogram corresponding to the total amount of barium (standard solution).

Percentage sulfur is calculated from the equation:

$$\% S = \frac{16.033 \times A \times N \times (B - C) \times 100,}{B \times W}$$

where, A = volume of barium chloride solution added (1 ml); N = normality of the barium solution; B = wave height of the standard solution (mm); C = wave height of the sample solution (mm); and W = weight of sample (mg).

The standard deviation σ is computed from the formula (*2*);

$$\sigma = \{[\Sigma(x - \bar{x})^2]/(n - 1)\}^{1/2}.$$

RESULTS AND DISCUSSION

As in any indirect method, maximal precision is attained when the amount of barium added in excess is kept small compared to that precipitated. The results in Table 1 show that the use of 1 ml of ca. 0.2 N barium chloride solution (slightly more than required for quantitative precipitation of 5 mg of a compound containing 50% sulfur) proved quite satisfactory for the analysis of all the available 12 sulfur compounds. The average error for 28 determinations is $\pm 0.3\%$; the maximum error amounted to $+0.63\%$ (Table 1).

Comparison between direct and indirect (or comparative) polarographic methods of analysis indicate that the latter eliminates many of the experimental difficulties (*11*). The limiting factors are the reproducibility of the waves and the precision with which comparison of wave heights may be made. The latter is nearly independent of the method of measurement provided similar polarographic conditions are applied for both the unknown and the standard solution. Taylor (*12*) recommended the use of indirect methods not only in routine work but where analytical results of high precision are desired.

It is already known that barium gives well-developed normal waves in 0.05 M calcium chloride supporting electrolyte (*13*). In the present work, a 0.025 M solution was found equally well. Plot of the barium ion concentration versus the wave height (mm) gave a straight line passing by the origin showing that the system satisfies the Ilkovic equation (*14*) over the range of 1.34×10^{-3} to 3.66×10^{-3} N(Fig. 1). This encouraged the use of barium, as a pilot ion, in the indirect polarographic microdetermination of sulfur; a method which proved simple, accurate, and fairly rapid.

Absorbing medium. Bromine water has been preferred, as absorbent, over hydrogen peroxide because of the ease with which it can be expelled. The use of 1 ml of saturated bromine solution, added after ignition of the sample, was found sufficient for quantitative oxidation of the sulfur oxides to sulfate. The slight excess is removed by heating for a few minutes.

Removal of phosphorous. Unfortunately, only two phosphorous-containing organic compounds were available (Table 1). Weakly acidic

TABLE 1

ANALYSIS OF ORGANIC SULFUR COMPOUNDS

Sample	Sample wt (mg)	Sulfur (%) Calc	Found	Error (%)	SD σ
p-Toluenesulfonic acid	3.845	16.86	17.25	+0.39	
	5.030		17.12	+0.26	
	8.230		16.71	−0.15	±0.30
	3.500		17.24	+0.38	
	6.265		16.91	+0.05	
Diphenylthiocarbazone (Dithizone)	4.725	12.51	12.35	−0.16	
	5.105		13.08	+0.57	±0.52
4-(*p*-Nitrophenylazo) chromotropic acid (Na salt)	6.412	12.49	12.35	−0.14	±0.32
	7.079		11.89	−0.60	
2-Nitroso-1-naphthol-4-sulfonic acid	8.561	12.66	12.60	−0.06	±0.16
	3.792		12.83	+0.17	
6-Chloro-5-nitro toluene-3-sulfonic acid	7.213	11.72	11.50	−0.22	±0.57
	7.748		12.30	+0.58	
Tetrabromophenol sulfophthalein	6.134	4.79	4.69	−0.10	±0.25
	9.437		4.33	−0.46	
Dibenzoyl disulfide	5.829	23.37	23.73	+0.36	+0.21
	6.742		23.43	+0.06	
Nitroso-R-salt	7.575	17.00	17.54	+0.54	±0.06
	6.075		17.63	+0.63	
l-Cystine	7.220	26.69	26.15	−0.54	±0.78
	3.836		27.26	+0.57	
o-Mercaptobenzoic acid	3.932	20.80	20.66	−0.14	±0.03
	6.787		20.71	−0.09	
Diphenylphosphine dithioic acid	4.457	25.62	25.14	−0.48	
	3.896		26.07	+0.45	±0.49
	8.392		25.33	−0.29	
Triphenylphosphine sulfide	4.450	10.89	11.01	+0.12	±0.25
	7.638		10.66	−0.23	

medium, ca. 0.01 *N* in acetic acid, prevented coprecipitation of phosphate provided the organic sample contains as much as 1 mg of phosphorous (in case of 8.392 mg of diphenylphosphine dithioic acid; Table 1). The acetic acid can be dispensed with if phosphorous is known to be absent.

Precipitation of sulfate. Complete and rapid precipitation of sulfate as the barium salt has been achieved following the method of Ogg (7)

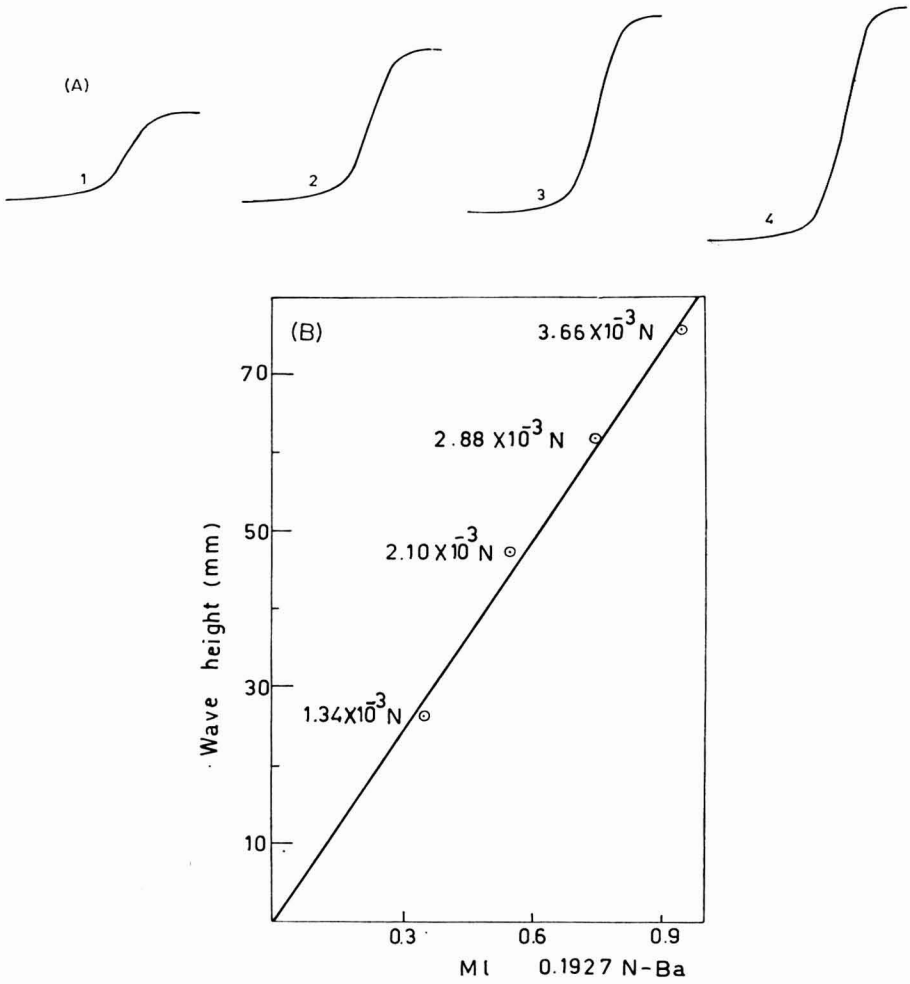

FIG. 1A. Cathodic waves of barium ion in 0.025*M* calcium chloride: concentration of barium ion was (1) 1.34 × $10^{-3}N$; (2) 2.10 × $10^{-3}N$; (3) 2.88 × $10^{-3}N$; and (4) 3.66 × $10^{-3}N$. (B) Variation of wave height with concentration of barium ion (calibration curve).

who advocated digestion of the reaction mixture (total volume not more than 11 ml) for at least 15 minutes. In the present work, digestion for 20 minutes gave accurate values for all the compounds analyzed. The procedure (*7*) is much less time-consuming compared with that of Lysyj and Zarembo (*8*); they recommended leaving the precipitate undisturbed for 2 hours.

Determination of barium. (i) After precipitation, the unreacted barium ions were determined polarographically in the same solution

containing the barium sulfate. The small amount of the precipitate, a few milligrams, present in 50-ml total volume exerted but negligible influence on the limiting diffusion current (or wave height). This, besides saving time, eliminates many of the difficulties associated with the filtration process.

(ii) Barium, like the other alkaline earth metals, is characterized by very negative reduction potential (*13*). Zlotowski and Kolthoff (*15*) reported that the cathodic reduction wave of barium has a half-wave potential of −1.94 V vs. SCE. This suggested the possibility of recording the polarograms even without bubbling nitrogen; the oxygen wave has a half-wave potential of −0.94 V vs. SCE. (*13*), i.e., well separated from that of barium.

Interference. The available organic compounds contain carbon, hydrogen, nitrogen, chlorine, bromine, phosphorous, oxygen, and sodium. None of them interfered with the procedure; carbon dioxide is polarographically inactive, nitrogen oxides present in amounts arising from organic compounds did not interfere, chloride and bromide give anodic oxidation waves, phosphate is kept in solution by using 0.01 *N* acetic acid medium, and sodium (or potassium) in quantities not exceeding that of barium should not interfere (*13*). Iodine, if present, would be oxidized to iodate by the bromine water. The half-wave potential of the iodate wave falls between −0.50 and −0.65 V vs. SCE (*16*), i.e., would not interfere with that of barium.

An advantage of the proposed method worth mentioning lies in the fact that after recording the polarogram, the amount of barium present in solution can be redetermined by any other method, e.g., titration with 0.02 *M EDTA* solution (*17*). In this way, one can easily carry out two determinations on the same sample weight.

SUMMARY

An indirect polarographic method has been developed for the microdetermination of organically-bound sulfur using barium as pilot ion. The analysis of 12 organic compounds gave accurate results with an average error of ±0.3%. Nitrogen, chlorine, bromine, and sodium do not interfere. Interference due to phosphorous is simply eliminated by using an acidic medium prior to precipitation. Iodine, if present, should not interfere. The method is simple, accurate, and relatively rapid. One determination takes ca. 40 to 45 minutes.

ACKNOWLEDGMENT

I express my thanks to Mr. George Toma for his help in a part of the experimental work.

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Direct Microdetermination of Oxygen by Static Flash Combustion Pyrolysis [1]

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INTRODUCTION

The direct microdetermination of oxygen in organic materials based on the Schütze–Unterzaucher method (*1*) or its modifications has not been universally accepted as a routine method of analysis because high, and often variable blank values were obtained. Besides the problem of blank values the fact that the accuracy claimed by Unterzaucher could not be reproduced by other investigators was an additional reason for the changes proposed in numerous modifications of the original procedure (*2–5*). The blanks have been attributed to a variety of causes: trace amounts of oxygen in the carrier gas which escape retention in the gas purification system (*6*), air inclusion during the insertion of the sample into the pyrolysis tube, the performance of the furnace that ought to maintain constant the high temperature required (*1*), the impurity of the reagents, the quality of quartz (*6*) and the type of carbon used (*7,8*). These can be quoted as external factors which can be eliminated more or less satisfactorily. On the contrary, the reaction of the quartz of the pyrolysis tube with the carbon layer (*8*), graphitization of the carbon (*9*), the retention of carbon dioxide on the carbon surface by the formation of carbon complexes low in oxygen content (*10*), and the reaction of reactive pyrolysis products with the quartz tube (*6*), cause variable blanks inherent in the principle of the method itself and as such cannot be eliminated completely.

In this study the blanks were minimized by excluding the carbon layer as permanent packing of the pyrolysis tube. The conversion to carbon monoxide of the oxygen contained in organic samples is performed by covering the sample in the platinum boat all over with car-

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bon. The pyrolysis and the conversion are simultaneously effected by static flash combustion at 1120°C in an empty quartz tube. The results of such a procedure are low and remarkably constant blank values, prolonged life of the pyrolysis tube, and the elimination of an extensive conditioning required whenever the combination tube is repacked.

MATERIALS AND METHODS

A detailed description of the oxygen microprocedure will be omitted here apart from a brief outline of distinguishing particulars.

Alterations in Apparatus and Procedure

The usual apparatus for microdetermination of oxygen described by Unterzaucher (*1*) has been used with both gravimetric and volumetric end determination (Fig. 1). Two columns of elementary copper in the purification system ensure better retention of oxygen present in the

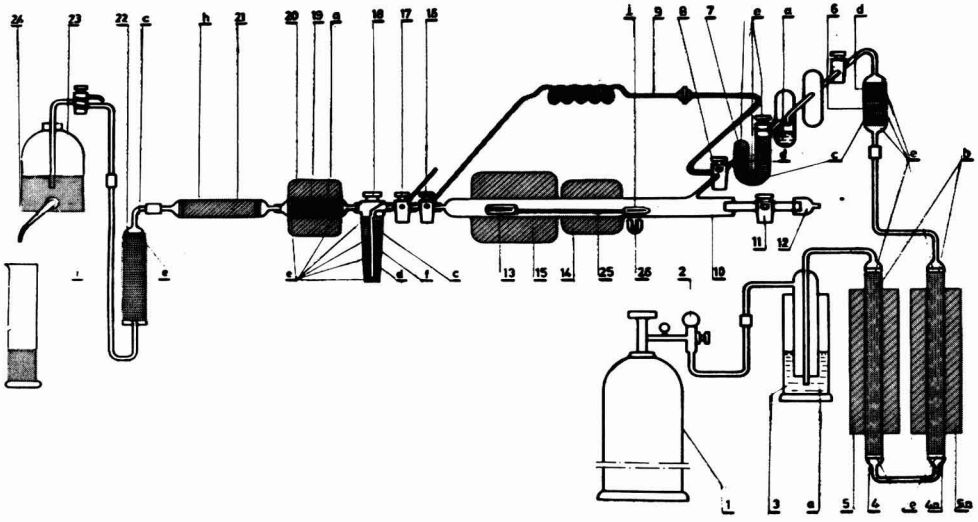

FIG. 1. Oxygen microdetermination apparatus: (1) compressed nitrogen in cylinder; (2) reducing valve; (3) pressure regulator; (4) and (4a) purification tube; (5) and (5a) preheater, 500°C; (6) absorption tube filled with dessiccant; (7) bubble counter with white oil; (8, 16, and 17) three-way stopcocks; (9) reverse-flow circuit: (10) quartz combustion tube; (11) Wide-bore stopcock; (12) protecting cap; (13) platinum boat; (14) movable combustion furnace; (15) main furnace, 1120°C; (18) absorption tube; (19) oxidation tube; (20) heating block, 118°C; (21) absorption tube; (22) guard tube; (23) Mariotte-bottle; (24) graduate (25) sample holder; (26) magnet; (a) white oil; (b) reduced copper; (c) anhydrone; (d) ascarite (registered trade mark of the Arthur H. Thomas Co. for sodium hydroxide on asbestos fibers); (e) quartz wool; (f) molecular silver (g) iodine pentoxide; (h) sodium hydroxide; (i) distilled water and (j) piece of iron.

nitrogen carrier gas. The three-way stopcock (*17*) enables the escape of nitrogen in the atmosphere during work-pauses and overnight to protect the filling of the oxidation tube.

The main difference is the omittance of carbon packing in the pyrolysis tube. One to 2 mg of organic substance covered with carbon (ca. 45 mg) in a platinum boat is introduced into the pyrolysis tube of 15-mm bore with the aid of a quartz sample holder (Fig. 2). Through the 10-mm bore stopcock (*11*) provided with a protecting cap (*12*) the holder is moved within 15 cm of the main furnace to prevent premature decomposition or volatilization of the sample during the purging with reverse stream of nitrogen (20 ml/min). After 10 minutes the tube is closed and the direct flow of nitrogen is adjusted to 10 ml/min.

The pyrolysis train is separated from the rest of the apparatus by turning the stopcocks (*8*) and (*16*), and the sample holder is moved to the heated part of the pyrolysis tube with the aid of a magnet. The position of the platinum boat corresponds to the zone within the furnace with constant temperature of 1120°C. The static flash combustion of the sample is aided by a movable furnace or burner placed near the inlet end of the furnace to prevent the upstream escape of the pyrolysis products. After 10–15 minutes, the flushing stream of nitrogen is adjusted to flow through the oxidation tube for 10 minutes more, and the determination is performed in the usual way.

Blank determinations were carried out in the same way only the organic sample was omitted. The runs thus included the inserting of a platinum boat loaded with about 45 mg of carbon, the purging and the combustion procedure performed as with the sample. Apparent oxygen values from oxygen-free substances are not essentially higher compared with the values obtained by other oxygen determination procedures. At the end of a working-day the sample holder is cleaned by heating in the flame of a Bunsen burner.

The maximum amount of substance quantitatively pyrolyzable under these conditions is 2 mg. Larger samples were led to incomplete decomposition, unburned sample escaping upstream and forming deposits in front of the burner.

Reagents

Carbon (CK Gassruss Degussa) was prepared by heating for 8 hours

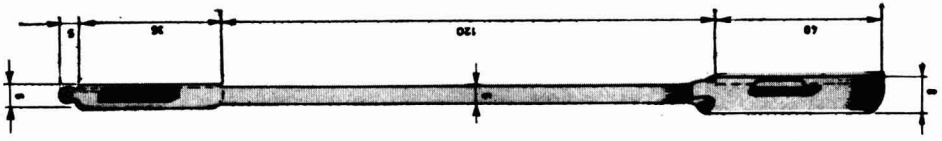

FIG. 2. Quartz sample holder.

TABLE 1

RESULTS OF OXYGEN DETERMINATIONS

Compound	Wt of sample (mg)		Oxygen (%)			No. of determinations	SD
	Minimal	Maximal	Theor.	Found	Difference		
Isatin	1.005	1.934	21.75	21.75	—	82	0.34
Benzoic acid	1.024	1.588	26.20	26.20	—	24	0.38
Salicylic acid	1.150	1.217	34.75	34.79	+0.04	3	0.46
p-Bromobenzoic acid	1.213	1.457	15.92	15.83	−0.09	5	0.66
Phenacetin	1.032	1.440	17.86	18.11	+0.25	10	0.44
Acetanilide	1.006	1.479	11.86	12.16	+0.30	8	0.40
Cinnamic acid	1.104	1.519	21.60	21.60	—	11	0.46
Vanillin	1.064	1.500	31.55	31.61	+0.06	7	0.33
p-Chlorobenzoic acid	1.092	1.310	20.44	20.31	−0.13	8	0.58
Atropine	1.091	1.958	16.95	16.58	−0.37	4	0.32

in each of the following atmospheres: nitrogen at 1100°C, hydrogen at 800°C, and nitrogen at 1100°C. The conditioning of pulverized carbon before use is performed by pyrolysis of three unweighed samples with ca. 45 mg of carbon in a platinum boat. Such an amount of carbon is sufficient for 10–12 determinations. All other chemicals, except nitrogen, were of the type and purity grade usually used for this determination.

DISCUSSION OF RESULTS

The results of a series of determinations carried out by the procedure described above are listed in Table 1. The deviation of mean values from the theoretical is given in column 5. Combination of standard deviations (column 7) results with $s = 0.39$ ($\phi = 152$). The blank values corresponding to 30–75 μg of oxygen were consistent for several months. They are due to deposition of carbon in the pyrolysis tube and on the sample holder as well as to the attack of active pyrolysis products. The extent of both reactions, and herewith the blanks, depend upon the quality of quartz used. By the usual Unterzaucher procedure with the same apparatus and the same quality of quartz, blank values of 60–217 μg of oxygen were obtained with significant variations even from day to day. Decrease of blank values below 30 μg was as a rule accompanied by erratic low results. This deficiency is removed by cleaning the graphite layer in the combustion tube after 20–30 runs by heating it for 20–30 minutes in an oxygen stream. The deficiency persisted if devitrification of the quartz occurred. Consistent and minimized blank values insure better accuracy and reproducibility of results so that further efforts seem justfied particularly in consideration of an ultramicro procedure for the direct oxygen determination.

SUMMARY

The pyrolysis and the conversion to carbon monoxide of the oxygen contained in organic samples are simultaneously effected by static flash combustion at 1120°C in an empty quartz tube of the sample covered with carbon. Consistent and minimized blank values, prolonged life of the pyrolysis tube, and the elimination of an extensive conditioning whenever the combustion tube is repacked justified further investigations of this preliminary procedure particularly in consideration of performance on the ultramicro scale.

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Applications Involving the Iodide Ion

V. Determination of Small Amounts of Platinum and Analysis of Its Mixtures with Some Metal Ions

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INTRODUCTION

Platinum, as well as other platinum metals, finds wide applications in industry, important among which, are its use as catalyst either in form of surface coating on other materials or commonly as platinum–alumina reforming catalysts, and its use as alloying metal with 5 to 10% of rhodium, iridium, ruthenium, nickel, or with 4% tungsten.

The simple, rapid and accurate procedure using mercury(II) as back titrant for excess iodide which was recently applied to the determination of metal ions, for which formation of adequately insoluble iodides exists, as well as to the analysis of mixtures involving them (*11–13*), is extended to the determination of platinum (IV) and analysis of its mixtures of industrial importance. The probable insolubility of platinum(IV) iodide promoted the present investigation.

There are no known selective gravimetric reagents for platinum. However, it is reported (*15*) that ammonium chloride and sodium formate (*2*) are most acceptable ones, despite the many adverse literature criticisms concerning them. Among the insoluble hexa chloroplatenate(IV) salts are to be mentioned those of potassium, rubidium, cesium, and thallium(I). The latter's solubility is reported to be 0.064 g/liter (*4*) which was employed in a spot test detecting 0.025 mg of platinum (*6*). Lloyd (*5*) precipitated and separated platinum quantitatively from iridium with hypophosphorous acid and mercury(II) or (I) chloride from boiling dilute HCl acid and volatilized the mercury by ignition.

Volumetric procedures are based on oxidation or reduction of platinum between its two stable valence states, such as its potentiometric titration with copper(I) (*7*), permanganate (*8*), bromate (*16*) cerium (IV) (*9*), and iodine (*10*).

Despite the fact that for micro amounts of platinum(IV) the colori-

metric method using tin(II) chloride is reported to provide a sensitive and accurate one (*1*) it involves some drawbacks, namely its sensitivity to a minimum of 3 ppm, the use of ketones and alcohols as extractants and the colloidal nature of tin(II) chloride solutions.

The present potentiometric method provides a rather simple means of determining with requisite accuracy platinum(IV) in the concentration range of several milligrams down to 1 ppm.

EXPERIMENTAL METHODS

The water used was always twice distilled. The chemicals were all of the highest purity available. These were potassium iodide; platinum and palladium metals; nitrates of mercury(II), calcium, strontium, barium, copper(II), zinc, iron(III), cobalt(II), and nickel; sulfates of manganese(II) and thallium(I); disodium ethylenediaminetetraacetate (EDTA); nitric and hydrochloric acids; Eriochrome black T(EBT), murexide, and methylthymol blue indicators.

Solutions

The 0.025 *M* platinum(IV) solution was prepared by dissolving, by aid of gentle heating, 2.465 g of a pure metal sample into a suitable volume of aqua regia, fuming the orange red solution twice with conc. HCl acid to remove nitrosyl compounds and excess acidity, and finally diluting the syrupy solution with water up to 250 ml in a volumetric flask. The pH of the resulting chloroplatinic acid solution was measured to be 1.4. It was standardized gravimetrically following the ammonium chloroplatinate method. From this solution the 0.00414 *M* solution was prepared by appropriate dilution and standardized gravimetrically. The 0.0005 *M* solution was prepared by accurate dilution from the 0.00414 *M* solution. The 0.00204–0.0977 iodide solutions were prepared normally and standardized potentiometrically against mercuric solutions using silver amalgam as the indicator electrode. The 0.001–0.05 *M* mercuric solutions were prepared as mentioned elsewhere and standardized potentiometrically against iodide and volumetrically with standard EDTA solution in urotropine and methylthymol blue as indicator. The 0.05 *M* EDTA solution was standardized against a standard 0.05 *M* zinc solution prepared from an oxide sample and nitric acid. The 0.02875 *M* palladium was prepared from a BDH Palladium foil (106.7) as described elsewhere (*13*) and standardized as dimethyl glyoximate and potentiometrically by titrating excess iodide with mercury(II) and the silver amalgam electrode. The 0.05 *M* solutions of the above metal nitrates or sulfates were prepared and standardized by recommended procedures.

The titration cell consisted of a 150-ml beaker; a 1/50 graded microburette; a mechanical stirrer; calomel and silver amalgam electrodes fitted to a three-cell Cambridge potentiometer NO. L-28795 connected to a spot galvanometer. Automatic Zintle 1/50 graded microburettes were used for storing and delivering the solutions, keeping the iodide-storing bottle out of direct light by wrapping it with black paper.

Procedures

A. To determine platinum alone transfer into the titration vessel 0.25 to 6 ml of Pt, 4 to 12 ml of iodide, heat to just before boiling to precipitate black PtI_4, and titrate excess iodide with mercury(II) and the silver amalgram electrode. Similarly determine Pt in presence of a variety of cations which do not interfere with iodide, and use EDTA as masking agent for any interfering cation.

B. Determine platinum in its binary mixtures with divalent copper, palladium, cobalt and mercury, nickel and iron(III) as in (A), using, with the latter, EDTA as masking agent. In an identical mixture determine Cu with EDTA and murexide, Pd as dimethyl glyoximate, Co or Hg with EDTA and methyl thymol blue in hexamine, and Ni by potentiometric back titration of excess EDTA with Hg(II) in hexamine, pH 10 after masking Pt(IV) by precipitation with Tl(I) as Tl_2PtCl_6.

C. Determine the total of platinum and palladium in a ternary mixture of Pt(IV), Pd(II), and Cu(II) as in (A); in two identical mixtures determine Pd and, respectively, Cu as in (B).

RESULTS AND DISCUSSION

Table 1 lists the results of determining milligram amounts of platinum(IV) either alone or in presence of relatively large amounts of Fe(III), Co(II), Ni, Cu(II), Zn, Mn(II), and In(III). The results show high accuracy and precision in almost all cases. The above cations do not interfere, with exception of iron(III) which is successfully masked with EDTA. Titrations are attended with fairly large breaks ranging from 215 to 84 mV/ 0.1 ml of 0.045 to 0.005 *M* Hg(II) respectively.

Table 2 lists the results of determining microgram amounts of platinum(IV) down to 24 μg/25 ml, i.e., 1 ppm, either alone or in the presence of milligram amounts of Ca, Ba, and Sr, with fair accuracy and precision. The end point potential breaks ranged from 168 to 89 mV/0.1 ml of 0.005 and 0.001 *M* Hg(II), respectively.

In Table 3 are recorded the results of analysis of binary mixtures which show that the above procedures for such an analysis are simple, accurate and reliable.

Table 4 lists the results of analysis of ternary mixtures of Pt(IV),

Pd(II), and Cu(II), showing the reliability of the above procedure for such an analysis.

Addition of excess iodide to solutions of 0.025 down to 0.004 *M* hexachloroplatinic acid results in the formation of the reddish brown hexaiodoplatinate complex, which on heating precipitates black platinum tetraiodide:

$$PtCl_6^{2-} + 6I^- \rightarrow PtI_6^{2-} + 6Cl^-,$$
$$PtI_6^{2-} \rightleftarrows PtI_4 + 2I^-.$$

Though it was reported (*3*) that tetraiodide tends to dissolve in excess iodide, the addition of Hg(II) as back titrant for excess iodide will always shift the above equilibrium towards formation of the

TABLE 1

DETERMINATION OF MILLIGRAM AMOUNTS OF PLATINUM [a]

No.	Pt (mg)		Error (±%)	Titrant (mV/0.1 ml)
	Taken	Found		
1	29.279	29.050	0.78	142
2	19.519	19.289	1.20	166
3	12.200	12.245	0.37	162
4	9.759	9.738	0.22	181
5	7.320	7.310	0.14	200
6	4.880	4.880	0.00	182
7	3.637	3.611	0.73	110
8	2.829	2.829	0.00	149
9	2.425	2.425	0.00	184
10	1.617	1.601	0.97	128
11	1.414	1.408	0.42	119
12	1.212	1.210	0.17	105
13	1.010	1.003	0.68	130
14	9.759	9.738	0.22	194
15	7.320	7.369	0.67	84
16	4.880	4.843	0.76	109
17	4.880	4.842	0.78	210
18	7.320	7.282	0.52	200
19	9.759	9.664	0.97	215
20	4.850	4.826	0.49	168
21	4.042	4.032	0.25	115
22	3.233	3.232	0.03	106
23	2.425	2.428	0.12	106
24	1.617	1.605	0.73	84

[a] Nos. 1–6 and 14–19, 0.0967 *M* I^- × 0.045 *M* Hg(II); 7–13 and 20–24, 0.0116 *M* I^- × 0.00506 *M* Hg(II); 14, in presence of 58 mg of In(III); 15–16, in presence of 30 mg of Zn + 21 mg of Mn(II) + 14 mg of Fe(III) + 5 ml of 0.05 *M* EDTA; 17, in presence of 18 mg of Ni + 16 mg of Cu(II); 20–24, in presence of 20 mg of Co + 20 mg of Ni

TABLE 2

DETERMINATION OF MICROGRAM AMOUNTS OF PLATINUM [a]

No.	Pt (mg)		Error (±%)	Titrant (mV/0.1 ml)
	Taken	Found		
1	808.300	808.300	0.00	138
2	606.200	600.300	0.97	102
3	404.200	403.200	0.25	153
4	202.200	203.500	0.64	168
5	97.615	97.615	0.00	90
6	73.211	72.988	0.31	91
7	48.807	47.779	2.11	105
8	24.304	24.304	0.00	95
9	97.615	94.942	2.73	94
10	73.211	71.747	1.99	89
11	48.807	46.875	3.9	90
12	24.304	23.704	2.47	95

[a] Nos. 1–4 0.0116 *M* I^- × 0.00506 *M* Hg(II); 5–12, 0.00207 *M* I^- × 0.001 *M* Hg(II); 9–12, in presence of 10 mg of Ca + 34 mg of Ba + 44 mg of Sr.

TABLE 3

ANALYSIS OF BINARY MIXTURES

Pt (mg)			M (mg)	
Taken	Found	Error (±%)	Taken	Found
4.880	4.822	1.07	4.188 Fe	4.171
9.760	9.683	0.79	2.792 Fe	2.792
14.639	14.525	0.78	1.396 Fe	1.385
4.880	4.939	1.21	9.458 Cu	9.374
9.760	9.879	1.22	6.305 Cu	6.248
14.639	14.582	0.39	3.153 Cu	3.124
4.880	4.900	0.41	7.250 Co	7.232
9.760	9.683	0.79	4.833 Co	4.798
14.639	14.582	0.39	2.416 Co	2.416
14.639	14.639	0.00	27.609 Pd	27.465
29.279	29.167	0.38	18.406 Pd	18.310
43.918	43.918	0.00	9.203 Pd	9.155
4.880	4.880	0.00	25.167 Hg	24.976
9.760	9.683	0.79	20.101 Hg	20.262
12.199	12.104	0.78	30.152 Hg	30.152
4.880	4.850	0.62	9.097 Ni	8.980
7.320	7.239	1.11	10.916 Ni	10.811
12.199	12.173	0.21	13.645 Ni	13.557

TABLE 4

ANALYSIS OF TERNARY MIXTURES

Pt (mg)		Pd (mg)		Cu (mg)	
Taken	Found	Taken	Found	Taken	Found
14.639	14.629	27.609	27.609	28.320	27.842
29.279	29.281	18.406	18.355	18.880	19.315
43.918	43.915	9.203	9.219	9.440	9.434

tetraiodide, and hence the quantitative transformation of the hexachloroplatinic acid and subsequently the high accurracy of the present method. In harmony with the above, is the fact that addition of Hg(II) to cold hexaiodoplatinate solutions, excepting 5×10^{-4} *M*, immediately precipitates the black tetraiodide.

Despite the fact that the literature does not refer to the solubility of the tetraiodide, our quantitative determinations of iodide with Hg(II) in the presence of PtI_4 indicate that the solubility of the latter is equal to or even less than that of HgI_2 which was referred to by Kohlrausch and Rose (*14*) to amount to 0.0004 g/liter at 18°C corresponding to 8.8×10^{-7} mole/liter. Under such conditions the dissociation $PtI_4 \rightleftharpoons Pt^{4+} + 4I^-$ will never tend to be shifted towards right, in accordance with the possible reactions

$$PtI_4 + 4I^- + 2Hg^{2+} \rightarrow Pt^{4+} + 2HgI_4^{2-},$$

and

$$PtI_4 + 2I^- + 3Hg^{2+} \rightarrow Pt^{4+} + 3HgI_2.$$

The quantitative determination of 24 μg/25 ml of titrated solution shows that the present potentiometric method is comparable with colorimetric ones as applied to microamounts of platinum.

Among other platinum metals, palladium(II) interferes with the present method by forming the black insoluble diiodide, but it is possible to determine their total in presence of some other platinum metals.

It is established that EDTA is not likely to form a stable platinum complex. We exploited this fact in regard to the use of EDTA as an efficient masking agent for iron(III).

SUMMARY

The stoichiometric reaction between mercuric and iodide ions is the basis underlying a new simple, rapid, and reliable potentiometric method for platinum. By its aid platinum(IV) in the range of 29 mg down to 1 ppm, in pure solutions, in presence of a cluster of cations including, iron, cobalt, nickel, and palladium, or in binary and ternary mixtures was determined with fair accuracy and pre-

cision. The end point potential breaks ranged from 84 to 215 mV/0.1 ml of lower and higher concentrations of the titrant, respectively, which are large enough to permit accurate determination of the end points.

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An Ultramicromethod for the Determination of Conjugated and Total Bilirubin in Serum or Plasma

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INTRODUCTION

Most chemical methods used for the estimation of billirubin are based on reaction of the pigment with diazotized sulfanilic acid to yield colored azobilirubin compounds. Conjugated (direct-reacting) bilirubin couples directly with the reagent in an aqueous medium. Many substances promote the coupling of diazo reagent with free bilirubin: methanol (*1*) and caffeine sodium benzoate (*2*) are the most commonly used solubilizing agents, but methyl Cellosolve (*3*), urea (*4*), and other compounds have also been tried. Winsten and Cehelyk (*5*), in a recent investigation of the diazo method developed by Smith Kline Instrument Company, reported that dimethyl sulfoxide (DMSO) could also be used as the solvent in the assay for total bilirubin.

For the management of hemolytic disease of the newborn, it is ideal to have an accurate micromethod for bilirubin that is highly sensitive so that small volumes of sample can be used. Many microchemical methods that have been used for the estimation of bilirubin are scaled-down versions of standard colorimetric macroprocedures, and either lack sensitivity or require long reaction times for the formation of azobilirubin. These specific objections were overcome in the procedures described by Hogg and Meites (*6*) and by Michaelsson (*7*). In all diazo procedures, however, each assay is time consuming to perform because there are many microvolumetric transfers to make before actual testing is begun.

Microprocedures for the direct spectrophotometric determination of bilirubin have also been described (*8–11*). In these procedures, dilution of specimen is the only treatment required prior to measuring absorb-

ances of the solution. A method using the Unopette[1] System for diluting the sample was recently developed by the authors (*12*). A limitation of all spectrophotometric procedures is that conjugated bilirubin cannot be measured.

It has been well documented that the Unopette System, consisting of a self-filling capillary pipette and a reservoir containing premeasured reagent, is a simple device that can be used for making precise microanalyses (*13–17*). This study describes the adaptation of a standard diazo method for the determination of bilirubin using the Unopette System. The two components of diazo reagent are stored in stable form by placing sulfanilic acid in a reservoir, and dry sodium nitrite in the overflow chamber of a capillary pipette (Unopak[1]). Acidified sulfanilic acid is in aqueous solution for the determination of conjugated bilirubin, and in DMSO for the determination of total bilirubin. By inserting the Unopak into a reservoir and dissolving the sodium nitrite, diazo reagent is freshly prepared for each analysis.

METHODS AND MATERIALS

1. Blood Collection and Processing

Blood specimens in this study were collected by venipuncture from adult patients. An aliquot of each serum specimen was transferred to a stoppered conical plastic AutoAnalyzer[2] cup for the micro assay. Samples not analyzed the day that blood was collected were stored overnight at +4°C.

2. Preparation of Bilirubin Standards

A stock solution of pure bilirubin[3] was prepared with albumin as described by Shinowara (*18*). Additional known concentrations of bilirubin were made by preparing different mixtures of the stock standard and a diluent. Concentrations of the stock and of the diluted standard solutions were verified spectrophotometrically (*18*). The solutions were used in establishing standard curves for both the colorimetric procedures described below.

3. Macromethod for the Determination of Bilirubin

The classical Malloy and Evelyn method (*1*) requiring 2 ml of serum was used as the standard technique for the determination of bilirubin.

[1] Becton, Dickinson and Company, Rutherford, N. J.

[2] Technicon Instruments Corp., Chauncey, N. Y.

[3] Distillation Products Industries, Div. of Eastman Kodak Co., Rochester, N. Y. (No. 2101).

4. Unopette Micromethod for the Determination of Bilirubin

The Unopette method consists of the following materials:

(a) 1.0 ml of sulfanilic acid (SA) in a Unopette reservoir: This solution contains 5 g of sulfanilic acid and 15 ml of concentrated HCl diluted to 1 liter with distilled water. The pH of this solution is 1.2.

(b) 1.0 ml of dimethyl sulfoxide–sulfanilic acid (DMSO-SA) in a Unopette reservoir: This solution was prepared by mixing 700 ml of sulfanilic acid prepared as above (a) with 300 ml of dimethyl sulfoxide. The pH of this solution is 1.4.

(c) 100 μg of solid sodium nitrite in a Unopak overflow chamber.

Standard technique for adding serum to a reservoir. Serum is collected in a self-filling capillary pipette and excess serum is removed from the outside of the glass by carefully wiping it with gauze. The Unopette reservoir is squeezed slightly and the capillary holder is fitted into the reservoir. Rinsing the capillary is accomplished by alternately squeezing and releasing the sides of the reservoir. Serum and solution are mixed by inverting the reservoir several times.

Preparation of specimen reference. To a Unopette reservoir containing 1.0 ml of sulfanilic acid (SA), 25 μl of serum is added with a capillary pipette.

Procedure for conjugated bilirubin. After removing the paraffin closure from a Unopak, the 25-μl capillary pipette is filled with serum and is delivered into a reservoir containing 1.0 ml of sulfanilic acid (SA). Rinsing the overflow chamber 5–6 times dissolved all of the sodium nitrite. Exactly 10 minutes after addition of serum, absorbance of the

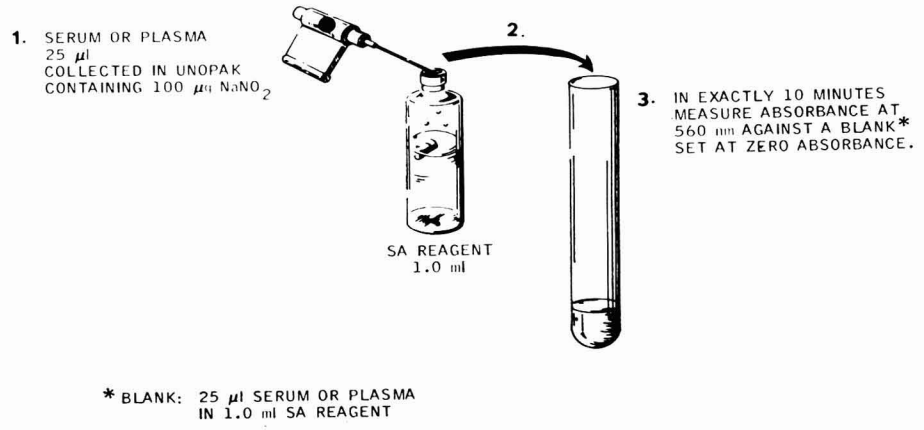

FIG. 1. Flow diagram showing Unopette method for determination of conjugated bilirubin.

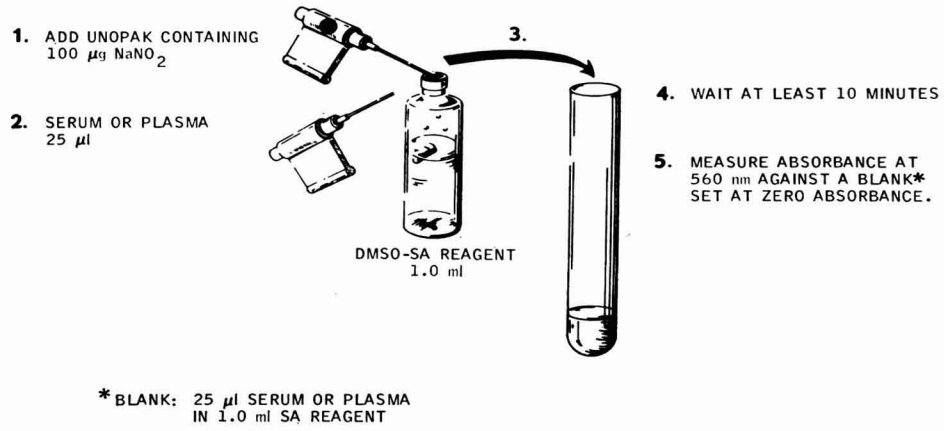

FIG. 2. Flow diagram showing Unopette method for determination of total bilirubin.

solution is measured at 560 nm, using the specimen reference as the optical reference (Fig. 1).

Procedure for total bilirubin. The sodium nitrite contained in a Unopak is dissolved in a reservoir containing 1.0 ml of DMSO-SA. It was noted that in rinsing the Unopak overflow chamber, a small area became wetted. When the wetted area disappeared (5–6 rinses), all of the sodium nitrite had been transferred to the solution in the reservoir. Using a clean capillary pipette, 25 μl of serum is then added to the mixture. The absorbance of this solution is also measured in 10 minutes at 560 nm, using the same specimen reference as the optical reference (Fig. 2).

$$\text{Bilirubin (mg/100 ml)} = \frac{A_u}{A_s(\text{DMSO})} \times C_s,$$

where A_u = absorbance of unknown[2]
A_s = absorbance of standard[2] and
C_s = concentration of bilirubin in the standard (mg/100 ml).

5. Spectrophotometry

A Coleman Jr., Model 6D,[4] was used throughout this study. For the macromethod, cuvettes with a 19-mm light path were used. For the Unopette procedure, 10-mm cuvettes were used with an adapter that had been modified to accommodate 1-ml minimum volume (*12*). The instrument was calibrated daily with a didymium glass standard.

[4] Coleman Instruments Div., The Perkin-Elmer Corp., Maywood, Ill.

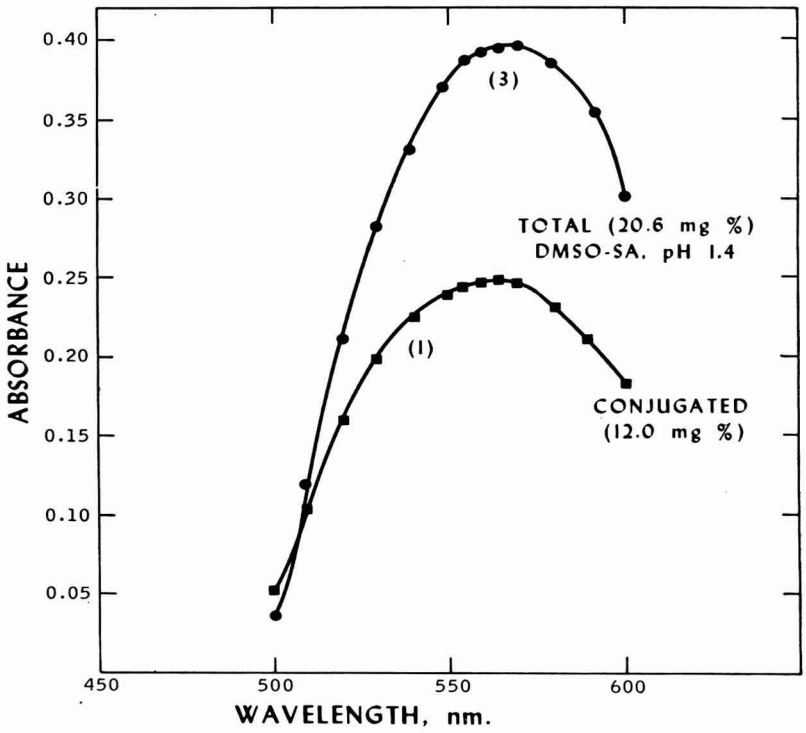

FIG. 3. Wave length–absorption curves of reactions for conjugated and total bilirubin.

EXPERIMENTAL METHODS

Wavelength-absorption studies were done on the conjugated and total bilirubin reactions. The results, shown in Fig. 3, reveal that maximum absorption for both azo compounds occurred at 560 nm.

Absorbances of the conjugated bilirubin reaction were measured at various time intervals, beginning at 1.5 minutes and extending up to 30 minutes. In a specimen containing 12.8 mg of conjugated bilirubin/100 ml, results (Fig. 4) showed that the reaction was essentially complete in 10 minutes, but that color slowly continued to develop throughout the entire time interval studied. Because absorbances found at 10 and 15 minutes differed by only 3%, all subsequent measurements for conjugated bilirubin were made at the 10-minute interval. The rate of development of color in the total bilirubin reaction was studied using Pediatric Versatol*.[5] The results obtained are presented in Fig. 4. It

[5] Warner-Chilcott, Morris Plains, N. J.

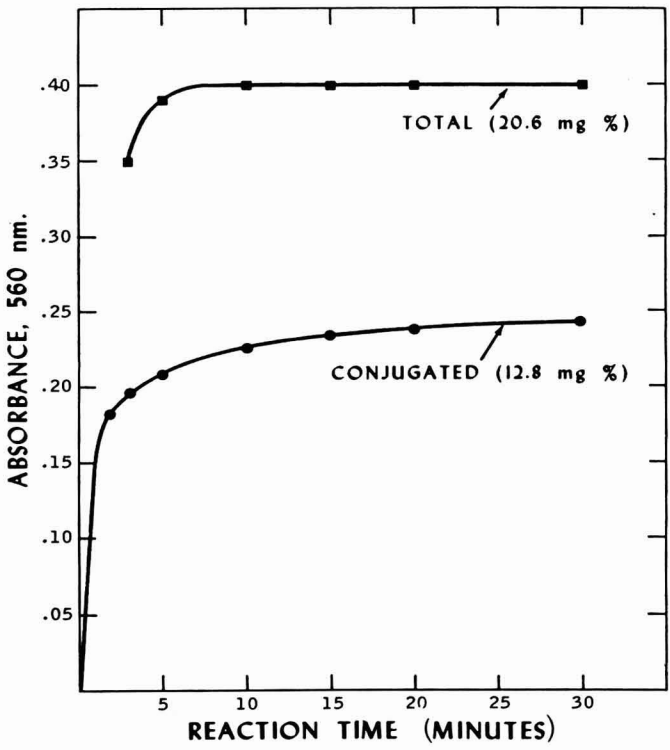

FIG. 4. Development of color in conjugated and total bilirubin reaction.

was noted that color developed more slowly with Pediatric Versatol than with human adult serum specimens containing comparable amounts of bilirubin. Nevertheless, even with Pediatric Versatol, absorbances were maximal at 10 minutes and the color was stable for at least 60 minutes. In the total bilirubin reaction it was found that reproducible results could be obtained only when diazotized sulfanilic acid was present prior to the addition of serum. In an experiment done to determine the stability of diazotized sulfanilic acid in DMSO, sodium nitrite was added to a number of reservoirs. Serum was added to one immediately after preparation of the diazo reagent, and to others at 5-minute intervals up to 60 minutes. Absorbances of all solutions were read 10 minutes after the addition of serum. Absorbances at all intervals agreed within ± 0.009 indicating that it is possible to prepare diazo reagent in Unopette reservoirs in advance of its use.

Assays for total bilirubin were done using standards prepared from pure bilirubin. The concentration range studied was from 10 to 50 mg/100 ml. Results plotted in Fig. 5 show that the reaction is linear over the entire concentration range. Absorptivities, *a*, were calculated from

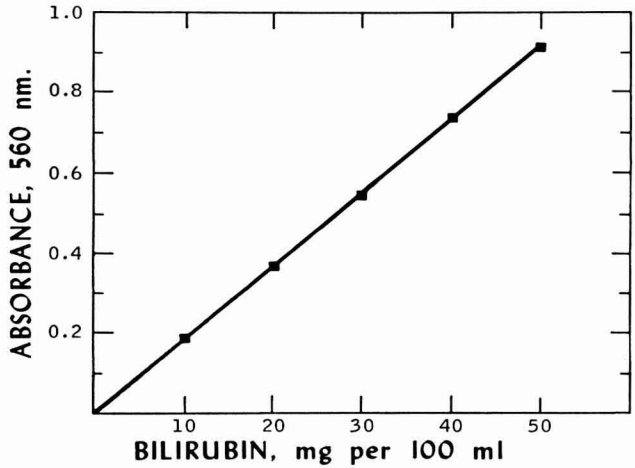

FIG. 5. Standardization curve prepared from the colorimetric reaction of purified bilirubin solutions using a Coleman, Jr., Model 6D spectrophotometer, cells with a 10-mm light path, and a specimen reference as the optical reference.

the equation: $a = A/C$, where A is absorbance for the reaction, and C is the concentration of bilirubin (mg/100 ml). The mean a, $\pm$ SD, found in analyses of 12 standard solutions was 18.31, $\pm$ 0.65 $\times$ 10^{-3}. The coefficient of variations for this series was 3.5%. Absorptivities were also computed from results obtained in 18 consecutive analyses when Pediatric Versatol had been used as the standard. The mean a was 18.86 $\times$ 10^{-3}, and agrees closely with the value established using the laboratory standards. Analytical data comparing the two standard solutions are shown in Table 1.

An evaluation of the effect of hemoglobin on the bilirubin reaction was done using a concentation of 420 mg/100 ml of heme pigment. Purified hemoglobin solution was added to different bilirubin standards. The results of the analyses are presented in Table 2.

Using human adult serum specimens, conjugated and total bilirubin

TABLE 1

COMPARISON OF ABSORPTIVITIES USING TWO TYPES OF BILIRUBIN STANDARDS

Bilirubin source	Conc range (mg/100 ml)	Absorptivity, a [a] ($\times$ 10^{-3})			
		n	Mean	$\pm$SD	CV [b]
Pure bilirubin albumin	10–50	12	18.31	0.65	3.5
Pediatric Versatol	20.6	18	18.86	0.77	4.0

[a] $a = A/C$; A = absorbance at 560 nm; C = concentration of bilirubin (mg/100 ml).
[b] Coefficient of variation = (SD/mean) $\times$ 100.

TABLE 2

EFFECT OF HEMOGLOBIN (420 mg/100 ml) ON BILIRUBIN DETERMINATION

Total bilirubin (mg/100 ml)	
Known	Found
40.0	37.4
30.0	28.0
20.0	18.0
10.0	9.0

concentrations were simultaneously determined using the standard macromethod and the proposed Unopette micromethod. A comparison of results obtained with the two methods on 44 specimens is presented in Table 3. Mean values for conjugated bilirubin were: 8.36 mg/100 ml with the macromethod; and 7.44 mg/100 ml with the Unopette method. The correlation coefficient, r, for values obtained with the two procedures was +0.979. Mean total bilirubin concentations on these specimens were: 12.25 mg/100 ml with the macromethod; and 12.13 mg/100 ml with the Unopette method. In this comparison, $r = 0.997$. Using the microprocedure, duplicate analyses for total bilirubin were done on all specimens. The mean difference between paired values was 0.18 mg/100 ml. In only two specimens did results differ by more than 0.5 mg/100 ml, and the concentration of bilirubin in each sample was in excess of 21 mg/100 ml.

DISCUSSION

In comparing concentrations found using the chemical microprocedure developed for the Unopette System with those of a standard macroprocedure, correlation between results was excellent for both conjugated ($r = +0.979$) and total ($r = +0.997$) bilirubin. Duplicate analyses were done using the microtechnique and the mean difference between pairs was 0.2 mg/100 ml. When 18 consecutive analyses were performed on a simple sample, the coefficient of variation was only 4%. These findings indicate that bilirubin concentrations obtained using the Unopette procedure are accurate and highly reproducible. Results presented in Fig. 5 and in Table 3 show that the colorimetric reaction is linear between 2 and 50 mg bilirubin/100 ml. The extensive concentration range that can be analyzed using 25 μl of undiluted specimen eliminates many potential sources of error.

It is possible to proportionally reduce the volumes of all reagents and of the specimen and adapt a micromethod directly from the classical Malloy and Evelyn (*1*) procedure. The modification has all of the dis-

TABLE 3

COMPARISON OF BILIRUBIN ANALYSES ON PATIENT SPECIMENS USING THE UNOPETTE METHOD AND THE MALLOY AND EVELYN MACROMETHOD

	Bilirubin (mg/100 ml)					Bilirubin (mg/100 ml)			
	Conjugated		Total			Conjugated		Total	
Spec. no.	Unopette method	Macromethod	Unopette method	Macromethod	Spec. no.	Unopette method	Macromethod	Unopette method	Macromethod
1	1.1	0.7	1.5	1.4	23	6.3	7.4	9.9	10.2
2	1.9	1.7	2.7	2.6	24	6.4	7.6	9.9	10.3
3	1.7	1.1	2.8	2.6	25	3.9	7.6	9.0	10.6
4	1.5	2.0	2.9	2.8	26	8.2	7.2	10.9	10.6
5	2.2	1.7	3.6	2.9	27	2.2	1.8	11.7	11.0
6	2.4	0.8	3.7	3.0	28	6.1	8.0	9.3	11.6
7	2.4	3.1	3.9	3.7	29	8.4	9.4	11.4	12.2
8	2.6	2.4	3.9	3.8	30	8.2	9.6	13.6	13.6
9	3.0	2.4	4.3	4.2	31	10.5	10.4	13.4	15.0
10	3.1	3.4	5.3	5.2	32	11.2	10.9	16.4	16.1
11	3.7	3.9	6.5	5.7	33	11.6	11.7	18.0	16.9
12	3.3	4.7	5.8	6.1	34	12.6	12.0	18.0	17.4
13	4.1	2.5	6.8	6.4	35	11.0	12.8	18.0	17.8
14	4.6	4.4	7.3	6.4	36	13.6	13.6	20.1	19.0
15	4.1	4.6	7.6	7.7	37	10.6	13.4	19.4	19.2
16	5.0	5.8	7.2	7.7	38	12.9	15.8	20.4	19.6
17	3.8	4.1	7.2	7.8	39	13.1	14.5	20.6	20.7
18	2.7	2.8	8.5	8.2	40	14.0	12.0	21.5	20.8
19	5.4	4.2	9.9	8.2	41	13.0	15.2	20.6	22.0
20	4.9	6.3	7.4	8.4	42	11.1	12.6	21.5	23.6
21	6.4	6.3	9.0	8.8	43	14.4	15.2	23.9	24.8
22	6.4	6.0	9.0	9.4	44	42.0	60.0	69.6	73.0

TABLE 4

A SUMMARY OF ABSORBANCE VALUES FOR A STANDARD SOLUTION OF BILIRUBIN REPORTED FOR VARIOUS MICROMETHODS

Procedure	Serum (μl) used for:			Reaction time (min)	Total vol, reaction mixture (ml)	Absorbance (20 mg/100 ml of bilirubin)
	Specimen reference	Conjugated bilirubin	Total bilirubin			
Unopette–chemical	25	25	25	10	1.0	0.4
Unopette–spectrophotometric (*12*)	None	None	50	None	1.0	0.5
Michaelsson (*7*)	50	50	50	12	2.0	0.5
Hogg and Meites (*6*)	50	50	50	10	1.0	0.5
Malloy and Evelyn[a] (*1*)	50	50	50	30	2.0	0.2

[a] Standard procedure but using 0.1 vol of reagents and of specimen.

advantages of the original procedure in that it requires 30 minutes for the formation of azobilirubin and is not accurate for measuring low levels of pigment. Hogg and Meites (*6*) were able to increase the sensitivity of the reaction and to shorten the reaction time when they modified concentrations of the components of diazo reagent. The Commission on Continuing Education (*19*) has recommended use of the bilirubin method of Jendrassik and Grof (*2*) because of its reported sensitivity at low bilirubin concentrations. Michaelsson (*7*) developed a micromethod from this procedure. Table 4 is a summary comparing features of the above-mentioned chemical methods, the authors' spectrophotometric method, and the Unopette chemical procedure described here. As shown, the present chemical method uses one half the amount of serum yet yields almost the same absorbance values for total bilirubin as were found by Hogg and Meites or by Michaelsson.

It has been reported that hemoglobin interferes with diazotization of bilirubin (*6, 11, 20*). Most investigators who have studied this effect used up to 500 mg of hemoglobin/100 ml of serum. Under conditions of extreme hemolysis, bilirubin values obtained with the proposed Unopette procedure were depressed by only 10%.

From evaluation of time reaction curves, such as the one shown in Fig. 4, it is evident that a wide range of values for conjugated bilirubin can be obtained solely by measuring color at different time intervals. Should one use the 1-minute interval to measure "prompt direct-reacting" bilirubin (*21*), a 15–30-minute interval to measure a maximum reaction (*1*), or some intermediate interval (*22*)? Henry (*23*) reviews this problem and points out that of necessity, the choice must be based on historical and comparative factors since there still is no conjugated bilirubin standard available to use in establishing optimum conditions. In the reaction for conjugated bilirubin using the Unopette procedure, absorbances were measured at an arbitrarily designated 10-minute interval, and the results obtained were in close agreement with those found using the reference macroprocedure.

The Unopette procedure described here for the chemical assay of bilirubin is unique. Its simplicity is in strong contrast to the multiple operations that are required for all other chemical methods for bilirubin. Diazo reagent is formed by dissolving preweighed dry sodium nitrite in a premeasured volume of sulfanilic acid–solvent solution. The analysis for conjugated bilirubin is done in one step: formation of diazotized sulfanilic acid and of azobilirubin begin when sodium nitrite and serum are added simultaneously. An additional step is required in the assay for total bilirubin because diazotized sulfanilic acid must be prepared prior to the addition of serum. It is a distinct advantage that when analy-

ses are done on blood specimens from newborn infants, the total time required for setting up and completing a test is less than 12 minutes. Because diazo reagent is stable in DMSO for at least 1 hour, the procedure can also be utilized for routine work where many specimens are analyzed at the same time.

SUMMARY

An ultramicromethod for the determination of conjugated and total bilirubin has been described. The colorimetric reaction for bilirubin is linear from 2 to 50 mg/100 ml using a standard 25-μl aliquot of undiluted sample. Diazotization of bilirubin is not significantly depressed by excessive concentrations of hemoglobin. The test can be set up and the analysis completed in less than 12 minutes. All of the reagents are premeasured and stable at room temperature for at least 2 years.

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Relationship Between Sensitivity and Precision in Atomic-Absorption Flame Photometry

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Since its inception, atomic-absorption spectroscopy (AAS) has been acclaimed for its high sensitivity. In fact, considerable time and effort has been devoted to this parameter whereby continued improvement has been made in both *percentual sensitivity,* related to size of the output signal, and *fluctutational sensitivity,* related to noise level. Preoccupation with these two factors is not surprising in view of the widespread popularity of AAS for trace analysis. However, this has overshadowed another important factor, namely the correlation of sensitivity with precision. Greater attention is now being given to this area which is the main subject of the discussion here.

Importance of the sensitivity–precision correlation is amplified by the fact that sensitivity of a given instrumental system usually can be changed at will over a wide range. Thus, a better understanding of the related variations in precision will aid in the selection of conditions best suited to a given analysis. Although the sample itself, as well as its preparation, also contributes to the actual precision attained, only the instrumental factors are considered here.

EXPERIMENTAL METHODS

All numerical data given in this communication were obtained with a Beckman Model 979 atomic-absorption spectrophotometer equipped with laminar flow burner (air–acetylene flame) and 10-inch potentiometer recorder. All solutions used were aqueous solutions. Calculations have been carried out by the use of a computer according to techniques previously described (*1*).

RESULTS AND DISCUSSION

Concepts of standard deviation, relative standard deviation, range or spread, confidence levels, etc. commonly used in other analytical methods can be used in the treatment and interpretation of atomic-

absorption data. No new precision terminology is proposed or used in this paper. However, a brief summary of several relationships seems useful for our discussions here.

Readings in atomic-absorption spectroscopy are typically observed as percentage absorption, %ABSN, which can be converted into absorbance, A, by electronic devices or by means of tables or simple calculations. Analytical working curves correlate absorbance with analyte concentration.

Since the primary signals are percentage of absorption, they can be used for any first approach to precision studies. Values of standard deviation, σ, as well as the RSD values (relative standard deviation or coefficient of variation) calculated should read in %ABSN units. However, %ABSN and absorbance are not related by a linear function, so these precision data cannot be directly related to analyte concentration. As a matter of fact, if values obtained from %ABSN and from A are compared, those for %ABSN are more favorable but less realistic. This is shown in Table 1. However, if σ values are obtained from absorbance values and for linear portions of analytical working curves, it can be established that

$$\sigma_A = m\,\sigma_C, \tag{1}$$

where σ_A is the standard deviation calculated from absorbance values, σ_C is the standard deviation calculated from concentration values, and m is the slope of the analytical working curve. This is based upon the relationship,

$$A = m\,C, \tag{2}$$

where A is absorbance, C is analyte concentration in the test solution, and m again is the slope of the analytical working curve. The correlation is not valid for nonlinear systems where m changes at different values of C.

TABLE 1

ABSORBANCE AND PERCENTAGE ABSORPTION VALUES

Analyte and wavelength (Å)	Conc (ppm)	No. of readings	Av	σ	RSD
Mg 2852	0.1	40	0.3485	0.0052	1.5
		40	55.2	0.53	0.97
Hg 2537	100.	10	0.3010	0.0044	1.5
		10	50.0	0.51	1.0

If the condition in Eq. 1 is met, RSD values calculated from sets of absorbance values can be directly referred to the corresponding concentrations.
Actually,

$$\mathrm{RSD} = \frac{\sigma}{A}\,100. \tag{3}$$

Then, according to Eqs. 1 and 2, the RSD_A, the RSD for absorbance, should be

$$\mathrm{RSD}_A = \frac{\sigma_A}{A}\,100 = \frac{\sigma_C m}{C m}\,100 = \frac{\sigma_C}{C}\,100, \tag{4}$$

or, in other words,

$$\mathrm{RSD}_A = \mathrm{RSD}_C, \tag{5}$$

where RSD_C is the RSD for concentrations.

Correlation of Precision with Signal Size

A larger signal size for a given analyte concentration is synonymous with higher slope, and, therefore, of higher sensitivity, expressed as percentual sensitivity. Percentual sensitivity can be measured in terms of the corresponding percentual concentration limits (PCL) in ppm, i.e., ppm of analyte necessary to obtain under given experimental conditions a signal of 1% ABSN (0.0044 absorbance units).

Large signals can be obtained with high analyte concentrations or also with low analyte concentration, but operating at high sensitivity conditions (hot spray chamber, long absorption path, for instance, long slot burners and/or multiple light passes through the flame).

At different signal levels a given difference in %ABSN readings, expressed as divisions of the %ABSN scale, have significant diverse values when calculated in absorbance values; see Table 2.

For simplicity several signals of different magnitude have been chosen for a solution of 1 ppm of analyte. Table 3 gives the corresponding A values—in this case $A = m$, as $C = 1$ ppm—and the calculated percentual concentration limits (PCL) in ppm. RSD_A has been calculated in each case for σ values of 0.1 to 3 div. A zone has been selected in which RSD_A is less than 5% (italicized values in Table 3).

In Table 3 it is clearly shown that better RSD_A values can be obtained between 25% and 75–90% signals. This is similar to the best region considered for better accuracy in terms of readability capabilities. For reference, observe the column corresponding to $\sigma = 1$ div in Table 3.

TABLE 2

DIFFERENCES IN ABSORBANCE UNITS

% Absorption level (scale divisions) (div):	% Absorption differences (scale divisions)				
	0.1	0.5	1	2	3
1	0.0004	0.0022	0.0044	0.0088	0.0132
5	0.0005	0.0023	0.0046	0.0092	0.0138
10	0.0005	0.0024	0.0048	0.0096	0.0145
25	0.0006	0.0028	0.0057	0.0106	0.0175
50	0.0009	0.0044	0.0087	0.0174	0.0264
75	0.0018	0.0087	0.0175	0.0348	0.0533
90	0.0045	0.0218	0.0437	0.0874	0.1384

Values of RSD_{ABSN} follow a different pattern as shown in Fig. 1, which reflects the more favorable appearance mentioned when including Table 1.

A general view can be obtained by representing the calculated values in a three-dimension graph. Only a region of the limiting theoretical surface enters under the level corresponding to $RSD_A = 5\%$; see Fig. 2.

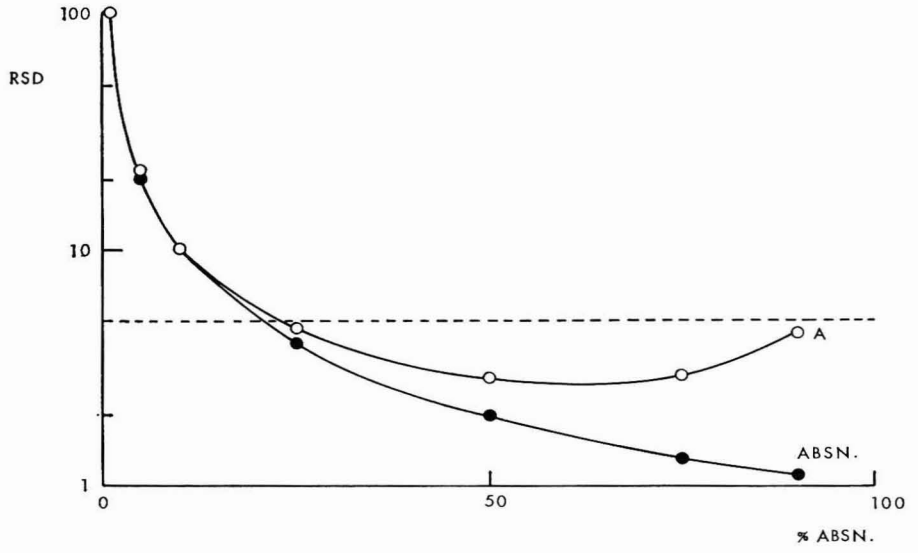

FIG. 1. Variation of the relative standard deviation (RSD) as a function of the reading in % absorption scale units (%ABSN scale). Values have been calculated for $\sigma = 1$ scale division (1% absorption). The broken line shows the level RSD = 5%. (○; A curve) indicate the variation of RSD values calculated in absorbance units, and (●; ABSN curve) indicate the variation of the RSD values calculated (% absorption units).

TABLE 3

RELATIVE STANDARD DEVIATION VALUES SIGNALS FOR 1 ppm ANALYTE

Signals in % absorption	A (or m)	PCL (ppm)	σ Values; % absorption (scale units)				
			(div): 0.1	0.5	1	2	3
1	0.0044	1.0	9.1	50.	100.	200.	300.
5	0.0223	0.20	*2.2*	10.	22.	41.	62.
10	0.0458	0.096	*1.1*	*4.8*	10.	21.	32.
25	0.1249	0.035	*0.48*	*2.2*	*4.6*	8.5	14.
50	0.3010	0.015	*0.30*	*1.5*	*2.9*	5.8	8.8
75	0.6021	0.0073	*0.30*	*1.4*	*2.9*	5.8	8.8
90	1.0000	0.0044	*0.45*	*2.2*	*4.4*	8.7	14.

The different signal sizes used here to illustrate precision data have been obtained by the following ways: (a) by using different analyte concentrations, and (b) by varying the instrumental operating conditions (by varying percentual sensitivity).

In Table 4, results on copper are summarized which correspond to 1, 2, and 5 ppm Cu. Results for 5 ppm are also included when using 22° burner rotation. These results show how RSD_A slightly decreases with concentration, and therefore with signal size. When a 5-ppm solution is read at lower percentual sensitivity (burner rotated), and the signal becomes smaller than that of 5 ppm, RSD_A for this condition becomes larger. In this particular case a higher noise and flame boundary zone instability is associated as a consequence of the angle formed by the optical beam and the flame.

Results for a 1-ppm solution with and without scale expansion are represented in Table 5. Actually, the use of scale expansion does not

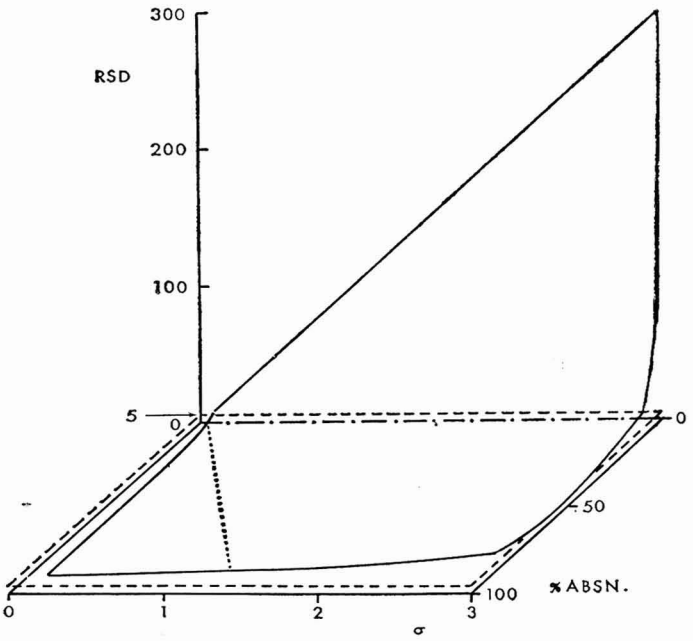

FIG. 2. Three-dimensional representation of the variation of the relative standard deviation (RSD) as a function of the variation of readings in a % absorption scale (0 to 100%) and of σ values (0 to 3) expressed in % absorption units. The surface extends from 1 to 90 divisions (% ABSN scale) and from 0.1 to 3 divisions (σ scale). Only a small part of the surface, limited at the lower left side by a broken line, goes under the level of 5% RSD. Also a small zone of the surface goes under the level of 5% RSD at midscale readings.

TABLE 4

COPPER VALUES: Cu 3248 Å

Conc (ppm)	No. of readings	Av (absorbance)	σ	RSD
1	20	0.1272	0.0027	2.1
2	20	0.2581	0.0025	0.96
5	20	0.6042	0.0097	1.6
5[a]	20	0.2832	0.0067	2.4

[a] Burner rotated 22°.

increase percentual sensitivity. It only increases *relative signal size,* and, therefore, *readability.* However, results in Table 5 demonstrate that moderate scale expansion does help to obtain signals which can be read with better readability, which in turn results in better precision. By all means it is desirable to avoid the use of too small signals, whenever it is possible.

When operating with signals of small size, two phenomena can distort precision data: (a) fluctuations of the signal due to residual effects, such as memory or small contaminations, and (b) small fluctuations of the zero adjustment (drifts of the zero position due to electronics or to source lamp). High scale expansion will not cure this situation. On the contrary, residual effects and drift is actually exaggerated on the recorder charts.

Correlation of Precision with Noise Level

A lower noise level for a given analyte concentration is synonymous with higher sensitivity, expressed as fluctuational sensitivity. Fluctuational sensitivity can be measured in terms of the corresponding fluctuational concentration limits (FCL) in ppm, i.e., ppm of analyte which produce, under given experimental conditions, a signal equal to the peak-to-peak noise at the 0% ABSN level (blank zeroed), which correspond to about 4 σ. The σ value can be calculated for fluctuations observed

TABLE 5

COPPER VALUES: Cu 3248 Å

Conc (ppm)	Scale expansion	Time constant (sec)	No. of readings	Av (absorbance)	σ	RSD
1	1×	2	20	0.1197	0.0030	2.5
1	2.5×	2	20	0.1148	0.0013	1.1
1	5×	2	20	0.1139	0.0009	0.78

while aspirating the blank solution zeroed, and measured at the chosen set of experimental conditions.

An easy way of decreasing noise level is by instrumental noise suppression devices. In spite of the fact that experimental values can show some improvement of precision by increasing time constant, an extreme time constant leads to other problems such as: (a) excessive sample consumption, (b) accumulation of deposits after long sample aspirations, and (c) lengthening of the operator's working time.

CONCLUSIONS

In atomic-absorption flame photometry precision achievable is related to sensitivity, both percentual and fluctuational. If sensitivity is increased in both directions—larger signal size and smaller noise level—precision is improved, as signal-to-noise ratio is maximized.

Precision also improves if a *moderate* scale expansion is used to achieve better readability on the chart when dealing with small original signals. *Moderate* noise suppression can also result in benefit of precision as signals, because of less noise and more stability.

Precision studies should discriminate between contribution by the instrumental system alone and contribution by the sample preparation preliminary steps. Thus repeatability and reproducibility tests should be performed with simple analyte solutions of known concentration and composition to get a preliminary idea of the analytical instrumental system under a set of working conditions, thus giving the opportunity of varying the working conditions until getting a compromise between sensitivity and precision desired. Precision studies of the whole analytical process can then be done involving both sample preparation and instrumental measurement.

SUMMARY

Precision results, standard deviation or relative standard deviation, can be expressed in terms of either reading or analyte concentrations. The latter is perhaps more useful when expressing analytical data, but both are based on instrumental readings obtained with the measuring instrument. An important consideration is the quality of the signal itself (signal size and noise level). Signal size for a given concentration is directly correlated with the corresponding percentual sensitivity obtained under the chosen working conditions. Signal noise, also for a given concentration, is in turn directly correlated with the values known as fluctuational sensitivities.

Three main cases are considered here: (a) *small signal size,* (b) *medium signal size,* and (c) *large signal size.* At low signal size two effects have been observed which can impair precision: (a) fluctuations of signal size due to residual effects, memory and similar forces; and (b) fluctuations of zero adjustment.

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Chemico-analytical Selectivity and Sensitivity in Atomic-Absorption Flame Photometry

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INTRODUCTION

Any quantitative analytical method should have a series of chemico-analytical characteristics: selectivity, sensitivity, accuracy, precision, reliability, and versatility. These are considered as essential and provide a basis for deciding if a method can or cannot be applied in a given analytical problem.

In this paper selectivity and sensitivity aspects are principally discussed. These are concepts which may be applied either to qualitative or to quantitative analytical methods. This is the reason why their definitions involve a double meaning, as both qualitative identification and quantitative determination should be estimated in terms of their selectivity and sensitivity.

Selectivity is a chemico-analytical characteristic which represents the capacity of an analytical *reaction* or *instrumental operation* to identify or to determine an analyte in the presence of other components of the tested sample—concomitants. Taking this definition into account, it is possible to distinguish between qualitative and quantitative selectivity.

A reaction or operation is considered as *general* when it produces the same type of reaction product or response with a number of analytes with a given technique. It is considered *selective* when it produces a reaction product or response with only a few analytes with a given technique. Finally, it is considered *specific* when it produces a reaction product or a response with *only one analyte* with a given technique.

When dealing with a given analyte, a careful choice of technique (in this case, of instrumental conditions) can convert a *selective* reaction or operation into a *specific* reaction or operation. Specific reactions and operations are much more reliable than selective or general ones. The reaction product or response obtained will be univocally correlated to the analyte searched.

In general terms chemists refer to reagents and instruments as being

selective—or even specific—when they know the reactions and operations have the potentiality of being selective or specific.

Sensitivity is a chemico-analytical characteristic which represents the capacity of an analytical *reaction* or *instrumental operation* to identify or to determine very small amounts or concentration of analyte. Qualitative sensitivity refers to identification reactions or operations; quantitative sensitivity refers to determination reactions or operations. Absolute sensitivity data are calculated when amounts of analyte are considered; relative sensitivity data are calculated when dealing with analyte concentrations.

It should be noted that reactions and instrumental operations are mentioned in the definition of sensitivity. It is meaningless to speak about sensitivity of an analyte or sensitivity or a reagent[1] or instrument. To establish and to measure sensitivity the whole reaction should be considered (analyte, instrument, medium, and technique).

WAYS TO MEASURE SENSITIVITY AND SELECTIVITY

Sensitivity can be very easily quantified. The smallest amount of concentrations of analyte that may be identified or measured are measured in units such as g or in g/ml, respectively. These values are called *limits*: absolute and relative limits:

Qualitative limits	absolute qualitative limit or absolute identification limit[2]
	relative qualitative limit or relative identification limit[2]
Quantitative limits	absolute quantitative limit or absolute determination limit
	relative quantitative limit or relative determination limit.

Sensitivity, which is a magnitude of inverse value compared with the corresponding limits, should be measured g^{-1} or in ml/g, if absolute or relative, respectively. Four different sensitivities can be considered, each one corresponding to the above-mentioned limits.

[1] For example, it is not adequate to say simply that copper is a very sensitive analyte. It will show good sensitivity when precipitated with sulfide ion or ferrocyanide ion but has no practical sensitivity when treated with chloride ion. In turn, it is not adequate to say that chloride ion is a sensitive reagent. It shows good sensitivity with silver as analyte, but poor sensitivity with lead. The same criterion can be applied when speaking about instrumental analysis.

Reference sensitivity values are usually obtained in ideal conditions, i.e., in absence of concomitants which may distort analytical data because of any kind of physical, chemical, and/or instrumental interferences. Here sensitivity values and the corresponding limits are calculated in highly selective conditions: selective identification and selective determination. This is the type of sensitivity values published in the literature when a new element is studied or when the performance of an instrument is tested and reported.

In practice, analytes frequently are encountered in samples accompanied by concomitants with a different nature which can modify in some extent the sensitivity achieved in ideal conditions. This leads to new sensitivity values which in fact are *realistic* sensitivity values and *realistic* limits. These new values show the actual sensitivity level which may be acheved when facing a given practical analytical problem.

As the presence of concomitants usually modifies the sensitivity achievable by a reaction or an instrumental operation, this modification represents the loss of selectivity due to sample composition (operation medium).

It is necessary to keep in mind that loss of selectivity can also be produced by any of the other factors involved in the reaction or in the operation: (a) reagent or instrument, and (b) technique (experimental conditions). As generally the reagent or instrument will be the same one, selectivity losses will depend on the experimental conditions, for a given operation medium.

If loss of selectivity produces changes in sensitivity, selectivity can then be measured by ratioing sensitivities or the corresponding limits, and by referring the ratio to percentage. Then, 100% selectivity, initial selectivity, will correspond to ideal conditions (no concomitants, optimized experimental conditions). Any loss of selectivity will be then represented by values smaller than 100%.

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[2] In some publications they are simply called *detection limits*. This criterion deserves some discussion: (i) They need some adjective to clear the term, for instance, they should be called at least *detection concentration limit,* if the limit is measured in concentration units. In other cases they might be called *detection dilution limits* or *detection dilution ratio limits*. (ii) Actually chemists, when working as analytical chemists, do not detect; they identify. They are not satisfied just detecting the formation of a fine precipitate or the appearance of a small signal; they look for a fine precipitate of a given color or a small signal at a given wavelength. This is not detection. This is identification. The analyte is more than detected; it is identified by means of a property univocally correlated to its nature and chemical and/or physical behavior. In other words, an identification is a *selective* detection.

In the special case that sensitivity is enhanced by the presence of concomitants the percentage over 100% can be calculated and this value can be taken as representative of the variation of selectivity.

With this way of measuring selectivity, losses of selectivity can be represented by log SL (logarithm of selectivity loss), which may be calculated as the absolute difference between the logarithms of the initial sensitivity (S_1) and the modified sensitivity (S_2). In this way log SL will be a positive number, $+n$:

$$+n = \log SL = \left| \log S_1 - \log S_2 \right| .$$

Some examples are reported in Table 1.

EXPERIMENTAL PART

Results reported in this paper were obtained at the author's laboratory with the help of Beckman Model 979 atomic-absorption spectrophotometer equipped with a potentiometer recorder. In some parts of the experimental work the following accessories were used: transmittance/absorbance display, linear concentration display, automatic sample sequencer, printer and control unit, and scale expander. With the instrument two types of burners were used: Mainly a laminar flow burner (air–acetylene flame and nitrous oxide–acetylene flame), and for some tests a turbulent flow burner (air–hydrogen flame). The instrument has been prepared for maximum performance for each analyte studied (by studying lamp current, support gas and fuel gas pressures, burner elevation, slit width and gain).

Solutions were aqueous, except when organic solvents were needed.

Most of the calculations have been performed by computer techniques (*2, 3, 7*). For some graphical representations a scope was used in connection with the computer, and the final displayed graphs were reproduced by means of an automatic plotter.

TABLE 1

SELECTIVITY LOSS

Limits; PCL (ppm) (ppm/1% ABSN.)	Sensitivities; Percentual sensitivities (1/PCL)	Selectivity losses; $+n$ (log SL)
2	0.5 (S_1)	0.7
10	0.1 (S_2)	
0.01	100 (S_1)	0.6
0.04	25 (S_2)	

SENSITIVITY IN ATOMIC-ABSORPTION FLAME PHOTOMETRY

Classical concepts of sensitivity can be fully applied to the case of atomic absorption flame photometry. The classical concepts used for many years in analytical chemistry do not change at all and do not need any drastic modification when applied to this instrumental method.

When considering the instrumental analytical response of the method, it is convenient to take advantage of the relative concepts of sensitivity, this is, the relationship between measurable response of the instrument (signal) and concentrations of the analyte. Thus, it is more useful to refer experimental data to concentrations as the method mainly deals with solutions that contain analytes with concentrations varying inside of a given concentration range.

In order to standardize the presentation of experimental values, performance tests for each analyte are scheduled in such a way that responses are measured by feeding the instrument with solutions of low analyte concentration. Then, concentration limits are calculated which correspond to concentrations necessary to obtain a previously established signal size.

For convenience, two different signal sizes have been established:

Signal equal to 1% absorption (1 div of the absorption scale with a total of 100 div, which corresponds to an absorbance of 0.0044).

Signal equal to peak-to-peak noise (noise fluctuations at 0% absorption level).

These signal sizes lead to establishing two concentration limits:

Percentual concentration limit and *fluctuational concentration limit,*

both measured in ppm of analyte and representing, respectively, the necessary concentration of analyte to reach a given signal level (the concentration value is related to the slope of the analytical calibration curves) and the necessary concentration of analyte to give a signal which may be detected and identified over the blank signal noise (the concentration value is related to the detectability achievable under the chosen operating conditions).

The corresponding sensitivities (respectively, percentual sensitivity and fluctuational sensitivity) are, as usual, the inverse of those limits. These can be considered as *qualitative* sensitivities. *Quantitative* sensitivities, correlated to quantitative limits, can be calculated after minimum usable signal sizes have been established. This provides the starting point at the lower end of the dynamic concentration range.

A complete discussion on qualitative and quantitative sensitivity in

atomic-absorption flame photometry has already been published elsewhere (*4, 5, 8*).

When considering sample requirements for obtaining identifiable and measurable signals, it is convenient to take into account the absolute sensitivities and the corresponding absolute limits. These limits provide the necessary information for choosing sample size, preliminary dilution, and preliminary concentration, and, when necessary, the use of scale expansion.

Comments of the relationship between sensitivity and accuracy and precisions have been included in other papers (*6, 9*).

Finally, it should be helpful to remember here that the value obtained by dividing slope of analytical calibration curves by precision values (represented by σ), will represent a sensitivity value very useful when trying to compare performance of two or more different instrumental conditions (different instruments or the same instrument under different instrumental settings) (*4*).

SELECTIVITY IN ATOMIC-ABSORPTION FLAME PHOTOMETRY

In spite of the fact that atomic-absorption flame photometry is recognized as a highly selective instrumental method of analysis, there are many factors which can cause a loss of selectivity in the analytical applications of the method. Some of these factors will be briefly reviewed here.

Instrumental Factors Affecting Selectivity

Measurements by atomic-absorption flame photometry are obtained through three main selective conditions:

(a) Discrete emitters (vapor discharge lamps or hollow–cathode lamps) emit characteristic sharp and well–defined radiations for each analyte. This characteristic emission spectrum is a first step of selectivity as only certain radiations of given wavelength reach the absorbing cell.

(b) The absorption process takes place at characteristic wavelengths (some absorption lines corresponding to some emission lines: resonance lines).

(c) The monochromator selects a narrow band of the whole spectrum, and the detector only views luminous intensity variations produced in this narrow band.

Any instrumental failure, especially in those systems of the instrument mentioned in (a) and (c), will cause losses of selectivity as detailed below:

In *discrete emitters* it is possible to find lines (with nonabsorbing

characteristics) very close to those analytical lines at whose wavelengths the instrument is set. The maximum resolution attainable with the instrument may not be enough to separate later these lines, and both lines (the absorbed line of the analyte and the line not suffering any absorption) are viewed by the detector. This situation produces a loss of linearity which in its turn can be considered as a loss of sensitivity (smaller signal than expected for a given concentration of analyte). Taking into account the statements included in previous paragraphs this effect can be interpreted as a loss of selectivity, and measured by the corresponding loss of sensitivity (percentual sensitivity). The same effect can be observed if the emitter has a high background emission also viewed by the detector.

With *continum emitters* operation the situation is different: (a) The monochromator is generally unable to select a sufficiently narrow spectral band to reject the unabsorbed light coming from the continuum emitter at both sides of the absorption line. (b) When two analytes are present in the same sample solution and they have very close absorption lines, the superimposed absorption produced by both analytes is measured with the corresponding lack of selectivity. Since nonselective measurements can result in both cases, they have been major limitations in the use of continum sources in atom absorption.

Some attempts have been made to use multiline hollow cathode lamps as universal sources for several elements, due to the proximity of some of the analytical lines of the analytes subject to study (*10*). In this particular case a nonspecific lamp imparts selectivity by virtue of the absorption process within the sample itself.

Some effects of losses of selectivity in the use of discrete emitters are illustrated in Figs. 1 and 2. Effects derived from neighbor lines or lamp background can be compensated in part by a proper increase of the selection by the monochromator, i.e., by the use of narrower slit widths.

Losses of Selectivity in the Absorption Cell[3]

The use of ac systems has become widespread because of the ability to increase selectivity of the instrument by rejecting dc signals coming from the flame. Thus, the photodetection system measures only the variations of ac light coming from the emitter, whose emitted beam is chopped before entering the flame. The electronics should be tuned at

[3] *Absorption cell* is the space occupied by absorbing entities and delimited by the height and width of the optical beam and the horizontal length of the flame (flame path).

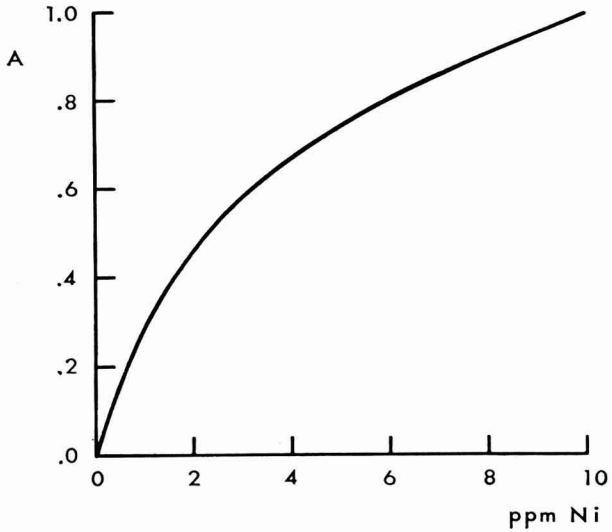

FIG. 1. Analytical calibration curve for nickel (Ni 2320 Å) obtained with a Beckman Model 979 atomic-absorption spectrophotometer, laminar flow burner (air–acetylene flame). A slight loss of selectivity is observed as loss of linearity due to nonselective isolation of the analytical line because of the closeness of neighboring lines emitted by the source lamp. This effect is observed with any atomic-absorption spectrophotometer unless a high intensity lamp is used and an extremely narrow slit width is utilized.

the same frequency. However, some ac signal components of the flame can be observed by the photodetection system superimposed upon the light of the chopped beam. In general, this circumstance produces an increase of noise, and a decrease of fluctuational sensitivity can be observed then. This, in turn, can be considered as decrease of selectivity. The situation becomes worse if the tuning is not perfect, i.e., if the instrument accidentally is unable to separate properly the ac signals and the dc signals, the latter ones produced in flame. This is most important when working with highly emitting flames or with emitting elements.

In certain cases, it is possible to work with dc instruments—with no modulated emitter beam—in which the photodetection system views all signals coming from both the emitter and the flame. The performance is fine if the emission from the flame and analtye or analytes is only a small fraction of the light received by the photodetection system. The emission can be compensated by proper correction (*4*). For instance, when working with high emitting elements, where the selectivity is actually poor, the selectivity can be improved by a proper adjustment of the burner elevation, until finding a flame section in which the maximum of the emission flame profile is avoided (*4*).

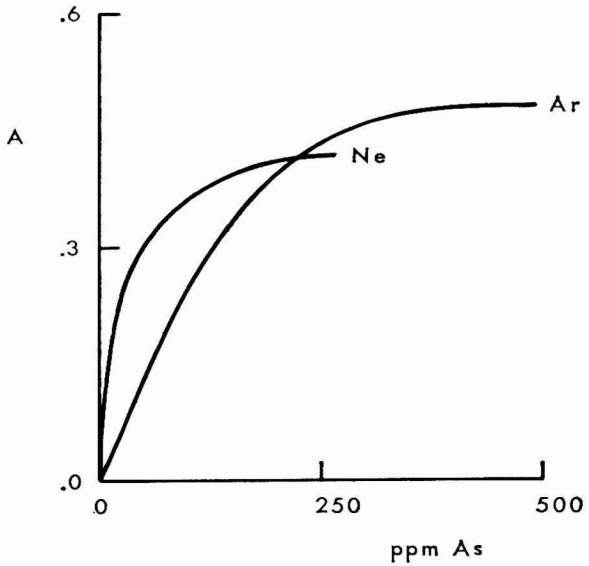

FIG. 2. Analytical calibration curves for arsenic (As 1937 Å) obtained with a Beckman Model 979 atomic absorption spectrophotometer and laminar flow burner (air–acetylene flame). With a neon filled lamp (Ne) better percentual sensitivity is obtained than with an argon filled lamp (Ar). However, loss of linearity is shown earlier in the case of the neon lamp because of the proximity of neon lines in that particular wavelength range.

Losses of Selectivity in the Selection System

The high selectivity achieved by the use of monochromators on the basis of their resolution is obvious in comparison with the poor selectivity that can be attained with selection by filters. Filters can only give acceptable selectivity when emission lines of the emitter used as analytical lines are sufficiently apart from other emitted lines.

Excessively wide slit widths is a frequent cause for losses in selectivity as observed as losses of percentual sensitivity and linearity. Therefore, a preliminary study of the effects of slit width on percentual sensitivity will always be of much help, e.g., see Fig. 3. On the other hand, narrower slit widths as well as smaller lamp current allow less energy to reach the photodetection system and high gain settings are necessary. This is detrimental for fluctuational sensitivity; see Fig. 4.

CHEMICAL FACTORS AFFECTING SELECTIVITY

As chemical composition of original samples and preliminary chemical preparation directly influence the final composition of sub-samples prepared for measurement, it is logical to comment here upon some specific chemical factors.

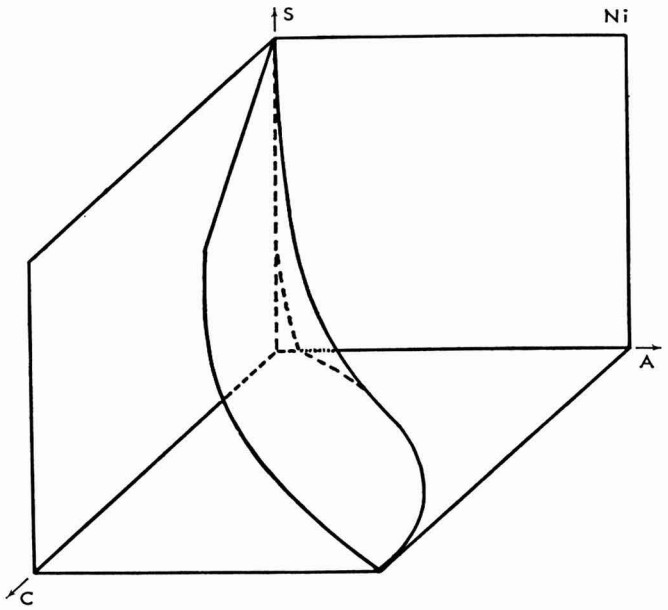

FIG. 3. Three dimensional representation of the variations of absorbance as a function of concentration and slit width (slit width profile representation) for nickel (Ni 2320 Å). Data were obtained with a Beckman Model 979 atomic-absorption spectrophotometer equipped with laminar flow burner (air–acetylene flame), operated in cold operation. Lamp 25 mA, and readings were taken at 0.2 inches above the burner head. Absorbance range (*A* axis) 0.00043–0.58; slit width range (*S* axis) 0.02–0.2 mm; concentration range (*C* axis) 0.5–100 ppm Ni. The decrease of signal size (percentual sensitivity) observed when increasing slit width is a consequence of the loss of selectivity in the selection of the analytical line.

Direct Measurements

The atomic absorption process carried out in the absorption cell is selective to a large extent because only the atoms of the analyte absorb light of the characteristic wavelength. If other atomic species are present in the absorption cell, they do not absorb light at the same wavelength. However, in many cases the presence of other entities in the flame—coming from concomitants present in the sample solution—results in a decrease of selectivity, mainly shown as a variation of the percentual sensitivity.

This effect is explained by considering that the active atom concentration of the analyte has varied in the flame (this concentration has increased or has decreased in comparison with the concentration when the analyte reaches the flame free of concomitants).

Several factors can lead to variations in the active atom concentration of the analyte when concomitants are present. Thus, modifications

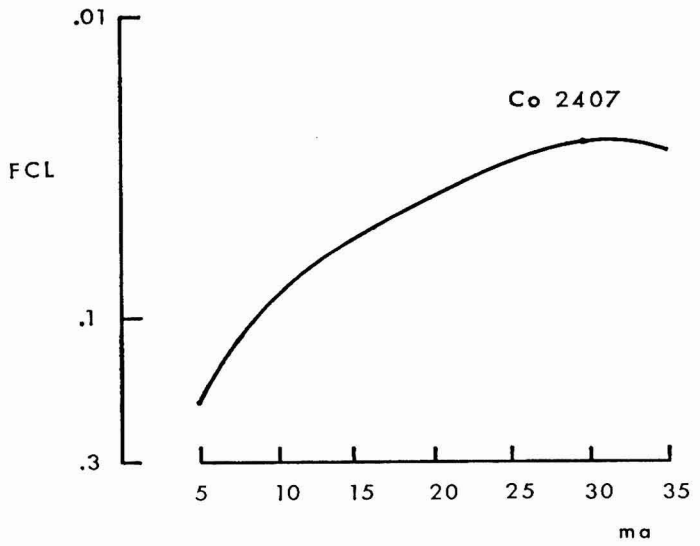

FIG. 4. Variation of the fluctuational concentration limit (FCL) as a function of the lamp current (mA) for cobalt (Co 2407 Å). Data obtained with a Beckman Model 979 atomic-absorption spectrophotometer with laminar flow burner (air–acetylene flame), with no scale expansion and 1-sec time constant. The increase of fluctuational concentration limit is an indication of the increase of noise level, due to higher gain settings necessary when decreasing lamp current. An increase of noise level leads to a loss of selectivity.

are produced before the analyte reaches the flame (changes of feeding rate because of changes in physical properties of the solution; changes in drop size; differences in drop vaporization rate). Other modifications occur in the flame itself (formation of oxide-type or salt-type compounds difficult to decompose at the flame temperature; changes of the diassociation equilibria; inhibition—masking-type—processes without any definite formation of stable compounds; ionization processes).

In this situation any means to restore percentual sensitivity can be considered effective as a means for restoring selectivity. Addition of wetting agents, releasers, protectors, counterionization agents, etc., converts a poor selective determination into high selective or specific determinations. Similar action is to be expected from the use of high temperature flames such as the nitrous oxide–acetylene flame; see Fig. 5.

Indirect Measurements

Unfortunately, in indirect measurements performed by precipitation procedures, the analysts are exposed to losses of accuracy and precision (as a consequence of the precipitation process at low analyte concentration levels) and also to dangerous losses of selectivity. Most of the

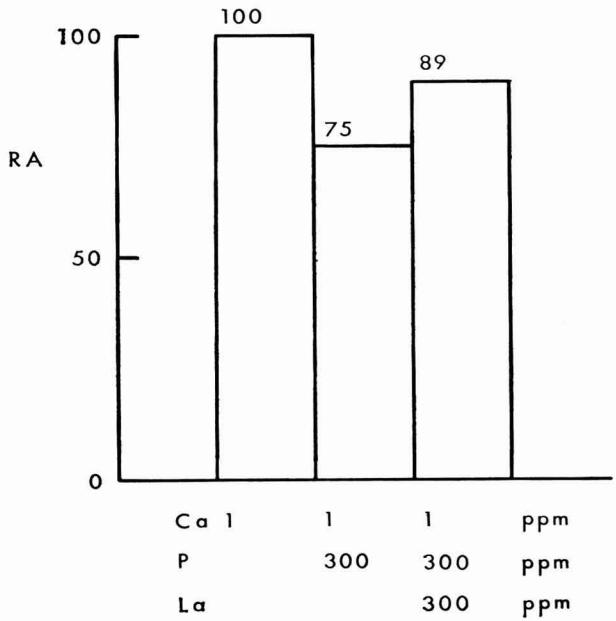

FIG. 5. Relative absorbances in the determination of calcium (Ca 4227 Å) in absence and in presence of phosphate (given in ppm of P). The addition of lanthanum, added as a releaser, contributes to a partial recovery of the initial sensitivity. In other words, the addition of lanthanum permits more selective determination of calcium in the presence of phosphates. Data obtained with a Beckman Model 979 atomic-absorption spectrophotometer with laminar flow burner (nitrous oxide–acetylene flame).

precipitations recommended are not selective. Precipitations with silver and precipitations with barium are group-selective precipitations. In the case of silver precipitations, background silver can be precipited by chlorides, bromides, and iodides, among others. If the procedure was planned for chloride determination, the chloride quantitative measurement is nonselective in the presence of other concomitants of similar chemical behavior (insoluble silver salt forming entities); see Fig. 6. The same comments apply to solutions containing two or more components which precipitate with barium. Of course, a precipitation with silver in the presence of an excess of ammonia or a precipitation with barium in the presence of an excess of HCl can appear as much more selective (for iodide and sulfate determinations, respectively).

LIMITATIONS OF SENSITIVITY AS A RESULT OF LOSSES OF SELECTIVITY

Looking at all these problems from the other side, any failure in any preparative process intended to be selective will result as a loss of sensitivity during measurement.

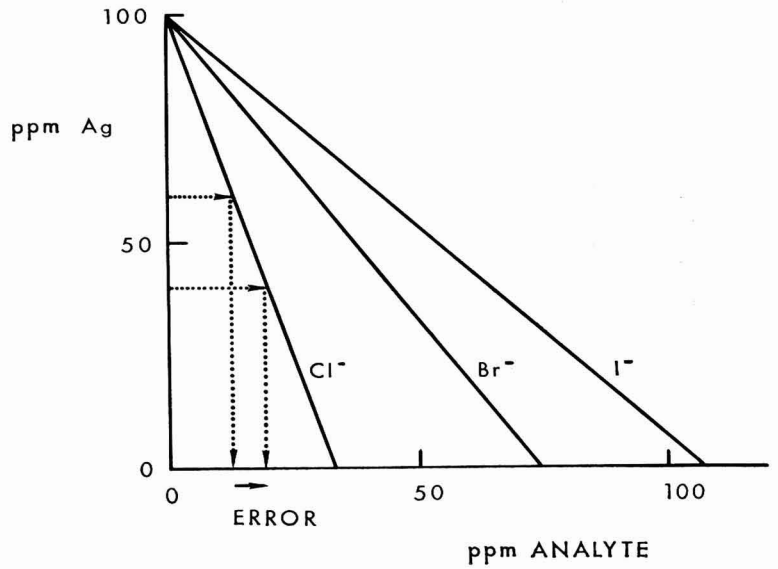

FIG. 6. Theoretical analytical calibration curves in the determination of chlorides, bromides, and iodides with silver as background element (indirect determination by difference). In chloride determination, the presence of amounts of the other halides can produce a smaller experimental reading which will give on the curve a higher value of the analyte. In this case the quantitative determination is not selective due to the lack of selectivity of the indirect technique.

Preparation procedures which do not include any preliminary separation of analytes from concomitants (or of concomitants from analytes) will provide the instrument with a low selectivity chemico-analytical medium which probably will show distorted percentual sensitivity values. In favor of speed, some procedures simply recommend preliminary dilution with no separations at all. Sufficient dilution will help to decrease condensed-phase and vapor-phase chemical interferences, but only to a degree.

Losses of sensitivity, as a consequence of chemical interferences, are clearly shown in sensitivity diagrams. Due to the correlation between sensitivity and selectivity, these diagrams can also be presented as selectivity as a function of the concentration of the interferent component, e.g., see Fig. 7.

Any separation of the diagrams from the horizontal line means a loss of selectivity. Additives used to prevent variations of sensitivity or the action of high temperature flames will be shown as a straightening of the selectivity diagrams toward the horizontal position; see Fig. 8.

If separations are included in the chemical preparation process, the solution processed can undergo losses of analyte. In fact, while the separation can increase selectivity (as the interferent concomitants are

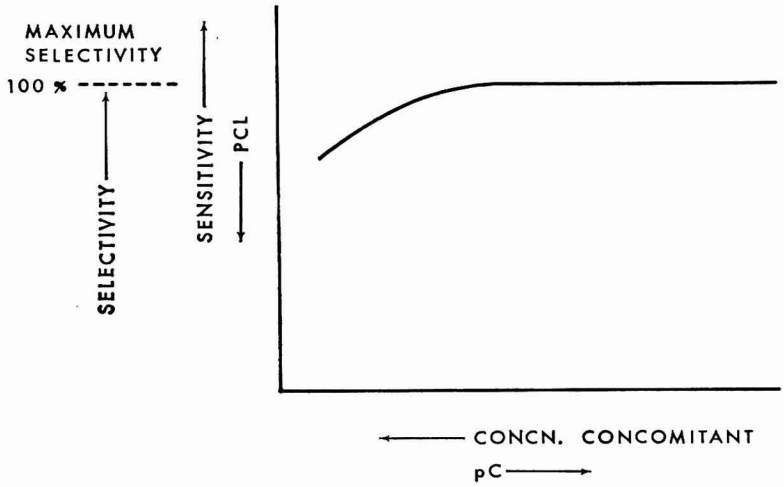

FIG. 7. Measurement of selectivity with the help of a sensitivity diagram. Maximum selectivity is attained when there are no losses of percentual sensitivity.

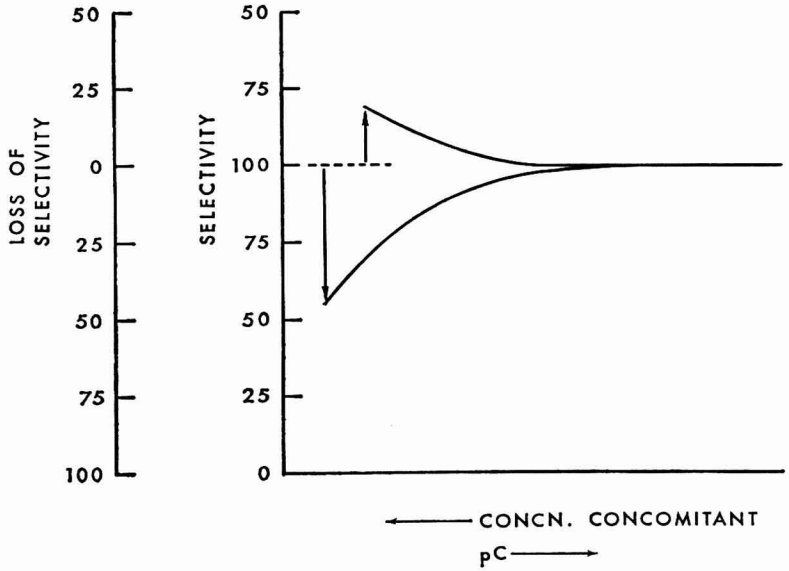

FIG. 8. Selectivity diagram showing selectivity scale and loss of selectivity scale. Any procedure which may bend the experimental diagrams toward the horizontal line will be a procedure which increases selectivity. Curvature (upward or downward) indicates loss of selectivity, which can be measured as percentage on the corresponding scale.

separate), losses will result in a decrease of the final concentration of the analyte. The signals will be smaller, and the selectivity will be found as smaller, if measured in terms of variations of percentual sensitivity. Going a little further, it is true that there are losses of selectivity, with separations which result in losses of analyte. These separations cannot be considered as *quantitatively selective*. These concepts apply to separations carried out by precipitation or by ion exchange procedures.

If the separation is made by organic solvent extraction, there is another factor to be considered besides any accidental loss of analyte. This other factor is the influence of the solvent. Solvents producing a decrease of sensitivity actually act as agents which decrease selectivity, e.g., see Fig. 9.

In any type of separation if contaminations are produced, anomalous percentual sensitivity values should be expected. In this case, the separation cannot be considered as a quantitatively selective separation.

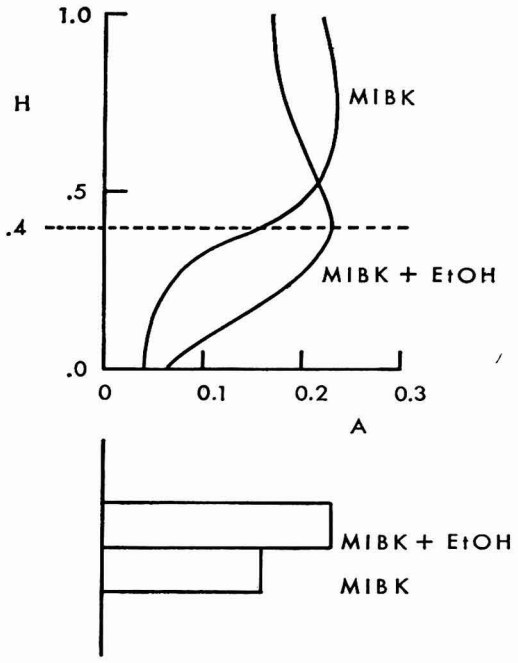

FIG. 9. Flame profiles for iron (Fe 2483 Å) obtained with a Beckman Model 979 atomic-absorption spectrophotometer and laminar flow burner (air–acetylene flame; very lean flame) in the presence of methyl isobutyl ketone (MIBK) and methyl isobutyl ketone and 25% ethyl alcohol (EtOH). By improper choosing of burner elevation, for instance, by reading both systems at 0.4 inches above the burner head, the signal obtained in presence of MIBK alone appear smaller. This smaller signal represents an apparent loss of selectivity.

In many separations an adjustment of pH may be mandatory both in simple extractions and in chelation extraction. This arises from the need to reach the optimum pH value necessary to achieve maximum analyte recovery at maximum separation ratio with respect to other components of the sample solution. Defective pH adjustment results in a loss of selectivity represented by a smaller analyte recovery, or by a group separation involving the analyte and other components, or both.

Use of Heated Spray-Chamber Laminar Flow Burners

The use of laminar flow burners equipped with heated spray-chambers (*1*) adds a new selective operation to the whole chemico-instrumental analytical system: The solvent is selectively separated in part before the moment in which the analyte reaches the flame. A relatively analyte-enriched spray goes to the flame. This pre-separation of the solvent results in an increase of the percentual sensitivity if compared with cold-chamber operation; see Fig. 10. In some cases condensed phase chemical interferents produce high effects when working with this type of

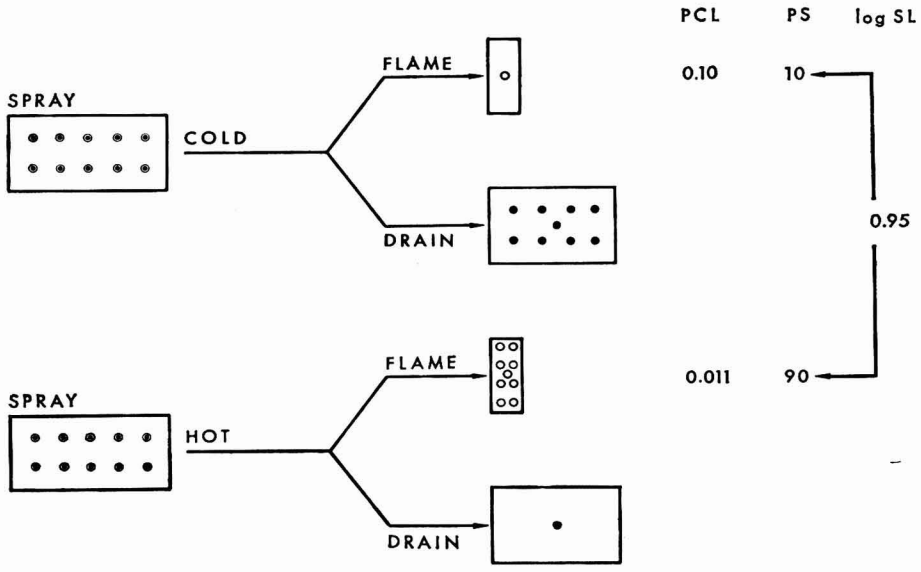

FIG. 10. Comparison of the hot and cold operation in a laminar flow burner with heated chamber. Under the supposition that equal amounts of liquid go to the flame and to the drain in both cases, as in hot operation the liquid going to the flame is enriched in analyte, cold operation represents a loss of selectivity in comparison with hot operation. In cold operation the analyte is not selectively separated from the spray obtained in the spray chamber. Loss of selectivity is represented by log SL.

dried sprays. However, the use of protectors and releasers restore to a great extent the original sensitivity.

CONCLUSIONS

Atomic-absorption flame photometry can be considered as a highly selective method of instrumental analysis. Main causes of loss of selectivity are improper choosing of instrumental parameters and matrix composition. A preliminary study of all parameters involved in the determination of an analyte can help to choose those instrument settings which correspond to the best percentual and fluctuational sensitivity and the best linearity. Then appropriate sample preparation can be a second step to avoid excessive loss of sensitivity. When separations are not advisable some additives act as interference suppressors. Also, high temperature flames permit more reliable results to be obtained, even if additives are necessary. If separations can be made prior to instrumental determinations, the isolation of the desired analyte or analytes from concomitants leads to higher selectivity in atomic-absorption flame photometric determinations. After separation, the analyte is determined in ideal conditions, i.e., in a medium liberated from interferent concomitants.

SUMMARY

Practical applications of atomic-absorption flame photometry should be established on the basis of the attainable analytical characteristics of the method both from the instrumental and chemical point of view. Main characteristics involved are sensitivity, selectivity, accuracy, and precision. The first two are discussed in this contributon. Sensitivity and selectivity depend on the analytical system (analyte or analytes, and concomitant components, i.e., chemical composition of the system subject to analytical determinations) and on the instrumental system. Factors involved are discussed and illustrated with examples based on experimental data. Lack of chemical and/or instrumental selectivity immediately affects sensitivity. Sensitivity changes can disturb accuracy and precision in determinations. In order to avoid this situation some operational ways of increasing selectivity are described and are correlated with effects in atomic-absorption sensitivity.

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The Dilution Process in Sample Preparation for Atomic-Absorption Flame Photometry

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INTRODUCTION

Original samples containing analytes at concentrations higher than the useful dynamic concentration ranges advisable for a given instrument should be diluted to an appropriate volume during sample preparation. Selection of pipet and flask volume for best accuracy can easily be calculated, once the final desired concentration or the optimal dilution is known (*1*). Dynamic concentration ranges can be established on the basis of the qualitative percentual concentration limit (PCL) (*2–4*) of the analyte under given conditions. From PCL values the size of signals for particular concentrations can be calculated (*5*). The optimum concentration range for maximum accuracy of measurement is well established in atomic-absorption methods (*6*).

A graph is presented that helps in choosing the appropriate dilutions to be applied to an original sample to bring the concentrations within the most convenient concentration range for instrumental measurement. It is also especially convenient for visualizing the general dilution schedule for sample preparation in the analysis of new types of samples. The graph can easily be reduced to simpler graphs applicable to each particular type of sample and analyte.

The choice of optimum dilution ratio is particularly critical in many situations where low original concentrations of constituents are encountered or where only a very small amount of sample is available.

DILUTION GRAPH

Log–log scales have been used to prepare the graph (Fig. 1) which correlates the decrease of concentration with increase of dilution (decrease of dilution ratio). This graph covers original concentrations from 100,000 ppm (10%) down to 0.1 ppm of analyte and dilution ratios from 1:1 down to 1:100,000.

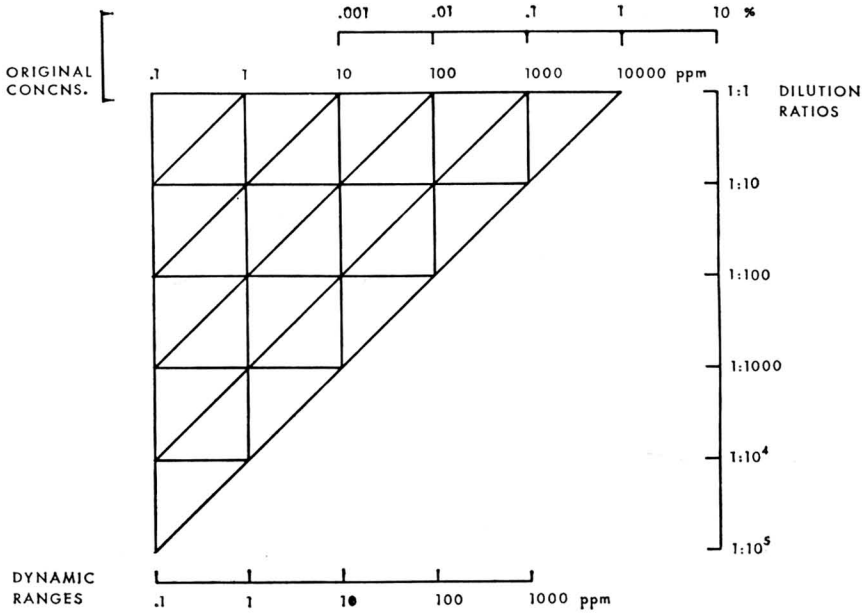

FIG. 1. Dilution graph.

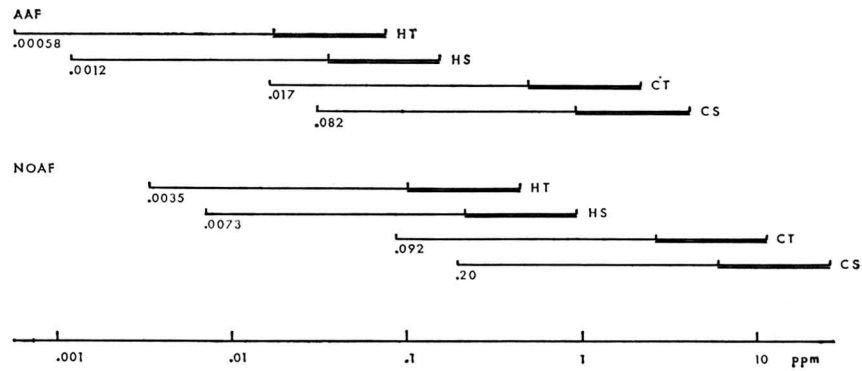

FIG. 2. Various dynamic concentration ranges found for magnesium by the use of the Beckman Model 979 atomic-absorption spectrophotometer and laminar flow burner: with air–acetylene flame (AAF); and with nitrous oxide–acetylene flame (NOAF), according to data published in Refs. (*7* and *8*). At the left of each range the corresponding percentual concentration limits (PCL) in ppm are given. The double line in each range brackets the restricted dynamic concentration range between 30 × PCL and 135 × PCL (0.132 to 0.549 absorbance). HT = hot mode, triple pass; HS = hot mode, single pass; CT = cold mode, triple pass; and CS = cold mode, single pass.

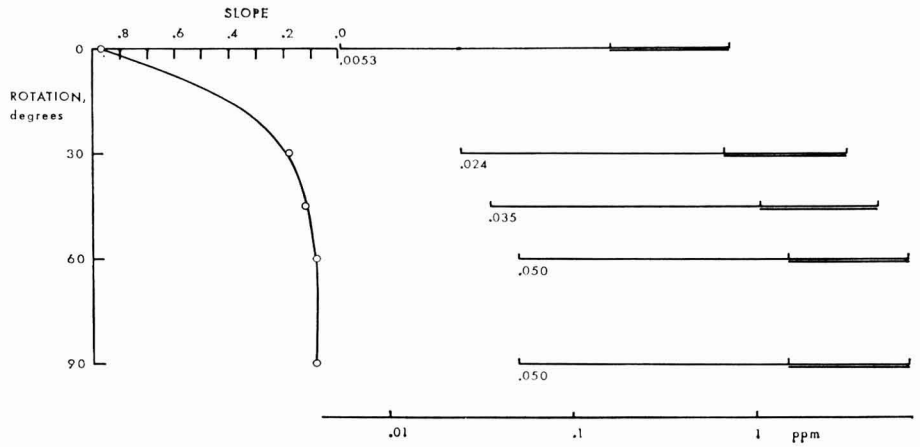

FIG. 3. Variation of dynamic concentration ranges as a function of the rotation angle of the laminar flow burner; data obtained for magnesium using a Beckman DU-2 Spectrophotometer with atomic-absorption accessory.

ESTABLISHMENT OF DYNAMIC RANGES

For systems in which the absorbance–concentration plot is linear, the upper end of the useful dynamic concentration range can be established by the concentration giving a signal of 0.5 to 0.7 absorbance (70–80% absorption). If the plot is nonlinear, the limit is set at the highest absorbance value permitted by the slope of the curve.

VARIATION OF DYNAMIC RANGES WITH INSTRUMENTAL SENSITIVITY

With a versatile instrument which allows *several steps* of instrumental sensitivity, several dynamic ranges can be established; see Fig. 2.

With an instrument which allows a continuous variation of instrumental sensitivity, for instance, by rotation of burner, the dynamic

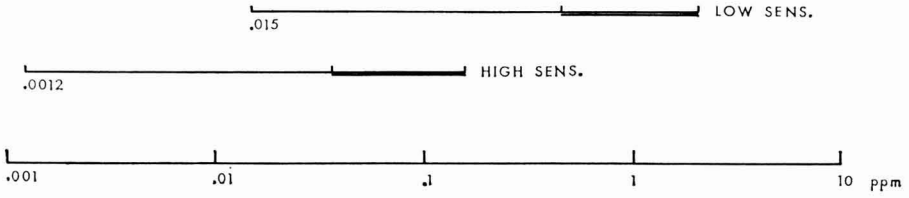

FIG. 4. Dynamic concentration ranges for two instruments of different sensitivity: PCL values correspond to magnesium. PCL of 0.0012 ppm is taken from Fig. 2 (hot mode, single pass).

ranges vary with the same pattern as the variable parameter; see Fig. 3.

If two instruments of different sensitivity are compared (each with a different PCL) the dynamic ranges will appear also at different positions on the concentration scale; see Fig. 4.

USE OF THE GRAPH

Figure 5 shows how to use the graph for a type of sample in which the analyte concentration varies within a definite range. The diagonals bracket the concentrations obtainable at different dilution ratios. By tracing perpendicular lines up from the ends of the known dynamic ranges, dilution ratios can be chosen to bring the final concentrations within the dynamic ranges.

A certain freedom is still available within an interval of dilution ratios. Using either the same or a different instrumental sensitivity for each analyte, the flame photometrist may choose a single dilution suitable for the determination of two or more elements.

In Fig. 6, both analytes can be determined at a single dilution which gives concentrations within their respective restricted ranges.

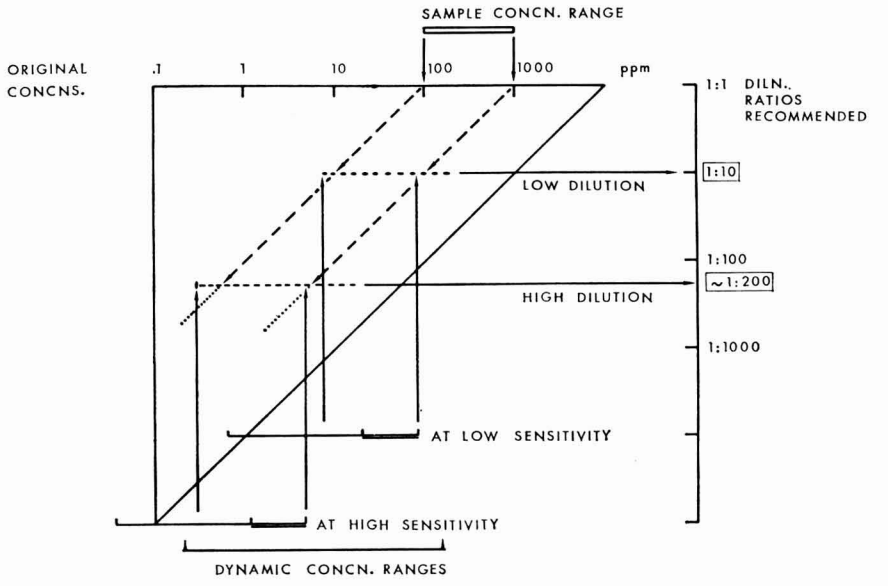

FIG. 5. Use of the dilution graph: from a single concentration range of the analyte in the original sample different dilutions can be selected according to the dynamic concentration ranges achieved with different instrumental sensitivity. Observe that original concentrations lie outside the useful dynamic concentration ranges.

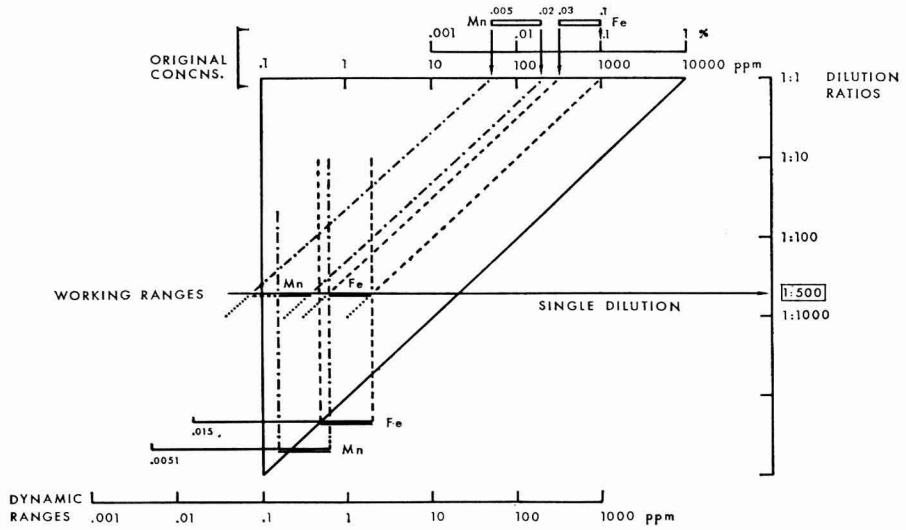

FIG. 6. Example of the selection of a single dilution for two analytes with different concentration ranges in the original samples: PCL values shown have been obtained with a Beckman Model 979 atomic absorption spectrophotometer and laminar flow burner, Air–acetylene flame, hot mode, single pass (7). This dilution schedule can be used, for instance, in the preliminary sample preparation of nonferrous alloys for determination of iron and manganese in the same diluted solution.

SIMPLIFIED SCHEMES

Taking advantage of the additive properties of log–log scales, the graph in Fig. 6 can be simplified as shown in Fig. 7. The horizontal

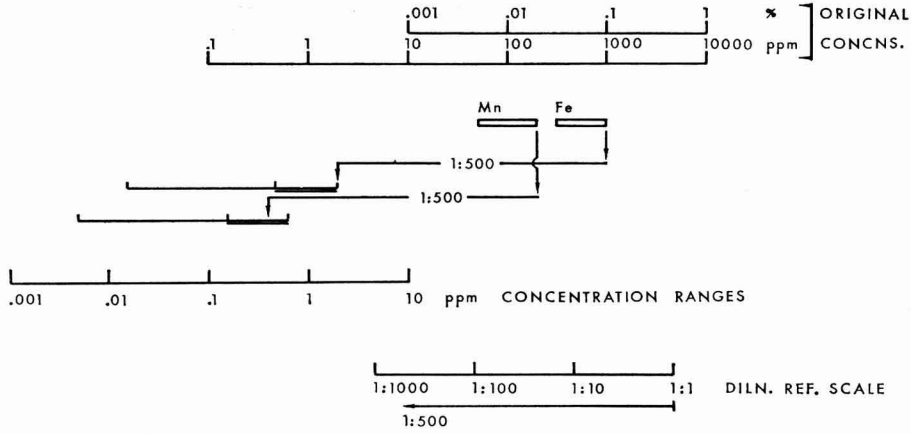

FIG. 7. Simplified graphical procedure corresponding to the example given in Fig. 6.

segments represent dilution ratios, which can be measured on the dilution ratio reference scale.

SUMMARY

A graph is presented and discussed to assist in choosing the appropriate dilutions for original samples in atomic-absorption flame photometry. It is based on the percentual sensitivity obtained with a given instrumental system. The additive properties of log–log scales permit even further simplifications of the scheme. The graph is also shown to be useful in choosing single dilutions for multielement analysis with analytes having different percentual sensitivities.

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MICROCHEMICAL JOURNAL 15, 277–280 (1970)

The Concentration Process in Sample Preparation for Atomic-Absorption Flame Photometry

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INTRODUCTION

If the analyte or analytes are present in original samples as micro-components, at concentrations lower than the dynamic concentration ranges advisable for an instrument, they should be concentrated by appropriate factors during sample preparation. This is an opposite situation to that faced by the flame photometrists wishing to lower the original concentration prior to analytical determination. Following a similar basis to that used for the preparation of the dilution process graph (*1*), a new graph has been prepared to be used in concentration processes. This new graph is to some extent a mirror image of the dilution graph, showing the correlation between concentration of analyte in solution and concentration factors. The graph helps in choosing the appropriate concentration factors which can be applied to an original sample to increase the analyte concentration to the most convenient concentration range for measurement with the instrument. The graph can also be used to plan and select the best general preliminary concentration schedule for sample preparation in the initial analysis of new types of samples.

PRELIMINARY CONCENTRATION GRAPH

The graph is shown in Fig. 1, covering original concentrations from 0.001 ppm up to 1000 ppm and concentration factors from 1 up to 10^6.

See comments published in Ref. (*1*) on establishment of dynamic ranges and their variation with instrumental sensitivity. The same concepts may be applied here.

USE OF THE GRAPH

The use of the graph is shown in Fig. 2. Diagonal lines bracket concentrations in solution obtainable at different concentration factors.

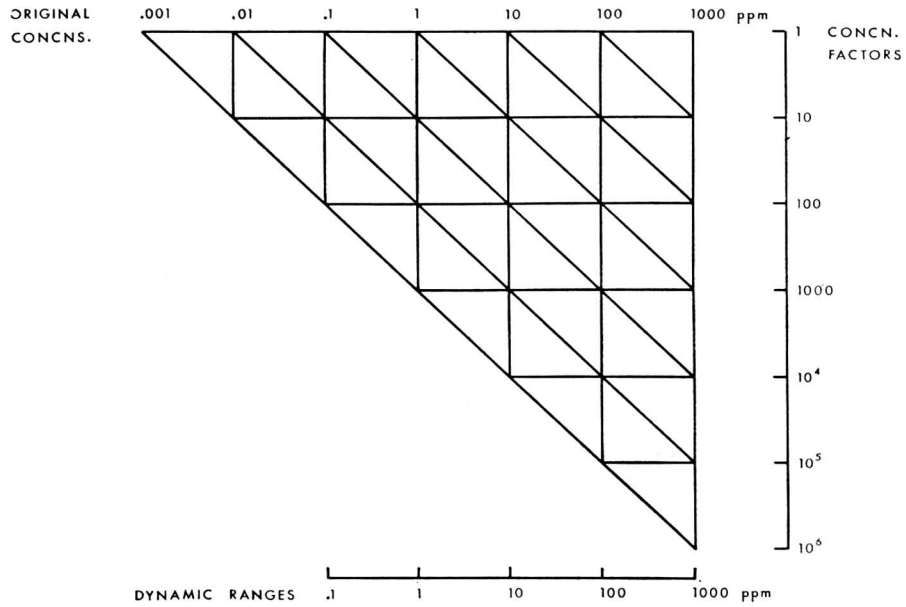

FIG. 1. Preliminary concentration graph.

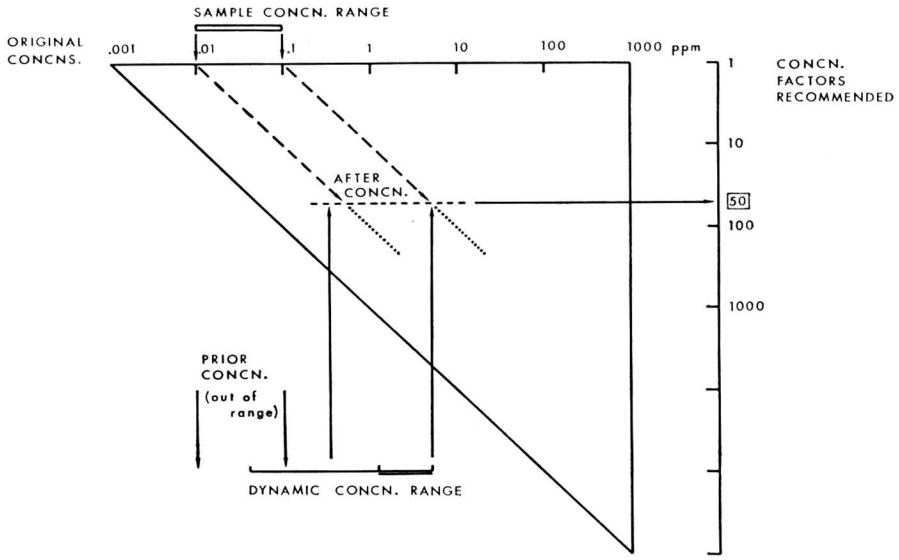

FIG. 2. Use of the preliminary concentration graph; observe that the sample concentration range corresponds to concentrations smaller than the dynamic concentration range established for given operating conditions. Upper original concentrations may overlap the lower end, but signals would be too small to allow reliable determinations.

SIMPLIFIED SCHEMES

By using log–log scales, and taking advantage of their additivity, the graph can be simplified as shown in Fig. 3. The magnitude of the horizontal shifts represent the concentration factors, which can be measured on the concentration factor reference scale.

USE OF ORGANIC SOLVENTS

PCL values obtained with organic solvents will be different from those obtained with aqueous solutions. When the concentration step is effected with an organic solvent, the dynamic concentration range should be referred to a new PCL determined with the extraction solvent. Thus the concentration factor will depend on the particular solvent used; see Fig. 4.

Group extractions (multielement extractions, single extractions) for separation and concentration can also be studied with the help of the above mentioned graph.

It is frequently possible to choose a single concentration ratio which yields an extract solution with several different analytes each within the proper dynamic concentration range for a given set of instrumental operating conditions.

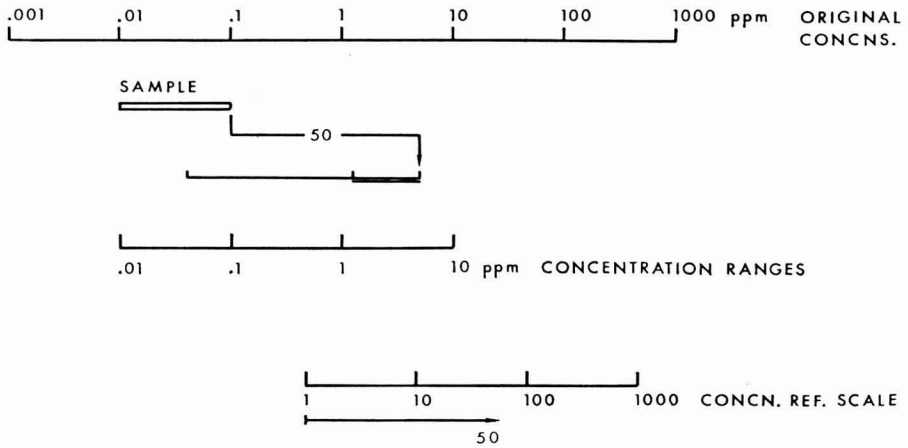

FIG. 3. Simplified graphical procedure corresponding to the example given in Fig. 2. The horizontal shift necessary to bring the analyte content of the sample within the dynamic concentration range corresponds to the concentration increase necessary for optimum analysis.

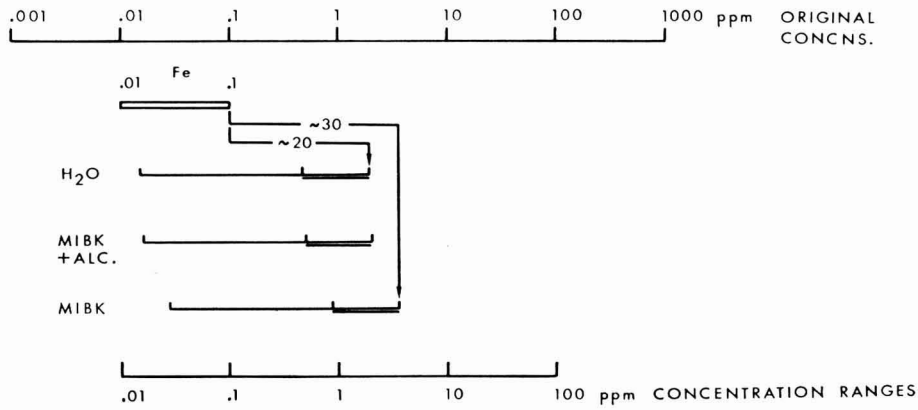

FIG. 4. Simplified graphical procedure showing the concentration factors (~30× and ~20×) necessary to bring iron concentrations of 0.01 to 0.1 ppm up to the most convenient concentration ranges when using different types of solvents (water; methyl isobutyl ketone + ethyl alcohol; and methyl isobutyl ketone, alone). Concentration ranges shown come from Ref. (*2*), obtained with a Beckman Model 979 atomic-absorption spectrophotometer and laminar flow burner, air–acetylene flame. When using organic solvents the necessary concentration factor can be achieved by proper selection of the liquid–liquid extraction procedure. In the particular case of iron the upper limit of the ranges can be slightly extended beyond the limits shown due to the nonlinearity of the analytical curve.

SUMMARY

A graphical representation can be used for choosing the appropriate preliminary concentration factors in preparing samples for atomic-absorption flame photometric determinations. The graph has been constructed on the basis of the percentual sensitivity obtained with a given instrumental system. The graph can be further simplified for practical applications. The use of the graph can also be extended to multielement concentrating extractions.

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MICROCHEMICAL JOURNAL 15, 281–284 (1970)

Microdetermination of Vitamin B_6: Gold Chloride as Oxidizing Agent

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Literature concerning the determination of vitamin B_6 is sufficient: it was determined microbiologically *(1, 2, 7, 8)*; fluorometrically *(3)* Chemically *(4)*; electrometrically *(6)*; titrimetrically *(5, 9)*, by synthetic ion exchange resins *(11)*; and colorimerically *(12, 13)*.

The present work deals with microdetermination of vitamin B_6 using gold chloride as oxidizing agent in alkaline medium. The reaction mixture consists in adding vitamin B_6 to a known excess of gold chloride and sodium hydroxide. Remaining excess of gold chloride is titrated *(10)* back after acidifying the alkaline mixture. Probably the following reaction takes place:

$$6C_8H_{11}O_3N + 74H^AuCl_4 + 296NaOH \rightarrow 48CO_2 + 3N_2 + 218H_2O + 74Au + 296NaCl.$$

From the above equation, it appears that vitamin B_6 breaks up into its components at 37 equivalence.

EXPERIMENTAL METHODS

Reagents. Vitamin B_6 and *N*-phenyl anthranilic acid (B.D.H. grade) gold chloride (Palmston's grade); sodium hydroxide (E. Merck grade); potassium ferrocyanide, ferrous ammonium sulfate and sulfuric acid (ANALAR B.D.H. grade); and ceric sulfate (Technical B.D.H. grade).

Apparatus used. Micropipette and burette had LC = 0.01 ml. The 0.0041 *M* ceric sulfate (in 8 *N* H_2SO_4) solution is standardized by titration against a standard solution of ferrous ammonium sulfate (in 1 *N* H_2SO_4) using *N*-phenyl anthranilic acid as indicator. The 0.0382 *M* potassium ferrocyanide solution is standardized against a standard solution of ceric sulfate (in 8 *N* H_2SO_4). The 0.0147 *M* gold chloride solution is standardized by adding a known excess of a standard solution of potassium ferrocyanide *(10)*.

Procedure

Known standard solution of vitamin B_6, known excess of gold chloride, and sodium hydroxide are placed in a beaker (from a micropipette) to form the reaction mixture. To the reaction mixture, 50 ml of distilled water is added and then put on a hot plate at full heat (keeping in mind that the reaction mixture may not evaporate) for 180 minutes. In case the volume of the reaction mixture is reduced to about 5 or 8 ml (much before 180 minutes) then again 20–25 ml of distilled water is added. After 180 minutes, the solution in the beaker is cooled at room temperature, and the precipitated metallic gold, (which corresponds to vitamin B_6 oxidized) is filtered off and thoroughly washed with distilled water. Remaining gold chloride and the washings are mixed together and acidified with 2 *N* H_2SO_4 solution. A known excess of potassium ferrocyanide solution is added, from a micropipette, to the above-mentioned acidified solution. The remaining excess of potassium ferrocyanide is titrated by running in a standard solution of ceric sulfate (in 8 *N* H_2SO_4) from a microburette, using *N*-phenyl anthranilic acid as indicator. At the end point a red-brown color appears sharply.

RESULTS AND CONCLUSION

Results are given in Table 1. Range in which vitamin B_6 is estimated varies from 8.3778 to 21.9379 mg/liter. The reaction between vitamin B_6 and gold chloride in alkaline medium takes place in the ratio of 1:37 complete rupture of the vitamin B_6 molecule results in the formation of CO_2, N_2, and water. This shows that 18.5 atoms of oxygen are required to complete the reaction under these conditions. It is observed that hydroxy acid, sugars, aminio acids, glycols, other vitamins, and alkaloids interfere. Large excess of sodium hydroxide inhibits the reaction and in that case different reaction products result. This reaction is advantageous from the kinetic study point of view and where pure vitamin B_6 is present it would be the best method for its determination.

SUMMARY

Vitamin B_6 was determined in micro amounts by oxidation with gold chloride in alkaline medium. Oxidation was done by adding a known excess of gold chloride and alkali solutions to vitamin B_6 solution. Ghe reaction mixture was heated and then the remaining excess of gold chloride was titrated back. The amount of gold chloride used corresponds to the vitamin B_6 oxidized, 18.5 atoms of oxygen are required for the complete rupture of the molecule. Traces of organic compounds did not interfere but excess alkali inhibited the reaction.

ACKNOWLEDGMENT

The author is grateful to the Council of Scientific and Industrial Research (Government of India), New Delhi for providing financial assistance.

TABLE 1

MICRODETERMINATION OF VITAMIN B_6

Vitamin B_6 0.001 *M* (ml)	NaOH 0.1 *M* (ml)	H_2O (ml)	$HAuCl_4$ 0.0147 *M* (ml)	$K_4FE(CN)_6$ 0.0362 *M* (ml)	$Ce(SO_4)_2$ 0.0041 *M* (ml)		Amount of Vitamin B_6 ($\times 10^3$ mg)		Error (%)
					Added	Consumed	Taken	Found	
—	—	—	—	1	9.10	—	—	—	—
—	—	—	1	1	5.60	3.50	—	—	—
0.04	13	20	1	1	5.96	0.36	8.2260	8.2008	0.3
0.06	13	20	1	1	6.15	0.55	12.3390	12.5290	1.5
0.08	13	20	1	1	6.32	0.72	16.4520	16.4016	0.3
0.10	13	20	1	1	6.60	0.90	20.5650	20.5020	0.3

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Analytical Reactions in Trifluoroacetic Acid
II. Organic Spot Test Parameters

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INTRODUCTION

Organic spot test reactions involving oxidation of aromatic compounds in trifluoroacetic acid by lead(IV) and by thallium(III) acetates were reported in Part I (*11*). A large number of compounds were screened with the reagents, but no extensive study was made of the effect of variables on color formation. An attempt at systematization of the test with respect to consideration of substrates, oxidants, and solvents, in relation to color, is reported in the current research. It is hoped that correlation will be obtained between color and certain parameters of the aforementioned system components.

MATERIALS AND METHODS

Reagents

Organic compounds studied were commercially available, practical or reagent grade. The trifluoracetic acid used was Distillation Product Industries' White Label grade. Lead(IV) acetate was prepared from red lead(IV) oxide, according to the directions of Feiser (*4*), and thallium(III) acetate was prepared from thallium(III) oxide by the method of Hecker and Lattrell (*6*). The other oxidants were obtained commercially.

Procedure

A drop of the organic substrate, in 0.5 mg/ml of a suitable solvent such as benzene, was treated with 1-3 drops of 1% oxidant in trifluoroacetic acid in a 10 × 75 mm test tube, and the color was noted.

RESULTS

Substitution for Trifluoroacetic Acid (TFA)

To determine whether TFA had a unique role in the spot test, it was replaced by a number of acids. No colors were formed in glacial acetic acid, difluoroacetic acid, dichloroacetic acid, and trichloroacetic

acid (1:1 by wt in benzene) with pyrene in benzene and lead(IV) acetate. However, the blue color was given in chlorodifluoroacetic acid and in perfluoropropionic acid. In addition, pyrene in benzene gave a purple color with thallium(III) perchlorate when TFA was replaced by 70% perchloric acid or by 80% sulfuric acid. The fact that the color was formed in the aqueous layer indicated the polarity of the oxidized species; however, TFA is still the acid of choice.

Oxidants

There are three general types of oxidants: metal salts, salts of oxyacids, and nonsalt oxidants. Because of favorable solubility parameters, the first group has been studied most extensively in TFA. Of this group, a number of metal salts were not satisfactory because of intense color [cobalt(III) acetate or manganese(III) acetate] or weak oxidizing power [iron(III) perchlorate]. Results with suitable reagents and a selected group of aromatic hydrocarbons are given in Table 1.

All oxidants were 1% in TFA except antimony(V) fluoride which was 10% in TFA. A number of other oxidants have been tested and, in general, the following pairs of oxidants behaved similarly: thallium(III) acetate and phenyliodosoacetate; lead(IV) acetate and nickel(III) acetate, the latter prepared from nickel(II) acetate and lead(IV) acetate; and thallium(III) perchlorate and lead(IV) perchlorate, prepared from lead(IV) acetate and mercury(II) perchlorate. Antimony(V) fluoride at the 1% level behaved similarly to thallium(III) acetate. Antimony(V) chloride gave colored precipitates with the more reactive polynuclear aromatic hydrocarbons.

Metathetical reactions involving various mercury(II) salts, and thallium(III) acetate in TFA with pyrene in benzene, indicated no difference when the salt was trifluoroacetate or nitrate but did form a different color when the salt was perchlorate. The initial colors formed by certain aromatics and mercury(II) perchlorate or antimony(V) fluoride may be due to charge–transfer complexes.

Brief studies made with some of the above oxidants and substituted benzenes indicated the following: phenol gave a characteristic color with both of the thallium(III) salts and with phenyliodosoacetate but not with lead (IV) acetate; anisole gave similar blue hues with the lead(IV) salt and with thallium(III) perchlorate, while *o*-chloroaniline gave a characteristic color only with lead(IV) acetate.

Cosolvents

Because of the relatively low solubility of many aromatic hydrocarbons in TFA, a cosolvent was required. As indicated in Table 1,

TABLE 1

OXIDATIONS IN TRIFLUOROACETIC ACID

Oxidant	Pyrene in		Anthracene in		Chrysene in	
	Benzene	Dichloromethane	Benzene	Dichloromethane	Benzene	Dichloromethane
Mercury(II) acetate	Yellow	Yellow→violet	NR	Pink	Yellow	Yellow
Mercury(II) perchlorate	Red→blue	Orange→violet	Red→blue	Red→violet	Orange	Orange
Thallium(III) acetate	Blue	Blue	Blue	Cyan	Magenta	Yellow→magenta
Thallium(III) perchlorate	Purple	Brown	Red	Blue	Violet	Violet
Lead(IV) acetate	Blue	Green	Blue	Yellow	Magenta	Magenta
Antimony(V) fluoride	Red→violet	Yellow→green	Yellow→blue	Yellow→green	Magenta	Orange→brown

different colors were often obtained in spot tests when benzene or dichloromethene was used as solvent. The former was of particular interest because it is a Lewis base and may combine with electrophilic oxidation products. Results with benzene, and substituted benzenes containing +I and −I groups, are given in Table 2.

The color transitions of antimony(V) fluoride with pyrene in toluene were more rapid than in benzene, as would be expected from a more reactive Lewis base. The greater stability of pyrene blue from thallium(III) acetate in the xylenes, as compared to benzene or toluene, may also be related to greater Lewis basicity of solvent. The greater stability (as well as intensity) of the color with pyrene and thallium(III) perchlorate, as compared to acetate, was also noteworthy and will be discussed later.

Very similar spectra were obtained from pyrene oxidized with thallium(III) perchlorate and with antimony(V) fluoride, indicating that similar products probably form.

The use of a benzene cosolvent is not always advantageous since fluorene gives a more characteristic color with various oxidants in dichloromethane.

Stability of Colors

The colors obtained in the above tests usually fade upon standing, and this phenomenon may serve to differentiate among various compounds. The following approximate fade-times have been observed with lead(IV) acetate and benzene cosolvent (Table 3).

As shown in the Discussion section, this order can be correlated with the predicted stability of the radical cation.

TABLE 2

EFFECT OF VARIOUS COSOLVENTS ON OXIDATIONS OF PYRENE IN TFA

Aromatic solvent	Tl(III) acetate	Tl(III) perchlorate	Sb(V) fluoride
Benzene	Blue	Purple	Red → violet
Toluene	Blue	Violet	Red → violet → blue
Ethylbenzene	Blue	Violet	Red → violet → green
o-Xylene	Blue	Purple → violet	Brown blank
m-Xylene	Blue	Violet	Yellow blank
p-Xylene	Yellow	Violet	Purple
Fluorobenzene	Blue	Purple → brown	Purple
Chlorobenzene	Blue	Violet brown	Orange → magenta

TABLE 3

FADE-TIMES FOR VARIOUS COLORS IN TFA WITH LEAD(IV) ACETATE

Time	Compounds
5–10 sec	Naphthalene, anthracene, phenanthrene
5–10 min	Pyrene, chrysene
20–30 min	Triphenylene, perylene, 1,2-benzanthracene
1 hr	9,10-dimethylanthracene and 9,10-diphenylanthracene [mercury(II) perchlorate oxidant]
1 hr	Rubrene [thallium(III) acetate]

Fluorescence

In addition to color, certain of the spot tests gave fluorescence which was observed when diluting the solution with benzene, such as that of pyrene with thallium(III) acetate. By contrast, the oxidation product of pyrene with thallium(III) perchlorate was not fluorescent. However, fluorescence has not been studied appreciably in this project because many of the aromatic compounds are also fluorescent. Nevertheless, the appearance of fluorescence in the spot tests may have application in differentiating various compounds.

DISCUSSION

Experimental results indicate that color formation with various aromatic hydrocarbons and a series of heavy metal salts in trifluoroacetic acid will depend upon: (i) the electron availability of the organic substrate and its oxidation products, (ii) the oxidizing strength of the heavy metal salt, and (iii) the Lewis basicity of the cosolvent.

The Electron Availability of the Substrate

Electrode potentials have been reported for a large number of aromatic hydrocarbons and their derivatives (*10*). Most of these values have been obtained in acetonitrile or glacial acetic acid. These values are a rough guide as to whether a substrate will require a relatively weak or a relatively strong oxidant. In organic spot tests, too strong an oxidant may often be as useless as one that is too weak.

Based upon experimental results, and taking electrochemical oxidative mechanisms as a model (*1*), the following scheme is proposed for oxidation of aromatic hydrocarbons in TFA. The substrate is oxidized initially with loss of an electron to a radical cation $Ar^{\cdot+}$. This species can oxidize further to, e.g., a quinoid type compound or else condense with unreacted substrate, or itself, to give the bis species

Ar–Ar which (because of the increased resonance) is both more readily oxidized than the starting material and more stable as the radical cation Ar–Ar$^{\cdot+}$ than is Ar$^{\cdot+}$. With the more powerful oxidants [e.g., thallium(III) perchlorate] the radical cation Ar$^{\cdot+}$ is oxidized further, perhaps with loss of an electron and a proton to the cation Ar^{+}. This species may then undergo aromatic substitution on the benzene present as cosolvent to give the species φ–Ar which, when further oxidized to φ–Ar$^{\cdot+}$, should be more stable than Ar$^{\cdot+}$ (*1*); not only because of increased charge delocalization but because one of the reactive positions is blocked by a phenyl group. A similar scheme has been proposed for sequential oxidation of toluene by tungstocobaltate(III) via radical cation, radical, and carbonium ion (cation), the last-named condensing with toluene to give phenyl-*p*-tolylmethane (*2*).

The unusual behavior of antimony(V) fluoride may be due in part to the fact that it is an extremely powerful Lewis acid and would be expected (aside from its oxidizing ability) to promote hydrocarbon condensations.

One indication of the proposed sequence is the order of stability of the colors obtained upon oxidation of the various aromatic hydrocarbons: naphthalene ~anthracene ~phenanthrene <pyrene <chrysene <triphenylene ~1,2-benzanthracene <perylene <9,10-diphenylanthracent ~rubrene. The stability sequence, according to highest-filled molecular orbital calculation (HFMO) of the electron density for the radical cation Ar$^{\cdot+}$, is: naphthalene <anthracene <phenanthrene <pyrene <1,2-benzanthracene <chrysene <triphenylene <perylene <rubrene (*1*). The agreement is generally good, with the exception of pyrene whose color is more stable than would be predicted. It is not certain whether the colors observed are due to the species Ar$^{\cdot+}$ or Ar–Ar$^{\cdot+}$. The former, for such substrates as naphthalene, pyrene, etc., are known to be quite unstable in solvents such as dichloromethane or nitrobenzene. However, their stability may be enhanced in TFA via possible hydrogen bonding. This type of bonding between the hydrogen atoms (of the amine and ether salts of TFA) and the fluorine atoms has been proposed (*5*), and might be expected in the ion pair Ar$^{\cdot+}$ CF_3OO^-. It is this possibility which may explain the difference in behavior between trifluoroacetic and trichloroacetic acids although both compounds have comparable polarity and acidity.

Because certain polynuclear aromatic hydrocarbons have phenyl groups at the reactive positions (e.g., rubrene), radical cations produced are appreciably stable since not only are the reactive positions blocked, but the phenyl ring adds stabilizing resonance (*1*). As mentioned previously, the increased stability of colors given by anthracene and py-

rene in benzene with thallium(III) perchlorate in TFA may be the result of cation formation, followed by aromatic substitution on the benzene, to give the aforementioned phenyl-substituted polynuclear aromatics.

The Oxidizing Strength of the Oxidant

The previous section indicated that different oxidative pathways are available for aromatic compounds. The pathway taken will depend considerably upon the inherent strength of the oxidant. The oxidizing strength, designated by the oxidation potential, E, is derived from the Nernst equation $E = E_0 + 0.06/n$ (activity oxidized/activity reduced). The E_0 values have been determined mainly in aqueous solution and are dependent upon the ionization potential, and the energies of sublimation and hydration of the species involved. Since TFA is not a Lewis base and would not be expected to solvate cations appreciably, large difference in E_0 values for heavy metal cations from those in water are not to be expected.

The difference in E values for different salts of a given heavy metal cation however, may frequently be larger in TFA than in water. The magnitude of E will depend upon the degree of association or complexation of the oxidized and reduced cation species with anions. In water, in a high dielectric liquid, or in aqueous acid a considerable leveling effect occurs due to strong disassociation or hydrolysis of many salts. In a low dielectric solvent (such as TFA) these salts may exist as unionized species (*3*), or as ion pairs, so that the influence of the anion on the activity, and hence oxidizing power of the heavy metal cation, is optimum. Two contrasting cases are provided by the acetate and perchlorate salts of metal oxidants. Acetate is a moderate complexer while perchlorate is an extremely weak one. Hence, heavy metal percholate salts in TFA should be much stronger oxidants than acetates, and this has been found to be the case. Further, reported literature results indicate that trifluoroacetates of thallium (III) and lead (IV) are more "powerful" reagents than are the acetates (*7–9*). Another factor that may enhance the oxidizing power of perchlorates is that their lower valency salts often are less soluble in TFA. Finally, the fact that perchlorates are much more polar than acetates may be significant in the assumed oxidants of radical cations to cations ("like oxidizes like").

Lewis Basicity of Cosolvent

Trifluoroacetic acid owes, in part, its success as a spot test solvent for oxidations to its extremely low basicity; however, a cosolvent is usually required in the test because of the relatively low solubility of

certain aromatics in TFA. If sufficiently basic, the cosolvent will enter into the reaction, and in certain instances the result will be a more intense and stable color. This ideal situation is exhibited by benzene and certain of its derivatives. As mentioned previously, the mechanism of color formation here probably involves aromatic substitution of a cation on the benzene ring followed by further oxidation. The substituted benzenes that exhibit this enhancement are in a limited range, since those containing strong $-I$ groups (e.g., nitro) are too inert to be attacked by the cation.

In conclusion, the following correlations appear to be generally true for spot tests involving oxidations in TFA:

1. Published electrode potentials of aromatic compounds serve as a rough guide to the strength of oxidant required for color formation. The weakest satisfactory oxidant selected will give a greater degree of selectivity. Too strong an oxidant may give nondescript colors ("over-oxidation").

2. Published HFMO calculations of radical cations will give an indication of the degree of stability of the colors formed by oxidation.

3. A series of heavy metal salts, useful as oxidants in TFA, in the order of increasing strength is: mercury(II), thallium(III), and lead(IV). For a given metal, the perchlorate salt will be a much more powerful oxidant than the acetate. Another useful oxidant is antimony(V) fluoride. The suitability of a given oxidant may depend not only upon its oxidation potential in TFA but also upon the extent of its Lewis acidity and its polarity.

4. The degree of Lewis basicity of the π-electron donor cosolvent will often affect the hue, intensity, and stability of the spot test. A variety of benzenes substituted with different $+I$ and $-I$ groups may be employed.

SUMMARY

Color formation observed in oxidation of aromatic compounds in trifluoroacetic acid will depend essentially upon three parameters, the latter two of which can be varied considerably: oxidation potential of the organic substrate and electrophilicity of its oxidation intermediates, oxidizing power of the reagent, and Lewis basicity of the cosolvent. The stability of the colors correlate well with theoretical predictions for radical cation stability.

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Solvent Effects in Photometric Analysis

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I. INTRODUCTION

One of the important tools in studies of the chemically altered human environment is photometric organic trace analysis. In biochemical and clinical studies this tool is used extensively. In pollution studies it is not yet used as extensively, probably because only in recent years has our poisoning of the human environment become recognized as a problem of some magnitude. Since solvent effects have proved useful in the photometric characterization and analysis of biochemical and clinical molecules and of the higher-boiling air pollutants (*1, 2*), these phenomena are believed to be important enough to warrant a more thorough study aimed at wider application to environmental problems, especially those concerned with air pollution. Of particular interest to health researchers is the composition of polluted atmospheres in terms of the families of aerotoxicants related to lung cancer, hay fever, asthma, and flu. For example, methods of characterization and assay for aeroallergens, co-allergens, anti-allergens, enhancers, sensitizers, and indicators would be of interest to all individuals concerned with understanding and controlling hay fever and asthma.

Since solvents are used extensively in ultraviolet absorption, colorimetric, fluorimetric, and phosphorimetric analyses, effects of the solvent on the wavelength position and intensity of the final chromogen, fluorogen, or phosphorogen become important. The solvent effect depends on the nature of the electronic transition of the final compound and on the properties of the solvent–solute system. The influence of a given solvent varies according to properties of the solute, such as the type of electronic transition of its long (usually) wavelength band, the dipole moment of the ground and excited states, and the acidity (basicity) of the ground and excited states. Electronic transitions can be some variety of $\sigma \rightarrow \pi^*$, $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, or charge transfer.

Attempts have been made to correlate macroscopic properties of solvents with their spectral effects. Some of these properties are dielectric constant, dipole moment, and refractive index. Other important factors that affect the spectra include hydrogen bonding, solvent–solute dipole interaction, and solvent–solute polarizability interaction (dispersion forces).

Present data indicate that solvent effects will be better understood once we know more about solvent–solute interaction in the microscopic volume of solvent concerned with the solute. This volume has been called "frozen" or an "iceberg" (*3*) and has been named the "cytobactic region" (*4*).

Before our main discussion we would like to depict the background of analysis of airborne particulates, Fig. 1. Polluted air is pulled through a glass-fiber filter. Particulate is extracted from the filter, and the extract is then separated. An example of an air pollutant that can be separated by thin-layer chromatography and then analyzed fluorimetrically is perylene. The sensitivity of analysis is strongly affected by solvent composition. On the other hand atmospheric phenols, which are best collected by impinger, are analyzed through the azo dye anion. The composition of these alkaline solvents strongly affects both wavelength position and intensity.

These and many other effects are discussed in this paper. Quenching techniques in fluorimetry are discussed only briefly, as are a few band types.

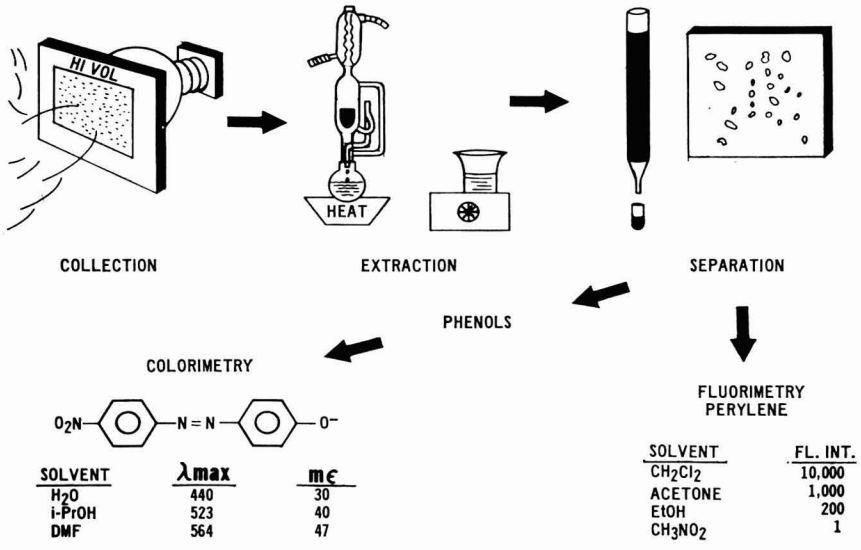


FIG. 1. Solvent effects—collection, extraction, separation, and photometric analysis of airborne particulates.

II. EXPERIMENTAL METHODS

Chemicals

All reagents, standards, and solvents were obtained in the purest form possible from commercial sources.

A 1 *M* solution of tetra-*n*-butylammonium hydroxide in methanol was obtained from Southwestern Analytical Chemicals, Inc., Austin, Texas.

Apparatus

Cary Model 11 recording absorption spectrophotometer with 1-cm path length 3-ml cells.

Aminco-Bowman spectrophotofluorimeters with the following settings: sensitivity 50; phototube RCA type 1P21; meter multiplier 0.01 or 0.03, dependent on the instrument, and slit arrangement no. 2.

Perkin-Elmer Hitachi spectrophotofluorimeter with the following settings: sample sensitivity reading 4; phototube R106; reference sensitivity direct; and 5-nm spectral bandwidth for both excitation and emission slits.

III. SOLVENT EFFECT FACTORS

A. Solute

The composition and the physical properties of the solute (e.g., chromogen, fluorogen, or phosphorogen) determine what type of solvent affects its spectral properties. The dipole moments in the ground and excited states affect the wavelength position and intensity of those molecules containing strong electron-donor and/or electron-acceptor resonance terminals. These are the types of molecules used as fluorescence or solvent polarity standards.

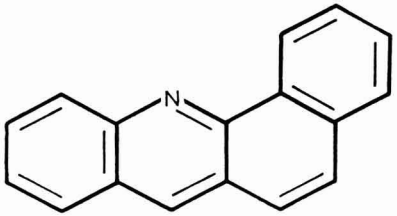
When a solute contains a heteroatom the polarizability effect can be enhanced, especially when the heteroatom is at one end of a conjugation chain. This type of molecule can bind with polar protic solvents by a hydrogen bond. This type of bond with a ring heteroatom can quench the fluorescence of a molecule, as shown by the effects of water and of phenol on the air pollutant, benz(*c*)acridine, Table 1.

On the other hand, in conjugated carbonyl compounds hydrogen bonding can enhance fluorescence, as shown by the spectrum of 1-pyrene aldehyde in ethanolic solution in a cell or on glass-fiber paper as compared with the spectrum of the compound in dioxane solution in Fig. 2.

Another important factor is the presence of active hydrogen in the solute. The effects of solvents containing heteroatoms on the fluorescence intensities of 5*H*-benzo(*b*)carbazole and 2-anilinonaphthalene are

TABLE 1

SOLVENT EFFECT AND FLUORESCENCE



Solvent	Factor	Relative fluorescence intensity
Dimethylformamide		10000
Ethanol		10000
p-Dioxane		4000
Acetone		4000
Ethanol–water (1:1)	Intermolecular hydrogen bond	800
Nitromethane	Excited state π complex	40
Dimethylaniline	Charge transfer fluor.	4
Phenol–water (9:1)	Intermolecular hydrogen bond	0

shown in Table 2. With acetone and pyridine the quenching effect is probably derived from the intermolecular hydrogen bond. The quenching effect is strong in pyridine because the hydrogen bond connects two conjugated systems. With nitromethane, benzo (*b*) carbazole probably forms a non-fluorescent excited-state pi complex.

A conjugated molecule that contains active hydrogen can also form anions in an appropriate solvent in either the ground or excited states, or both. Phenols (*5*), carbazoles (*6*, *7*), 9-acridanone (*8*), and aromatic amines (*9*) show this phenomenon.

In addition, compounds with active hydrogen can form tautomers in the ground and/or excited states. In this fashion the tautometric equilibrium and the derived spectrum can be drastically affected by solvent changes. Thus, 2,4-pentanedione is mainly in the keto form (85%) in water and in the enol form (96%) in hexane (*10*). In the latter solvent an intramolecular hydrogen bond stabilizes the enol; in the former solvent water breaks this bond to form an intermolecular hydrogen bond. The enol is favored with decreasing solvent polarity for 2,4-pentanedione, ethyl acetoacetate, and 1-phenylazo-2-naphthols. This is shown in Table 3 for ethyl acetoacetate, whose percentage enolization increases with a decrease in solvent polarity (increase in X_R) (*11*). These various tautomeric changes are reflected in the absorption spectra. A compound show-

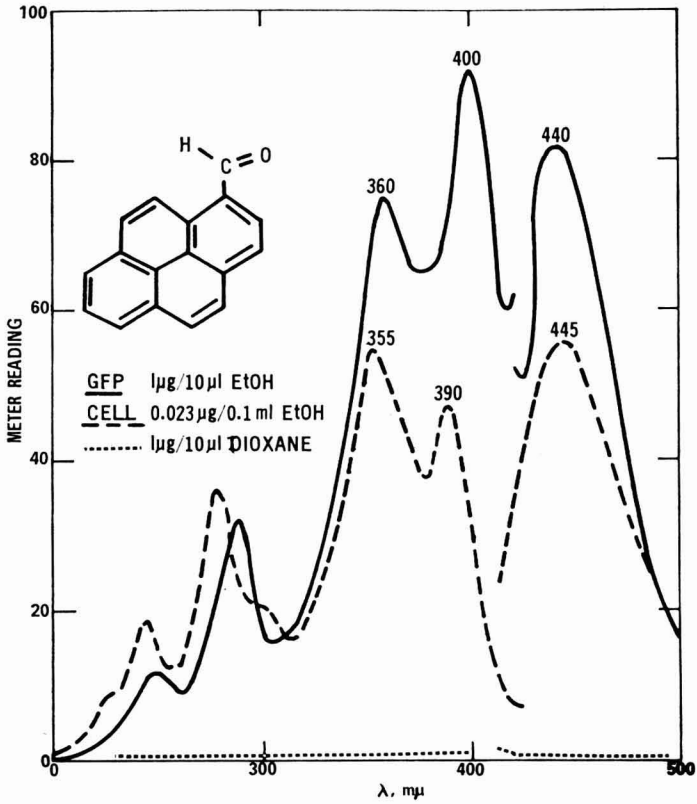


FIG. 2. Fluorescence excitation and emission spectra of 1-pyrene aldehyde on glass-fiber paper: spot wet with ethanol (——); spot wet with dioxane (. . . .); and in a cell in ethanol solution (– – –).

ing a drastic change in spectrum with solvent change is 1-hydroxyacridine, which is present in absolute alcohol as the pure enol (λ_{max} 400 nm) and in 20% aqueous alcohol in the keto form (λ_{max} 570 nm) (*12*).

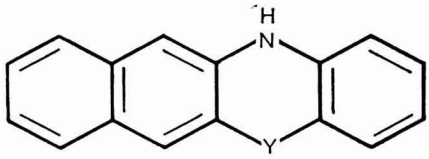
The phenylazonaphthols are useful analytical chromogens. The tautomeric equilibrium of 4-(*m*-tolylazo)-1-naphthol as affected by solvent changes is shown in Fig. 3 (*13*). With increasing solvent polarity the hydrazone tautomer absorbing at the longest wavelength increases at the expense of the azonaphthol tautomer.

The basicity or acidity of a molecule or of its resonance terminals or resonance nodes in the ground and excited states have varying effects on the spectra with variations in solvent.

The type of transition involved in the longest wavelength band affects the spectrum of the molecule, especially with solvent change.

TABLE 2

INTERMOLECULAR HYDROGEN BONDING AND FLUORESCENCE INTENSITY



Solvent	Relative fluorescence intensity	
	Y = —[a]	H_2
Dimethylformamide	1000	1000
95% Ethanol	350	800
Methylene chloride	300	350
p-Dioxane	300	300
Ethanol–water (1:1)	300	300
Phenol–water (9:1)	250	70
N,N-Dimethylaniline	100	80
Acetone	80	80
Pyridine	10	0.3
Nitromethane	0	0.0

[a] 5*H*-Benzo(*b*)carbazole.

B. Solvent

Reaction rates. Photometric analysis can be drastically affected by the effect of the solvent on the rate of reaction between the test substance and the reagent. Thus, in the determination of amino acids with ninhydrin, solvents such as alcohol, dioxane, 2-methoxyethanol, and pyridine–phenol accelerate the rate of formation of the final chromogen, λ_{max} 570 nm, mε 21.6 (*14*). In determination of cyanide with a *p*-benzoquinone, the rate of reaction is related to the dielectric constant of the solvent; dimethylsulfoxide is best, alcohol is worst (*15*). The reason is that reactions involving bases can go 10^{13} times faster in dimethylsulfoxide than they do in methanol (*16*).

TABLE 3

SOLVENT POLARITY AND PERCENTAGE ENOLIZATION OF ETHYL ACETOACETATE (*11*)

Solvent	X_R	Enolization (%)
Water	<35	0.4
Methanol	43.1	7.1
Ether	48.3	32.
n-Hexane	50.9	52.

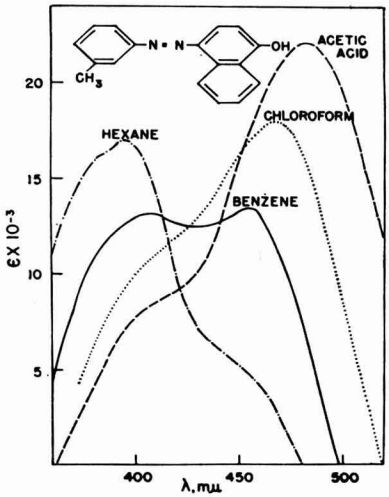


FIG. 3. Visible absorption spectra of 4-(*m*-tolylazo)-1-naphthol in hexane (——.; in benezene (——); in chloroform (. . . .); and in acetic acid (– –).

Dipole moment and dielectric constant. The physical properties of many solvents used in photometric analysis are shown in Table 4. The solvents are listed in the order of increasing solvent polarity of the *Zn* structure type (to be discussed).

Generally, the lower the temperature the higher the dielectric constant (*20*). The value of the dielectric constant decreases with increasing molecular weight of a homologous series (*21*). The relation between dielectric constant and structure has been thoroughly discussed (*22*).

Attempts to correlate the dielectric constant, the dipole moment, or the refractive index of a solvent with the spectral shift caused by changes in the solvent composition of the solutions of various chromogens have usually proved disappointing when studied thoroughly. However, these solvent properties do enable us to classify the type of changes taking place.

Amino acids have been found in every part of our environment. In trace amounts they are best analyzed as the fluorescent dansyl derivatives. The smallest amount thus measurable is about 3×10^{-13} mole (*23*). As Table 5 shows, the solvent with the lowest dielectric constant gives the best sensitivity in such an assay. The emission wavelength maximum position of dansyl-DL-tryptophan correlates better with solvent polarity, E_T, than it does with dielectric constant.

The 4-nitrophenylhydrazones of aromatic aldehydes are fluorescent in solvents of low dielectric constant such as dioxane, are nonfluorescent in alcohol at room temperature, and are fluorescent in alcohol and di-

oxane at liquid nitrogen temperatures (*24*). For example, *N,N*-diethyl-4-aminobenzaldehyde 4-nitrophenylhydrazone is fluorescent in *p*-dioxane, dielectric constant 2.2, and nonfluorescent in solvents of higher dielectric constant, such as chloroform, dimethylformamide, formamide, and *N*-methylformamide. Addition of methylcyclohexane, dielectric constant 2.02, to a dioxane solution of the hydrazone decreases the fluorescence intensity.

With acetophenone 4-nitrophenylhydrazone the dielectric constant of the solvent correlates fairly well with the positions of the excitation (absorption) spectral maxima and the fluorescence emission spectral maxima, and with the relative fluorescence intensity. In solvents of very high dielectric constant the molecule is not fluorescent at room temperature. Aniline is an exceptional solvent in this respect, probably because it forms a nonfluorescent excited-state complex with the hydrazone. These various results indicate the importance of factors other than the dielectric constant (Tables 5 and 6).

The compound, 4′-nitro-4-dimethylaminostilbene, has been recommended as a fluorescence standard (*25*). The dielectric constant appears to correlate with the fluorescence emission maxima, Table 7. This apparent correlation may be fortuitous, since the RCA 1P21 phototube used in the study is insensitive to energy at wavelengths greater than 600 nm. Thus, the data in Table 7 indicate that the stilbene derivative is nonfluorescent to the eye and to the 1P21 tube in solvents with a dielectric constant greater than $\sim$9. The data also show that the stilbene derivative can be used to calibrate the excitation source and the emission unit of a spectrophotofluorimeter.

Intermolecular hydrogen bonding. An intermolecular hydrogen bond can have a drastic effect on the absorption spectrum of a chromogen and a much more dramatic effect on the fluorescence spectrum of a fluorogen. Although the topic of hydrogen bonding has been covered in several reviews (*21, 26, 27*), the role of hydrogen bonding in photometry and photometric analysis has not as yet been thoroughly reviewed. Such a review would be extensive and would be invaluable to the analyst and the biochemist. A few aspects of hydrogen bonding are discussed in this section.

Ultraviolet-visible absorptiometric, fluorimetric, and phosphorimetric scales of empirical hydrogen-bonding values for solvents are needed in trace analysis since they would indicate the strength (in the ground and excited states) of the hydrogen bond between a particular class of test substances and various solvents. Infrared absorption spectroscopy has been the principle means for studying weak hydrogen-bonding interaction; absorption spectroscopy in the ultraviolet-visible region has been

TABLE 4
PHYSICAL PROPERTIES OF SOLVENTS USEFUL IN PHOTOMETRIC ANALYSIS

Solvent	Solvent polarity[a] *Zb* E_T(17,18)	*Zz* X_B(19)	*Zn* X_R(19)	Dielectric constant	Dipole moment	mp	Bp (°C; 760 mm)
Pentane	30.9		50.9	1.84	0.0	−130	36
Heptane	30.9		50.9	1.92	0.0	− 91	98
Methylcyclohexane			50.1	2.02	0.0		101
Cyclohexane			50.0	2.02	0.0		81
Triethylamine			49.3	2.42	0.77	−115	89
Carbon tetrachloride	32.5		48.7	2.24	0.0	− 23	77
Propyl ether			48.6	3.39	1.18	−122	90
n-Butyl ether			48.6	3.06	1.22	− 95	142
p-Dioxane	36.0		48.4	2.21	0.45	+ 12	101
Ethyl ether	34.6		48.3	4.34	1.15	−123	35
p-Xylene			47.7	2.27	0.00	+ 13	138
n-Butyl acetate			47.5	5.01	1.84		126
Toluene	33.9	41.7	47.2	2.38	0.39	− 95	111
Ethyl acetate	38.1		47.2	6.02	1.81	− 84	77
Benzene	34.5		46.9	2.28	0.00	+ 6	80
Tetrahydrofuran	37.4		46.6	7.39			
Acetonitrile	46.0	53.7	45.7	37.5	3.37	− 46	82
Acetone	42.2	50.1	45.7	20.7	2.72	− 95	56
1-Octanol			45.4		1.68		192
2-Butanone			45.4	18.5	2.75	− 87	80

Chlorobenzene	37.5		45.2	5.62	1.56	− 46	132
Methylene chloride	41.1	47.5	44.9	9.08	1.55	− 97	
Isopropanol	48.6	56.1	44.5	18.3	1.68	− 90	82
n-Butanol	50.2	56.8	44.5	17.1	1.68	− 90	118
Cyclohexanone			44.3	18.3	2.8	− 16	156
Chloroform	39.1		44.2	4.81	1.15	− 64	61
n-Propanol	50.7		44.1	20		−126	97
Nitromethane	46.3		44.0	35.9	3.17	− 29	101
Pyridine	40.2	50.0	43.9	12.3		− 42	116
Ethanol	51.9	60.4	43.9	24.3	1.68	−115	
Dimethylformamide	43.8	51.5	43.7	36.7		− 61	153
2-Methoxyethanol	52.3		43.5	16.0	2.04		124
Methanol	55.5	63.0	43.1	32.6	1.66	− 98	65
Dimethylacetamide			43.0	37.8			
Nitrobenzene	42.0		42.6	34.8	3.99	+ 6	211
Dimethylsulfoxide	45.0		42.0	46.4			
Aniline	44.3		41.1	6.89	1.51	− 6	184
Ethylene glycol[b]	56.3		40.4	37.7	2.28	− 13	198
m-Cresol			33.6	11.8	1.54	+ 12	203
Formamide	56.6			109.5			
Water	63.1	68.9		78.5			

[a] Solvent polarity = hc/λ_{max} (nm) = 28,600/λ_{max} (nm), where the λ_{max} is that of the chromogen in the solvent.

[b] Contains 2% of 2,6-lutidine.

TABLE 5

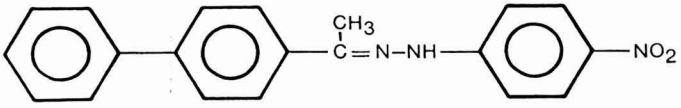
SOLVENT EFFECT ON THE FLUORESCENCE OF DANSYL DL-TRYPTOPHAN (*23*)

Solvent	Solvent polarity		Dielectric constant (D)	Emission λ_{max} (nm)[a]	Quantum yield
	E_T(*17,18*)	X_R(*19*)			
Water	63.1	~30	78.5	578	0.068
Glycerol			42.5	553	0.18
Ethylene glycol	56.3		37	543	0.36
Propylene glycol			32	538	0.37
Methanol	55.5	43.1	31.2	533	0.37
Ethanol	51.9	43.9	25.8	529	0.50
n-Butanol	50.2	44.5	19.2	519	0.50
Dimethylformamide	43.8	51.5	36.7	517	0.59
Acetone	42.2	45.7	21.5	513	0.35
Ethyl acetate	38.1	47.2	6.11	510	0.54
Chloroform	39.1	44.2	5.14	508	0.41
Dioxane	36	48.4	3.0	500	0.70
Cyclohexane	31	50	2.02	~468	

[a] Corrected.

TABLE 6

SOLVENT EFFECT ON FLUORESCENCE OF



Solvent	Dielectric constant (D)	λ_{exc} (nm)	λ_{em} (nm)	Relative fluorescence intensity
Toluene	2.4	400	460	40
p-Dioxane	2.2	410	485	90
Chloroform	4.8	410	525	25
t-Butanol	12.5	430	510	22
Benzyl alcohol	13.1	435	520	16
Dimethylformamide	36.7	460	540	2
Ethylene glycol	38.0	460	550	0.4
Aniline	6.9	—[a]		0.0
Ethanol–water (1:1)		—[a]		0.0
Phenol–water (9:1)		—[a]		0.0
Formamide	109.	—[a]		0.0
N-Methylformamide	182.	—[a]		0.0

[a] Nonfluorescent.

TABLE 7

ABSORPTION AND FLUORESCENCE EMISSION SPECTRA OF 4′-NITRO-4-DIMETHYLAMINOSTILBENE AS AFFECTED BY THE PHYSICAL PROPERTIES OF THE SOLVENT

Solvent	X_R	Dielectric constant (D)	λ_{abs} (nm)	λ_{em} (nm)
Pentane–cyclohexane (4:1)	50.8	~1.92	410	490fs[a]
Cyclohexane	50.0	2.02	417	500fs
Carbon tetrochloride	48.7	2.23	419	520fs
p-Dioxane	48.4	2.21	425	580
Toluene	47.2	2.44	428	545
Acetone	45.7	20.7	429	—[b]
Chloroform	44.2	4.81	437	600
Methylene chloride		9.08	436	—[b]
n-Propanol	44.1	20.0	427	—[b]
Ethanol	43.9	24.3	425	—[b]
Dimethylformamide	43.7	36.7	443	—[b]
Methanol	43.1	32.6	423	—[b]
Dimethylsulfoxide	42.0	48.9	449	—[b]
Ethylene glycol		37.7	441	—[b]
Water[c]	~30	78.5	403	—[b]
Formamide[c]		109.5	446	—[b]
N-Methylformamide[c]		182.4	441	—[b]

[a] fs = fine structure.

[b] Nonfluorescent to the eye or to the spectrophotofluorimeter equipped with an 1P21 phototube. With an RCA 7102 phototube λ_{em} 710 has been reported for this compound in an unspecified solvent (*25*).

[c] Contains 10% dimethylsulfoxide.

the principle method for studying interactions of strong or moderately strong acids and bases (*21*).

For preliminary studies approximate hydrogen-bonding strengths can be derived from the relative frequency shifts ($\Delta\gamma/\gamma \times 10^3$) in hydrogen bonding of pyrrole to protophilic organic solvents, Table 8 (*28*). Pyridine apparently is the strongest proton-acceptor, with ethers, ketones, and esters next in strength. Not unexpectedly, benzene and halogen derivatives show protophilic properties. There is no regular relation between the dielectric constant and the frequency shifts.

In studies of hydrogen bonding, log K_{BHA} has been used where the equilibrium constant, K_{BHA}, equals (B − HA)/(B)(HA). The larger the value of log K_{BHA}, the greater is the strength of the hydrogen bond. In a study of the association of pyridine with alcohols and phenols in carbon tetrachloride, log K_{BHA} decreases in the order *m*-chlorophenol > 1-naphthol > phenol > benzyl alcohol > methanol > ethanol > 1-

TABLE 8

RELATIVE FREQUENCY SHIFTS ($\Delta\gamma/\gamma \times 10^3$) IN HYDROGEN BONDING OF PYRROLE TO ORGANIC SOLVENTS

Solvent	$\Delta\gamma/\gamma \times 10^3$ (N–H)
n-Hexane	6.8
Carbon tetrachloride	8.5
Chloroform	12.5
Carbon disulfide	13.9
Chlorobenzene	14.7
1,2-Dichloroethane	18.4
Nitromethane	19.8
Benzene	20.4
Nitrobenzene	22.9
Mesitylene	25.8
Acetonitrile	30.6
Ethyl acetate	34.6
Acetone	39.6
Cyclohexanone	47.3
Dioxane	50.2
Ethyl ether	50.4
Pyridine	88.1

propanol > 1-butanol > 2-butanol > *t-butanol* (*21*). Acridine forms a more stable hydrogen bond with phenol in carbon tetrachloride than does pyridine (*29*). The association of phenol with various protophilic solutes in carbon tetrachloride solution decreases in the order di-*n*-butylamine > *n*-butylamine > dimethylpyridines > triethylamine > methylpyridines > acridine > pyridine > quinoline $\gg$ *p*-anisidine > aniline > *p*-chloroaniline $\gg$ *o*-fluoroaniline (*21*).

For an $n \rightarrow \pi^*$ transition, such as is found in many ketones, hydrogen bonding is considerably weakened in the excited state as compared with the ground state.

Phenols should associate more extensively in the excited state with protophilic solvents and solutes, since phenols are stronger acids in the excited singlet state, while protophilic solvents and solutes are stronger bases in the excited state. In this respect 1-hydroxypyrene associates with pyridine (pK_a 5.17) in methylcyclohexane with log K_{BHA} values of 2.34 in the ground state and 2.99 in the excited-singlet state (*30*).

With 2-chloropyridine, *p* K_a 0.72 in methylcyclihexane, the log K_{BHA} values are 1.27 in the ground state and 1.78 in the excited state. The stronger the base and the acid, the more extensive the association. The strength of hydrogen bonding between two solutes is greater in solvents of lower polarity.

Large amounts of data are scattered through the literature on the effect of the hydrogen bonding between solvent or solute and a chromogen. Thus, the amount of alkylation of the ammonium cation (and thus, the lack or presence of N–H groups) and the solvent composition determine the wavelength position of anionic resonance structures, such as bromophthalein magenta E or B (*31*). An intermolecular hydrogen bond between the ammonium cation and the dye causes a shift to violet.

As shown later, *Zn* resonance structures shift to red with increasing solvent polarity. Aqueous solutions produce an especially strong effect. However, with solutions of the fluorescence standard, 4′-nitro-4-dimethylaminostilbene, the wavelength absorption maximum shifts to red with increasing solvent polarity, Table 7. The exceptions are the alcohols and aqueous solutions; these absorb at the shortest wavelength, probably because of the intermolecular hydrogen bond.

In the same way the keto tautomer is stabilized most strongly by water and fairly strongly by methanol, Table 3.

Fluorescence intensity can be enhanced by intermolecular hydrogen bonding. Thus, chlorophyll shows little fluorescence in nonpolar solvents, such as anhydrous benzene (*32*). Addition of water or a protogenic solvent causes a remarkable enhancement of fluorescence. Concentrations of heptylamine or piperidine as low as that (5×10^{-6} *M*) of chlorophyll produce a marked increase in fluorescence intensity. The intermolecular hydrogen bond stabilizes the fluorescent keto tautomer.

The fluorescent keto tautomers of the 1-phenylazo-2-naphthols are favored in protogenic solvents (*33*). Here again the intermolecular hydrogen bond appears to be of some importance. The enol forms of 1-phenylazo-2-naphthol stabilized by an intramolecular hydrogen bond and 2-phenylazo-3-naphthol, which does not ketonize, are nonfluorescent.

Intermolecular hydrogen bonding can produce a dramatic effect on the fluorescence of conjugated carbonyl compounds. The atmospheric pollutant, *7H*-benz(*de*)anthracen-7-one, or benzanthrone, is not fluorescent in nonhydroxylic solvents such as dimethylformamide, cyclohexane, pyridine, acetone, and chloroform, Table 9. It is fluorescent in alcoholic solutions and is intensely fluorescent in aqueous solutions.

Many aromatic aldehyde 4-nitrophenylhydrazones and aromatic carbonyl compounds are nonfluorescent in dioxane and fluorescent in alcohol (*24*). As an example, Table 10 shows that 1-pyrene aldehyde fluoresces with greatest intensity in protogenic solvents. Even on glass-fiber paper this aldehyde fluoresces in alcoholic solution but not in dioxane, Fig. 2.

Acridine shows a similar phenomenon. In trifluoroethanol it is intensely fluorescent as the salt with relative fluorescence intensity (RFI)

TABLE 9

EFFECT OF INTERMOLECULAR HYDROGEN BONDING ON FLUORESCENCE OF BENZANTHRONE

Solvent	Relative fluorescence intensity[a]
Acetone	0.0
o-Dichlorobenzene	0.0
Chloroform	0.0
Dimethylformamide	0.0
Dioxane	0.0
Perfluorokerosene	0.0
Pyridine	0.0
Toluene	0.0
Phenol–water (9:1)	0.0
t-Butanol	2
Methanol	3
Acetone–water (8:2)	6
i-Propanol	20
Ethanol absolute	25
3-Phenyl-1-propanol	25
Ethylene glycol	35
Phenethanol	40
Benzyl alcohol	100
Acetone–water (1:1)	120
Methanol–water (1:1)	450
Acetone–water (3:7)	500
Acetone–water (5:95)	1000[b]

[a] In all cases the solutions were as dilute as possible. In all these solvents the excitation wavelength maximum varied from 390 to 400 nm, the emission wavelength maximum from 460 for weakly fluorescent solutions to 490 for strongly fluorescent solutions. For most hydroxylic solvents weaker excitation maxima are found near 250 and 310 nm.

[b] Product of meter multiplier and "transmittance" readings equals 1.7 at $5 \times 10^{-7} M$ benzanthrone.

= 1000. Other solvents show the following RFI values: alcohol–water (1:1), 100; alkanols, ~10 to 20; formamide and its *N*-methyl and *N,N*-dimethyl derivatives, 1 to 2; chloroform, methylene chloride, or tetrachloroethane, 1 or less; and triethylamine, mesitylene, toluene, cyclohexane, heptane, or phenol–water (9:1), 0. Figure 4 shows the superiority of aqueous solvents.

Intermolecular hydrogen bonding can also decrease the intensity of fluorescence. The fluorescence of acridine *N*-oxide is quenched in phenol, ethanol, or acetic acid, but not in anisole (*34*). Hydrogen bonds formed with the protogenic solvents in the excited state are weaker than those formed in the ground state

TABLE 10

EFFECT OF THE INTERMOLECULAR HYDROGEN BOND ON THE FLUORESCENCE INTENSITY OF 1-PYRENE ALDEHYDE[a]

Solvent	Relative fluorescence intensity
2,2,2-Trifluoroethanol	1000
Ethanol–water (1:1)	600
Formamide	600
Methanol	200
Ethanol, *n*-pentanol, or *n*-heptanol	100
t-Butanol or *s*-tetrachloroethane	80
Chloroform, *N*-methylformamide, or *t*-butylformamide	60
Methylene chloride	15
Phenol–water (9:1)	5
Dimethylformamide	2
Triethylamine or toluene	0
Cyclohexane or heptane	0

[a] Excitation wavelength maxima ranges from 390 to 395 nm; emission maxima from 415 (methylene chloride) to 460 [phenol–water (9:1)].

Traces of alcohol quench completely the fluorescence of thioindigo (*35*) or 4-amino-4′-nitro-stilbene (*36*) in benzene.

Water has unique properties as a protogenic solvent. Its molecule is much smaller than other hydrogen-bonding molecules; it has two active hydrogens and is probably a stronger hydrogen bonder than the alcohols. Thus, its effect on a chromogen or fluorogen can be drastically different from that of an alcohol.

This effect of water can be seen in the fluorescence properties of some types of compounds. One example is 4-hydroxy-1,5-naphthyridine, which exists as the fluorescent keto tautomer in polar solvents such as alcohol and acetonitrile and as the nonfluorescent enol form in nonpolar solvents such as dioxane and chloroform (*37*). The enol form has a long-life low energy $n \rightarrow \pi^*$ transition, which leaks out the absorbed energy through heat processes. The keto form is present in water as a nonfluorescent intermolecularly hydrogen-bonded molecule.

The dansyl amino acids are much less fluorescent in water than they are in the alcohols or in most other types of solvents. An example is dansyl DL-tryptophan, Table 5. In this molecule, classified as a *Zn* resonance structure, the excited state is more polar than the ground state, so that excited molecules would interact more strongly with polar solvents than would molecules in the ground state. Consequently, this molecule emits at longest wavelength in water.

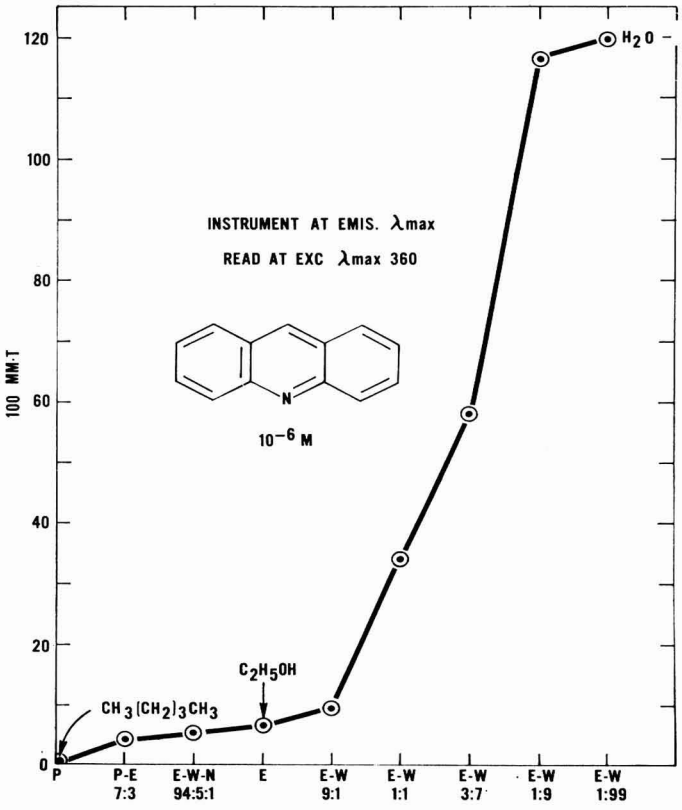


FIG. 4. Fluorescence intensity of acridine: P = pentane, E = ethanol, N = nitromethane, and W = water.

Somewhat similar molecules that are even more sensitive to water are the anilinonaphthalenesulfonic acids and analogous compounds. These compounds fluoresce weakly in water but fluoresce strongly in organic solvents or when bound to proteins. They emit at longest wavelength in water (*38*) and are used as fluorescent protein probes (*38, 39*). They are discussed more fully in the section on site effects.

Several properties of phenol make it of interest in fluorimetric analysis. It can form a strong intermolecular hydrogen bond with compounds containing an electronegative heteroatom. If the heteroatom is part of a conjugation system, the hydrogen bond connects two conjugated systems. The absorbed energy is then more readily dissipated through nonradiative processes and the fluorescence is partially or completely quenched. Thus, a minute concentration of phenol can quench the fluorescence of much larger concentrations of pseudoisocyanine in its polymerizate (*40*). *o*-Cresol quenches the fluorescence of the polynu-

clear aza arenes but not that of the arenes, imino arenes, and polynuclear aromatic amines (*41*). This quenching effect of aqueous phenol on benz(*c*)acridine is emphasized in Table 1, in which the phenolic solvent is compared with other solvents. In a similar fashion the fluorescence of acridine, 3,6-diaminoacridine, and 3,6-bis-(dimethylamino) acridine is quenched considerably by phenol, aniline, or pyrrol (*42*). Pyrrol (*41*) and 3-methylthioaniline (*9*) quench the fluorescence of the polynuclear aza arenes. Benzanthrone is not fluorescent in phenolic solution but is fluorescent in benzyl alcohol and phenethanol and is highly fluorescent in acetone–water (5:95), Table 9.

When compounds contain both carbonyl and hydroxy groups as key components of their fluorophoric systems, both phenols and pyridine tend to quench their fluorescence. Thus, scopoletin, a component of the atmosphere (*43*), shows a relative fluorescence intensity of 1000 in alkaline methanol (anion), 200 in benzyl alcohol, 5 in dioxane, 2 in phenol–water (9:1), and 2 in pyridine (anion). 1,4-Dihydroxyanthraquinone shows a similar phenomenon; it is fluorescent (RFI = ~1000) in acetone, nitromethane, alcohol, methylene chloride, and aqueous alcohol; less fluorescent in dimethylformamide (RFI 300) and *p*-dioxane (RFI 100); much less fluorescent in pyridine (RFI 3); and nonfluorescent in dimethylaniline and phenol–water (9:1).

Pyridine is also a powerful quencher; this is shown in Table 2 by the considerably decreased fluorescence of benzocarbazoles and of 2-anilinonaphthalene in pyridine solution as compared with fluorescence in other solvents.

Quenching of the fluorescence of fluorogens by phenols or pyridines can be ascribed to interaction between π electron systems of fluorescer and quencher molecules via hydrogen bonds between proton donor and acceptor (*42*).

Phenol itself forms a nonfluorescent complex with *N,N*-dimethylacetamide (*44*). Small amounts of phenols have also been determined through a method based on the quenching effect of phenols on the fluorescence of luminol in the presence of hydrogen peroxide, cupric ion, and aqueous ammonia (*45*).

Solvent polarity. In this paper, solvent polarity is defined as the sum of all intermolecular interactions between solvent and chromogen that affect the absorption spectrum of the chromogen, except those interactions that lead to definite chemical changes in the chromogen as a result of protonation, deprotonation, oxidation, reduction, complex formation, etc. Since the spectral effects of solvents on a solute cannot be described by a single parameter, attempts have been made to measure spectral effect empirically with appropriate chromogen standards whose

wavelength maxima are very sensitive to solvents. Ideally this standard would be soluble in all solvents; actually it should be soluble in a wide variety of solvents under experimental conditions. The value of solvent polarity for any solvent is arbitrarily selected to be inversely proportional to the wavelength maximum of the standard in that solvent, e.g., $E_T = hc/\lambda = 28{,}600/\lambda_{max}$, (nm). There is one other criterion for selecting the standard: the ΔE_T value for the solvent of highest polarity and the solvent of lowest polarity should be as large as possible so that the polarity of even fairly similar solvents can be readily differentiated.

Solvent polarity depends on the action of intermolecular forces between the solvent and solute, e.g., coulomb, directional, inductive, dispersion, and charge-transfer forces, as well as hydrogen-bonding forces. The interaction can be between solvent-solute dipoles and solvent-solute polarizability.

Nonabsorptiometric methods of determining solvent polarity are the *Y* values (an empirical measure of the ability of a solvent to solvate ions) (*46–48*), the (log *k* ion) values of the ionization of *p*-methoxyneophyl *p*-toluenesulfonate (*49*), X values from the reaction of bromine with tetramethyltin (*50*), and Ω-values from the addition of cyclopentadiene to methyl acrylate (*51*).

The standards used to determine solvent polarity by absorptiometric methods are shown in Table 11. Of the zwitterionic resonance, *Zn* type, number 1 has the largest ΔE_T value and so is most sensitive to solvent changes. Among the *Zz* types for which the dipole moment is larger in the ground state than the excited state, 6 has the largest ΔE_T. Number 7 can be used for colorimetric determination of pyridine in water or vice versa (*52*). Among the *Zb* types 9 has the largest ΔE_T. The solvent effect is so dramatic in this type that the color of the solution of this betaine is red in methanol, blue in isopropanol, and green in acetone. Its analog, 14, also shows striking color changes, Table 12 (*18*).

The standard, 10, shows even more striking changes to the eye, e.g., violet (λ_{max} 563) in methanol, blue (λ_{max} 610) in ethanol, and green (λ_{max} 707) in isopropanol.

Comparison of the intermolecular charge-transfer iodides shows that 12 has been most thoroughly investigated, while 13 has the largest ΔE_T.

Good comparisons with rate and spectroscopic data have been obtained for 1 (*19*), 6 (*19*), 9 (*17*), and 12 (*4*).

Unfortunately, few of these standards are readily available commercially, except for 11 and 12.

Solvent polarity could also be developed from the aspect of luminescence transitions, e.g., $\pi \leftarrow \pi^*$, $n \leftarrow \pi^*$, etc. The fluorescence probes could be used as singlet $\pi \leftarrow \pi^*$ type standards for investigation of sol-

TABLE 11

SOLVENT POLARITY INDICATORS

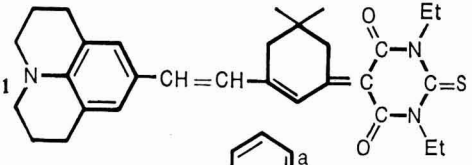
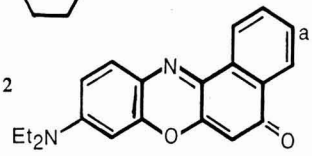
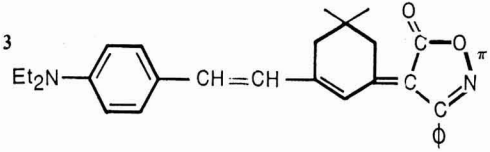
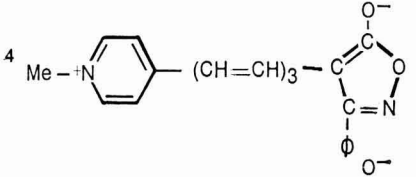
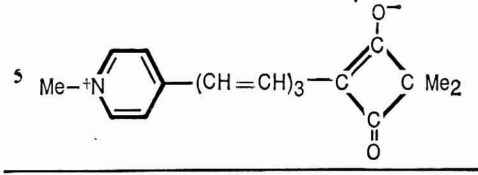
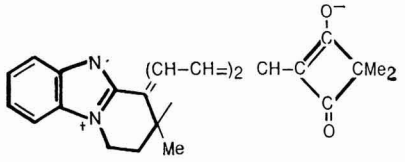
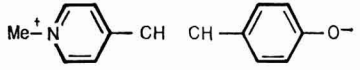
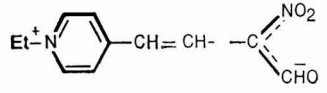
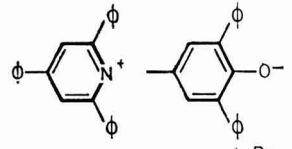
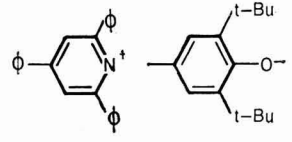
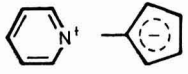
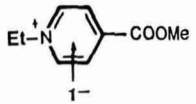
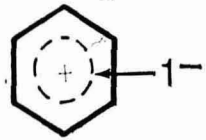
	Indicator structure	Type of transition	Dipole moment (D)		Nonpolar		Polar		ΔE_T (kcal/mole)	Ref.
			Ground	Exc. state	Solvent	λ_{max} (nm)	Solvent	λ_{max} (nm)		
1		$\pi \rightarrow \pi^*$ *Zn*	Moderate	High	Isooctane	562	Water–lutidine (8:2)	747	12.6	(*19*)
2		$\pi \rightarrow \pi^*$ *Zn*	Moderate	High	2,2,4-TriMe-pentane	508	Formamide	563	5.5	(*51*)
3		$\pi \rightarrow \pi^*$ *Zn*	Moderate	High	Isooctane	508	Water–lutidine, (9:1)	632	11.3	(*19*)
4		$\pi \rightarrow \pi^*$ *Zz*	High	Moderate	2,6-Lutidine	740	Water	488	19.8	(*19*)
5		$\pi \rightarrow \pi^*$ *Zz*	High	Moderate	Toluene	732	Water	458	23.3	(*19*)

TABLE 11 Continued

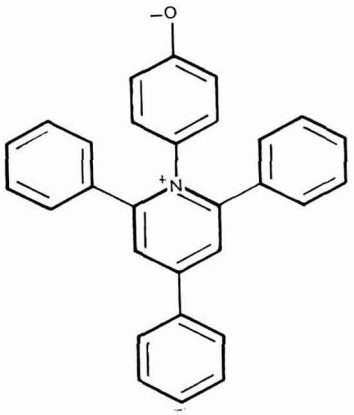
	Indicator structure	Type of transition	Dipole moment (D)		Nonpolar		Polar			
			Ground	Exc. state	Solvent	λ_{max} (nm)	Solvent	λ_{max} (nm)	ΔE_T (kcal/mole)	Ref.
6		$\pi \rightarrow \pi^*$ *Zz*	High	Moderate	Toluene	685	Water	415	27.2	(*19*)
7		$\pi \rightarrow \pi^*$ *Zz*	High	Moderate	Pyridine	605	Water	444	17.1	(*52*)
8		$\pi \rightarrow \pi^*$ *Zz*	High	Moderate	Benzene	519	Water	424	7.6	(*53*)
9		$\pi \rightarrow \pi^*$ *Zb*			Phenyl ether	810	Water	453	27.8[b]	(*17,18*)
10		$\pi \rightarrow \pi^*$ *Zb*	14.8	6.0	Toluene	867	Ethylene glycol	560	18.1	(*18*)
11		$\pi \rightarrow \pi^*$ *Zb*	High	Moderate	Heptane	557	Water	453	11.8	(*54*)

12		ICT[c]	~13.9	~8.6	Chloroform	453	Ethanol–water (7:3)	331	23.2[d]	(4)
13		ICT	High	Moderate	CH_2Cl_2	571	Water	346	32.5	(55)

[a] Nile blue A oxazone.
[b] Extrapolated to 32.2 with *n*-hexane, although dye insoluble in this solvent.
[c] Intermolecular charge-transfer complex.
[d] Extrapolated to 30.6 with water although insoluble in this solvent.

TABLE 12

HYDROLYLIC SOLVENTS AND THE ABSORPTION SPECTRA OF 2,4,6-TRIPHENYL-*N*-[4-HYDROXY-PHENYL]PYRIDINIUM BETAINE (*18*)



Solvent	λ_{max} (nm)(mϵ) Band 1	Band 2
Water	302 (34.1)	412 (2.48)
Glycol	311 (28.7)	437 (2.55)
Methanol	306 (38.7)	442 (2.75)
Ethanol	307 (31.5)	467 (2.33)
n-Propanol	307 (29.6)	477 (2.60)
n-Butanol	307 (29.5)	484 (3.16)
i-Propanol	307 (34.2)	501 (3.32)
i-Amyl alcohol	310 (34.0)	516 (3.30)
tert-Butanol	308 (35.4)	560 (3.50)

vent polarity. Thus, the fluorescence yield, emission maximum, and bandwidth of 1-anilinonaphthalene-7-sulfonate in a variety of solvents vary as a function of Z, Kosower's empirical solvent polarity scale (*4, 38*). In the same way the fluorescence emission maximum of dansyl DL-tryptophan correlates with the E_T solvent polarity scale, Table 5 (*23*). Dansylglycine has a ΔE_T of 11.6 in going from water to cyclohexane. The fluorescence standard, 4′-nitro-4-dimethylaminostilbene, is also worth investigating as a fluorescence solvent polarity standard, Table 7.

As discussed later the effect of a change in solvent polarity depends primarily on the nature of the electron transition under study.

Solvent acidity. In photometric analysis of a test substance it is necessary sometimes to form a cationic resonance structure that absorbs at longer wavelength, farther from the interfering substances. Acidity of the solvent is important in such a case. The common hydroxylic solvents

are very weakly acidic, e.g., methanol, pK_a 17.7; water pK_a 18.2; ethanol, pK_a 18.3; and isopropanol, pK_a 19.4 (*56*). The stronger acids are discussed in the section on types of solvents.

Solvent basicity. The basicity of a solvent increases with an increase in the solvent polarizability plus a decrease in the protogenic strength of the solvent. Anions in dipolar aprotic solvents have no general hydrogen-bonding interaction with the solvent, as they have in protic solvents, and are thus much less solvated and more reactive in the dipolar aprotic solvents (*57*). Since anions are much stronger bases, relative to neutral bases, in dipolar aprotic solvents than in protic solvents, much higher basicities can be obtained in dipolar aprotic solvents.

In trace analytical work in which the final chromogen or fluorogen is an anion, sensitivity may be much poorer than it should be because the anion is in equilibrium with the neutral compound. An example of this phenomenon is the fluorogen obtained in the analysis of malonaldehyde and its precursors, deoxyribose and DNA, with 4′-aminoacetophenone (*58, 59*). With increasing solvent basicity a larger amount of fluorogen anion is present, Table 13. In solvents such as trifluoroethanol, water, and methanol the compound is essentially nonionized. The bands at *F* 460/520 are either derived from the nonionized compound or from impurities.

Since the long-wavelength maximum of an anionic resonance structure moves to the red with increasing solvent basicity, Table 14, the authors believe that solvent basicity could be reported in terms of $E_T = hc/\lambda_{max}$ values. We are arbitrarily calling this the absorptiometric basicity or AB value. We have chosen 4-(*p*-nitrophenylazo)phenol as the standard. It is readily prepared pure and shows a very large shift with solvent basicity changes (*60*). The values obtained with the standard

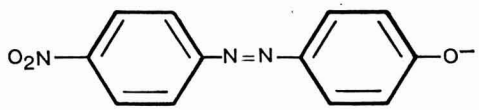
TABLE 13

EFFECT OF SOLVENT BASICITY ON IONIZATION AND FLUORESCENCE INTENSITY
Ac—C_6H_4—NH—CH=CH—CH=N—C_6H_4—Ac

+1% BAH	H_-	$F_{exc/em}$	Relative fluorescence intensity
$CF_3CH_2 \cdot OH$		460/520	0.3
$H \cdot OH$	12.01		0.0
$CH_3 \cdot OH$	12.66	460/525	0.2
$CH_3CH_2 \cdot OH$	14.57	485/570	0.3
$(CH_3)_2CH \cdot OH$	16.95	500/575	25.0
$(CH_3)_2C(Et) \cdot OH$	18.09	505/570	40.0
$(CH_3)_3C \cdot OH$	19.14	505/575	100.0

TABLE 14

CORRELATION BETWEEN SOLVENT BASICITY AND POSITION AND INTENSITY OF THE LONG-WAVELENGTH MAXIMUM OF



Solvent	H_-[a]	λ_{max}[b] (nm)	$m\epsilon$
Water	12.01	440	30.0
Methanol	12.66	485	31.0
Ethanol	14.57	505	35.6
i-Propanol	16.95	523	39.8
t-Pentanol	18.09	540	35.6
t-Butanol	19.14	538	38.6
Dimethylformamide	>20	564	47.3

[a] Containing 0.1 *M* alkali metal alkoxide.
[b] Containing 0.1 *M* tetrabutylammonium hydroxide.

dye are reported in Table 15. With the help of this dye and the standardized values the AB value of a basic solvent mixture can be determined.

The effect of base on the AB value was determined. One percent solutions of various organic bases in tetramethyl urea gave the following wavelength maxima: 23% methanolic tetra-*n*-butylammonium hydroxide, 603; 40% aqueous tetraethylammonium hydroxide, 603; 35% methanolic benzyltrimethylammonium hydroxide, 600; and tetra-*n*-propylammonium hydroxide, 600. A 24% methanolic solution of tetramethylammonium hydroxide precipitates in tetramethylurea.

TABLE 15

AB VALUES OF ALKALINE SOLVENTS CONTAINING 4-(*p*-NITROPHENYLAZO)PHENOL

Solvent[a]	λ_{max} (nm)	AB value[b]
Water	472	60.6
Methanol	483	59.2
Ethylene glycol	490	58.4
Formamide	500	57.2
Ethanol	505	56.6
Isoamyl alcohol	512	55.9
n-Propanol	513	55.8
Xylene	513	55.8
n-Butanol	514	55.6
Benzyl alcohol	514	55.6
Pyrrole	516	55.4
n-Methylformamide	516	55.4

TABLE 15 (Continued)

Solvent[a]	λ_{max} (nm)	AB value[b]
2-Methoxyethanol	518	55.2
Benzene	519	55.1
Phenylacetylene	528	54.2
Ethyl iodide	534[c]	53.6
Ether–Perfluoro(methylcyclohexane) (4:1; v/v)	535	53.5
Ether	537	53.3
Chloroform	539[d]	53.1
Nitromethane	539	53.1
tert-Amyl alcohol	542	52.8
1,2-Dichloroethane	543	52.7
Acetonitrile	546	52.4
p-Dioxane	546	52.4
tert-Butanol	547	52.3
Bis(2-ethoxyethyl) ether	550	52.0
Tetramethylene sulfone	551	51.9
Methylene chloride	551	51.9
2-Pyrrolidone	552	51.8
n-Butyl acetate	552	51.8
2-Nitropropane	554	51.6
Tetrahydrothiophene	556	51.4
Dimethyl phthalate	558	51.3
Aniline	562	50.9
Acetone	563[d]	50.8
Diethylamine	564	50.7
Tetrahydrofuran	565	50.6
Propylene oxide	572	50.0
n-Butylamine	573	49.9
2-Butanone	575	49.7
Pyrrolidine	582	49.1
1-Methylimidazole	585	48.9
1,1-Dimethylhydrazine	586	48.8
Tributyl phosphate	591	48.4
Dimethylformamide	591	48.4
Tetramethylene sulfoxide	596	48.0
Dimethylsulfoxide	596	48.0
Pyridine	597	47.9
1,3-Diaminopropane	597	47.9
N-Methyl-2-pyrrolidone	602	47.5
1,1,3,3-Tetramethylurea	603	47.4
Tetraethylenepentamine	606	47.2
Hexamethylphosphoramide	617	46.4

[a] All solvents contain 1% by volume of 1 *M* methanolic tetra-*n*-butylammonium hydroxide.

[b] Absorptiometric basicity value = $E_T = hc/\lambda_{max}$ (nm) = $28{,}600/\lambda_{max}$ (nm).

[c] Solution was slightly turbid.

[d] Rapid decolorization.

The usefulness in trace analysis of a knowledge of solvent basicity is demonstrated in the determination of aniline with *p*-nitrobenzenediazonium fluoborate (*60*). With water as the solvent, λ_{max} 490 nm and millimolar absorptivity ($m\varepsilon$) 14.0 are obtained; with dimethylformamide as solvent λ_{max} 570 nm and $m\varepsilon$ 55.6 are obtained. The fourfold increase in intensity and the stable brilliant purple color produced in the latter procedure make it the method of choice.

Adsorption and allied effects in aqueous solution. A biopolymer in aqueous solution can affect the spectra of an adsorbed fluorogen, chromogen, or phosphorogen through noncovalent interaction. Some of the most varied and complex biopolymers are the proteins, with their intricate three-dimensional structures. These compounds usually have hydrophobic amino acid residues clustered on the inside of the folded molecule and hydrophilic side chains present on the surface of the folded protein so as to favor interaction with aqueous solutes (*39*). Attempts are being made to understand the intricate three-dimensional or tertiary structure of proteins with the help of colorimetric, fluorimetric, and phosphorimetric probes. Fluorescence probes have seen the most use recently, Table 16. They may be defined as small, weakly fluorescent (in water) molecules that undergo large changes in wavelength and/or intensity as a result of interaction through space with a protein or other macromolecule. The requirements for optimum use of an extrinsic fluorophore are that it be bound or covalently attached to the protein at or near the active site, that it be sensitive to the environment of the amino acid side chains, and that insertion of the probe should not appreciably disturb those features of the protein that are being investigated (*75*).

Some knowledge of the diverse selectivities of various dye binders can be valuable also in analysis of aeroallergens; for example, albumin reacts with 1,8-ANS, whereas σ-globulin and the pollen protein from short ragweed do not react (Fig. 5). Because of this interaction, 1,8-ANS has been used for the fluorimetric analysis of serum albumin (*75a*).

Conclusive evidence has shown that many aeroallergens are present in indoor and outdoor atmospheres (*76*). The use of fluorescence and other types of probes will be invaluable in determining the active sites of those various aeroallergens that are protein or glycoprotein in nature. With knowledge of the active sites, the biochemical mechanisms of allergic reactions involved in hay fever and asthma will be better understood.

Colorimetric probes are not as well developed as the fluorescence probes. However, it is obvious that the various types of solvent polarity standards, and especially those of the zwitterionic resonance type, could be modified into sulfonic acid derivatives and could then be used as

TABLE 16

FLUORESCENT PROBES OF POLARITY IN PROTEINS

Probe[a]	$F_{em}(Q)$[b]	Protein	$F_{em}(Q)$[c]	Study	Ref.
1,8-ANS	515(0.004)	Apomylogobin	454(0.98)	Binding in heme crevice	*(61)*
1,8-ANS		Bovine serum albumin		Acid expansion of proteins	*(62,63)*
1,8-ANS		Bence-Jones proteins		Thermal denaturation of proteins	*(64)*
1,8-ANS[d]	55(0.0032)	19 Enzymes	479–491[e]	Polarity of protein binding sites	*(38)*
1,7-ANS	515(0.0091)	19 Enzymes	446–476	Polarity of protein binding sites	*(38)*
1,5-ANS	529(0.0099)	19 Enzymes	460–484	Polarity of protein binding sites	*(38)*
2,6-TNS	500(0.002)	19 Enzymes	422–464	Polarity of protein binding sites	*(38)*
2,6-TNS		Antibodies		Interaction	*(65)*
2,6-TNS	500(0.0008)	13 Proteins	426–462 (0.02–0.56)	Hydrophobic probe	*(66)*
2,6-TNS	500(0.008)	α-Chymotrypsin	446(0.18)	Competitive inhibitors	*(66,67)*
2,6-TNS		Chymotrypsinogen A	436(0.07)	Conversion to chymotrypsin	*(66,68)*
Dansylamino acids[f]	578-580 (0.053-0.091)	Bovine serum albumin Apomyoglobin (sperm whale) Apomyoglobin (horse heart)[g]	485	Hydrophobic probe	*(69)*
Dansyl L-proline	578(0.0.053)	Serum albumin[h]	480–505	Hydrophobic probe	*(69)*
5-(4′-Arsonoanilino)-2-chloro-7-methoxy-acridine					*(70)*
Dansyl amide		Carbonic anhydrase			*(71)*
e-Dansyl lysine	556(0.029)	Antidansyl antibody	500(0.85)	Antibody combining site	*(72)*
2,6-Mansyl chloride	Nonfluor.	Blood serum albumin	450	Denaturation of covalent albumin derivative	*(73)*
p-Nitrophenyl anthranilate	Nonfluor.	Chymotrypsin		Active site of chymotrypsin	*(74)*

[a] 1,8-ANS = 1-anilino-8-naphthalenesulfonate; 2,6-TNS = 2-*p*-toluidino-6-naphthalenesulfonate; Dansyl = 1-difethylamino-8-naphthalenesulfonyl; 2,6-Mansyl chloride = *N*-methyl-2-anilinonaphthalene-6-sulfonyl chloride.

[b] Fluorescence emission of probe in aqueous solution; Q = quantum yield.

[c] Fluorescence emission of probe in aqueous solution of protein.

[d] Some properties of 2,6-ANS and its *N*-methyl derivative, 2,6-MANS, described.

[e] Range in emission wavelength maxima.

[f] Six studied.

[g] And nine other proteins giving much lower fluorescence quantum yields with reagent.

[h] Of canine, porcine, bovine, human, equine, and rabbit species.

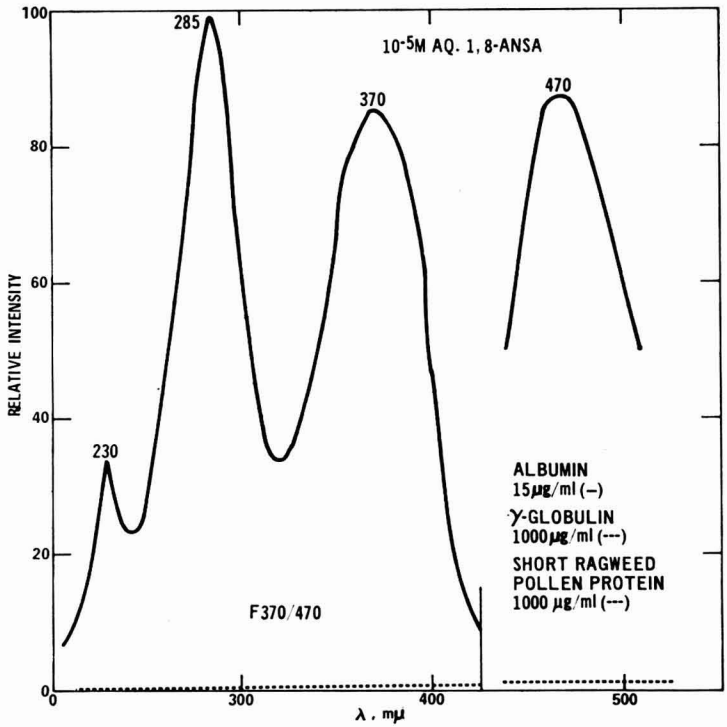


FIG. 5. Fluorescence excitation and emission spectra of 10^{-5} *M* aqueous 1-anilino-8-naphthalenesulfonic acid (1,8-ANSA): Solution or 1000 μg of γ-globulin/ml of solution or 1000 μg of short ragweed pollen protein/ml of solution (- - -), and 15 μg of albumin/ml of solution (——).

powerful probes of site polarity in proteins. This site polarity of the various proteins could also be used in photometric analysis in water, as shown in Fig. 5.

The binding of vividly colored cationic resonance structures in aqueous solution to anionic polymers results in a shift in the absorption spectrum and/or a change in the fluorescence properties of the dye. Nucleic acids have been analyzed and their structures have been studied with these types of dyes. When 2,7-diamino-9-phenyl-10-ethylphenanthridinium bromide binds to a nucleic acid, the fluorescence wavelength maximum shifts and the intensity increases by a factor of 50 to 100 (*77, 78*). With this method both nucleic acids and DNA (with the help of ribonuclease) have been determined. It is believed that this binding is specfic for double-stranded regions of polynucleotides wherein the dye is intercalated. Site effects have also been studied for the nucleic acids with other cationic resonance structures, e.g., acridinium salts (*79*) and 3,6-

diaminoacridinium salts (*79–82*). The spectral changes of acridine orange produced with DNA are stated to be due to binding of the dye with the deoxyadenosine and deoxyguanosine moieties of DNA (*83*).

Site effects in nucleic acids have also been studied phosphorimetrically. In the presence of proflavine, the DNA phosphorescence with a peak near 460 nm diminishes and two new peaks appear; the emissions around 580 and 495 nm have been ascribed, respectively, to the phosphorescence and delayed fluorescence of the dye bound to DNA (*84*).

Methods for the analysis of mucopolysaccharides have been developed that make use of the metachromatic reaction of acidic polymers with histological dyes such as toluidine blue (*85, 86*). A spectral shift of the cationic resonance structure, 1-ethyl-2-(3-(1-ethylnaphtho-(1,2-*d*)-thiazolin-2-ylidene)2-methylpropenyl)-naphtho(1,2-*d*)thiazolium bromide, can be brought about by small amounts of anionic polymers (*87*). This dye has been used to estimate acidic polysaccharides (*88*): Absorbance of the dye at 508 nm is reduced, while increased absorbance in the region of 600 to 645 nm is produced by 15 acidic polymers. The shift of the spectrum of a cationic resonance dye toward longer wavelengths is believed to be caused by aggregation of the dye molecules in a highly ordered array absorbed onto the polymer (*89*).

Fluorescence and phosphorescence can also be affected by binding of appropriate compounds to polymers in aqueous solution. When polymethylacrylic acid at pH 5.0 is added to a solution of crystal violet or ethyl violet at the same pH, the color shifts from purple to deep blue and the nonfluorescent solution becomes fluorescent (*90*). The fluorescence of bound ethyl violet shows concentration quenching even at concentrations of dye as low as $3 \times 10^{-6} M$. The aggregation of dye molecules can result in an increase in the quantum yield of phosphorescence at the expense of fluorescence (*91*). For example, the phosphorescence of acridine increases when it is bound to the nucleotide, adenosine 5′-monophosphate (*92*).

The spectral consequences of adsorption of chromogens, fluorogens, and phosphorogens are diverse in nature and extent and are promising in terms of further usefulness. The aggregation of dye molecules and the interaction of probes with biopolymers at, or near, active sites can result in changes in absorption and emission spectra, changes in polarization, and changes in the intensity of fluorescence and phosphorescence. The application of these phenomena to aeroallergens and other air pollutants needs to be investigated.

Solid adsorbent effects. Salicylaldehyde, a component of auto exhaust, is nonfluorescent as the anion in aqueous solution; on a dry paper chromatogram, however, the anion is intensely fluorescent, Fig. 6. Although

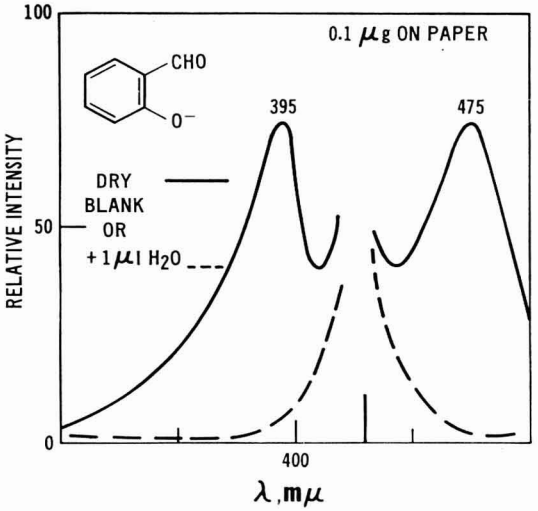


FIG. 6. Fluorescence excitation and emission spectra of 0.1 μg of salicylaldehyde anion: dry spot on paper (——); and spot wet with water or blank area of paper (– –).

acridine and 1-pyrene aldehyde are nonfluorescent in nonhydroxylic solvents, they are fluorescent in the dry state on cellulose, silica gel, and alumina TLC plates. One hundred ng can be readily characterized through the fluorescence spectra. Many examples of such differences in fluorescence properties on a chromatogram and in solution are known (*93*).

Solvent impurities. Since nanogram to microgram amounts of some compounds in a 1-ml volume of solvent can be intensely fluorescent, solvent purity is especially important in fluorimetric analysis. Whenever solvent concentration is necessary, fluorescent solvent impurities become a serious problem (*94*). Solvent impurities can affect the rate of reaction, the yield of chromogen, etc., and the forcing of a reaction in a different direction. Too many times attempts to duplicate some results obtained by photometric analytical methods have resulted in failure primarily caused by impurities in the solvent.

In many hydroxylic solvents aldehyde impurities are the main problem. Thus, in the spectrophotometric determination of aliphatic aldehyde 2,4-dinitrophenylhydrazones with 3-methyl-2-benzothiazolinone hydrazone, the use of 2-methoxyethanol contaminated with aldehydes will give a highly colored blank (*95*). In the determination of 17-hydroxycorticosteroids with phenylhydrazine and sulfuric acid, the use of *n*-butanol contaminated with aldehydes will result in a negative reaction (*96*). Another type of effect is shown in the determination of fructose with resorcinol (*97*). When pure alcohol or acetic acid is used as the

solvent, a wavelength maximum of 480 nm is obtained; when the alcohol or acetic acid is contaminated with traces of acetaldehyde, a wavelength maximum of 550 nm is obtained.

IV. TYPES OF SOLVENTS

Solvents have been classified on the basis of their acidic and/or basic properties, dielectric constants, and hydrogen-bonding strengths. On the basis of these properties Bronsted (*98*) subdivided solvents into eight classes. Solvents such as water and the alcohols were considered both protogenic (capable of supplying a hydrogen for a hydrogen bond) and protophilic (capable of accepting a hydrogen toward formation of a hydrogen bond) and so were termed amphiprotic. Nonpolar solvents represented the opposite extreme in properties and were classified as aprotic. On the other hand Popov (*99*) and Kratochvil (*100*) classified solvents on the basis of their Lewis acid–base properties and their dielectric constants.

In this paper solvents are classified on the basis of their effect on the absorption, fluorescence, and phosphorescence spectra of conjugated organic compounds. The classification is complicated because it is derived from differing and interrelated solvent properties such as dielectric constant; dipole moment; solvent polarity; acidity, basicity, or amphoteric properties; protogenic, protophilic, or amphiprotic properties; solvent polarizability; and solvent pi-acid or base strength.

The solvents have been divided into 12 classes; each of these classes has been further subdivided, and even some of the subclasses have been subdivided, Table 17. The first class consists of the nonpolar to weakly polar solvents. The least polar of these are the aliphatic hydrocarbons, of which pentane has the lowest polarity with a dielectric constant of 1.84. With this type of solvent the spectra of arenes and heterocyclic arenes show the narrowest bands and the most fine structure. For example in the column chromatographic (*101, 102*) and thin-layer chromatographic (*103, 104*) analysis of atmospheric benzo(*a*)pyrene (BaP), the absorption spectrum of BaP shows bands at wavelengths 377, 379, and 382 nm in pentane (refractive index, 1.3601 at 18°C) as compared to wavelengths 378 and 382 nm in methanol (refractive index, 1.3288 at 20°C and wavelengths 378s and 383 nm in cyclohexane (refractive index, 1.4266 at 19.5°C), Fig. 7. Here, the polarity and refractive index of the solvent appear to affect the fine structure of BaP. Thus, pentane is the ideal solvent for the spectrophotometric analysis of atmospheric BaP (*101*, *102*).

Even for more polar chromogens the vibrational fine structure is more prominent in a nonpolar solvent than in a polar solvent. Thus, Nile

TABLE 17

CLASSIFICATION OF ANALYTICAL SOLVENTS

Solvents	Dielectric constant, *d*	Dipole moment (D)	Misc. properties
1. Nonpolar to weakly polar			
a. Aliphatic hydrocarbons			
Pentane, heptane, cyclohexane	1.8–2.0	0.0	
b. Benzene and simple derivatives			
1. Alkylbenzenes	2.3–2.8	0–0.4	m-pp
2. Halobenzenes[b]	2–6	0–1.6	w-pp
c. Halogenated alkanes[b]			
1. Chloroform, methylene chloride	4.8–9.1	0–1.6	w-pg; w-pp
2. Alkyl halides	6–10	1.4–2.2	
3. Perchloroalkanes	2.2–	0–	m-pp
4. Perfluoroalkanes			
2. Aliphatic ethers and tertiary amines			
a. Ether			
Ether, dioxane, tetrahydrofuran	2.2–7.4	0.4–1.3	s-pp
b. Tertiary amines			
1. Triethylamine	2.4	0.8	s-pp
2. Dialkylanilines			s-pp
3. Dipolar aprotic			
a. Aliphatic ketones			
Acetone, 2-butanone, cyclohexanone	17–21	2.7–2.8	s-pp
b. Alkyl nitriles			
Isocapronitrile, acetonitrile	16–38	3.4–3.7	m-pp
c. Nitroalkanes and nitrobenzenes			
1. 1-Nitropropane, nitromethane	23–36	3.2–4	m-pp
2. Nitrobenzenes			
d. N,N,-Dialkylacylamides			
Dimethylformamide, dimethylacetamide, N-methylpyrrolidinone-2	36–38	3.8	s-pp
e. Dialkylsulfoxides and sulfones			
Dimethylsulfoxide, tetramethylene sulfone	44–47		s-pp
4. Dipolar amphiprotic			s-pg, s-pp
a. NH_2CO-R type			
Formamide			
b. RNH-CO-R type			
N-Methylformamide, 2-pyrrolidinone			
5. Weakly dipolar aprotic			
a. Esters			
Diethyl carbonate, ethyl acetate, etc.	2.8–8.5	1.8–1.9	m-pp
b. Carbon disulfide	2.6	0.0	w-pp
c. Aza arenes			
Pyridines, quinolines	12	2.2	vs-pp

TABLE 17 (Continued)

Solvents	Dielectric constant, d	Dipole moment (D)	Misc. properties
6. Amphiprotic			
a. Water	79		vs-pg
b. Alcohols			
Methanol, ethanol, ethylene glycol	12–38	1.7–2.3	s-pg
c. Fluorinated alcohols			
Trifluoroethanol			vs-pg
d. Primary aliphatic amines			
Isobutylamine, ethylenediamine	4.4–14.2	1.3–1.9	m-pg
d. Secondary aliphatic amines			
Diethylamine	3.6	1.1	m-pg
7. Dipolar conjugated amphiprotic			
a. Phenols			
Phenol, *o*-cresol, *m*-cresol	9.8–12		vs-pg, s-pp
b. Anilines	6.9		s-pg, s-pp
c. *N*-Alkylanilines			s-pg, s-pp
8. Weak acids (pK_a, 0–7)			
a. Formic, acetic, propionic acids	2.4–6.1	0.6–1.2	s-pg
9. Moderate acids (pK_a, −5–0)			
a. Perfluoro- and perchloro-alkanoic acids			
Trifluoroacetic acid, perfluoropropionic acid,	~40		s-pg
b. Sulfonic acids			
Methanesulfonic acid, benzenesulfonic acids			
10. Strong acids			pK_a −5 to −13
a. Sulfuric acids			
Fluorosulfonic, chlorosulfonic, and sulfuric acids; methyl hydrogen sulfate			
b. Halogen acids			
Perchloric acid, hydrogen fluoride (anhydrous)			
c. Lewis acids			
Aluminum chloride (anh.) in nitromethane			
11. Super acids			< −13
a. SbF_5 in fluorosulfonic acid, SbF_5–SO_2 in fluorosulfonic acid			
12. Basic solvents			
a. Aliphatic amines			9–11
b. Pyridines			5–6
c. Aqueous and alcoholic alkaline solutions			12–14

TABLE 17 (Continued)

Solvent	Alkali	Conc. (*M*)	H_-	Ref.
	13. Strongly basic solvents			
Methanol	NaOMe	5×10^{-4}–3.75	10.36–16.64	(*139*)
Water–Me_2SO(10.32–99.59 mole % of Me_2SO)	Me_4NOH	0.011	13.17–26.19	(*137*)
Dimethylsulfoxide	NaO-*t*-Bu		~30	(*140*)
	$NaCH_2 \cdot SO \cdot CH_3$		>30	(*141*)

[a] pg = protogenic = hydrogen bonding donor strength; pp = protophilic = hydrogen bonding acceptor strength; m = moderate; s = strong; vs = very strong; w = weak.

[b] With increase in atomic weight of halogen greater intersystem crossover and quenching of fluorescence.

blue A oxazone, a *Zn* resonance structure, shows the usual shift to red with increasing solvent polarity but also shows a fine structure effect (*105*). Thus, in 2,2,4-trimethylpentane, cyclohexane, and carbon tetrachloride a doublet is found at 487, 507.5; 490, 512.5; and 499, 519 nm, respectively. In the more polar solvents only one band is found, ranging from 507 nm in ether to 564 nm in formamide. Even in benzene or chloroform only one band is found at 521 or 537 nm.

A similar effect is shown in the fluorescence spectrum of 4′-nitro-4-dimethylaminostilbene, Table 7. This fluorescence standard shows bands at 470, 497, and 525s nm in ethylcyclohexane but only one band in carbon tetrachloride, toluene, or chloroform. The positions of the wavelength maxima of these various solutions also vary widely.

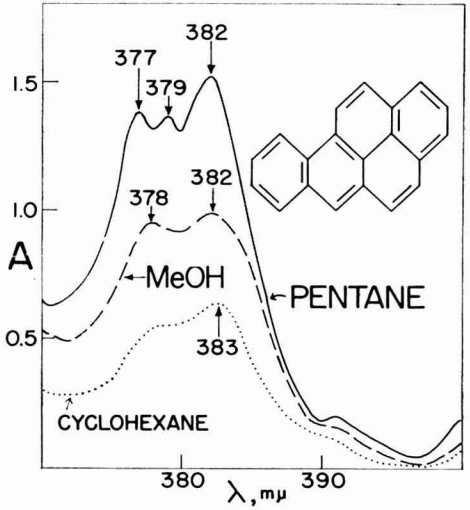


FIG. 7. Ultraviolet absorption spectra of benzo[*a*]pyrene in pentane (——); methanol (– – –); and cyclohexane (. . . .).

The benzene solvents are easily differentiated from the alkanes, since their solvent polarity is greater and they are weakly pi basic and weakly protophilic. Thus, the *Zn* standard, 1,3-diethyl-5-(5-(2,3,6,7-tetrahydro-1*H*,5*H*-benzo(*ij*)quinolizin-9-yl)-1,3-neopentylene-2,4-pentadienylidene)-2-thiobarbituric acid, absorbs at 562 nm in hexane and at 610 nm in benzene, essentially an X_R value of 50.9 in *n*-hexane and of 46.9 in benzene, Table 4.

Heavy-atom solvents, such as bromoform, the alkyl iodides, and iodobenzene tend to cause intersystem crossing (from the excited-singlet state to the excited-triplet state). This results in fluorescence quenching and an increase in the phosphorescence quantum efficiency of the solute under investigation.

The quenching effect of methyl iodide on many ordinarily fluorescent molecules has been used to determine perylene (whose fluorescence is relatively unaffected) in mixtures as complex as coal tar pitch extracts (*106*). The external heavy-atom effect has also been applied to the luminescence analysis of other types of organic compounds (*107*). A solution of 35% phenanthridone, 20% triphenylene, and 45% 11*H*-benzo(*a*)carbazole in EPA (ethanol–isopentane–ether; 2:5:5, v/v) at liquid nitrogen temperatures gave the phosphorescence spectrum of phenanthridone when excited at 355 nm. In EPA–methyl iodide (10:1) the equally intense phosphorescence spectral bands of phenanthridone and the benzcarbazole are present. In the same way >0.2% of 2-naphthol in 1-naphthol can be detected phosphorimetrically in the EPA–methyl iodide solvent. The phosphorescence spectral intensity of many conjugated compounds is increased by the addition of methyl iodide to EPA (*107*). Arenes, aza arenes, imino arenes, phenols, and aromatic amines show this heavy-atom effect. The absorption spectra are relatively unaffected by the addition of methyl iodide to EPA. These results show that the various subclasses of the nonpolar to weakly polar solvents can be readily differentiated spectrally.

Molten polyethylene is also a useful solvent of this class. The test substance is stirred into the polymer, which is then pressed to form a film. Examination by spectrophotometer is possible to 220 nm (*108*).

The second class of solvents consists of tertiary amines and aliphatic ethers. These solvents are strongly protophilic. The amines are more basic solvents. They tend to form excited-state complexes. Thus, triethylamine efficiently quenches the fluorenone emission (while pyridine does not), the quenching efficiency increasing with the increasing polarity of the solvent (*109*). Deactivation of the perylene excited-state singlet via complex formation with amines has also been postulated (*110*). With *N*,*N*-dialkylanilines polynuclear hydrocarbons form fluorescent charge

transfer complexes stable in the excited state only, Table 18. Other solvents of this type that have been used include *N,N*-dimethyl-*p*-toluidine and *N,N*-dimethyl-β-naphthylamine (*111*).

Pyrene shows behavior somewhat different from that of some of the other arenes. In a rigid solvent at 77°K the rearrangement motions of molecules during the lifetime of the excited state, motions that are necessary for charge-transfer complex formation, are more or less hindered. Because of the very long fluorescence lifetime of pyrene as compared to perylene and anthracene, the CTF complex of pyrene is stable at 77°K while the complexes of perylene and anthracene do not form at this temperature. The fluorescence quantum yields of pyrene and its complex at room temperature in *n*-hexane are 0.76 and 0.48, respectively; those of perylene and its complex are 0.89 and 0.01, respectively (*112*). In polar solvents such as acetonitrile and ethanol, the pyrene fluorescence is strongly quenched by dimethylaniline and the fluorescence of the complex does not appear except at very high concentration of dimethylaniline (mole fraction ~0.5). The fluorescence spectra of perylene in methylene chloride, in dimethylaniline, and in a 1:1 mixture of these solvents are shown in Fig. 8. In dimethylaniline the monomer emission of perylene is completely quenched. Dimethylaniline also quenches or decreases the fluorescence of 3-nitro-*N*-methylcarbazole, 4′-nitro-4-dimethylaminostilbene, 1,4-dihydroxyanthraquinone, 2-hydroxydibenzofuran, and benz(*c*)acridine. For example, the latter compound is 10,000 times more

TABLE 18

CHARGE-TRANSFER FLUORESCENCE BANDS OF ARENE-DIMETHYLANILINE COMPLEXES IN *N,N*-DIMETHYLANILINE

Compound	λ_{em} (nm)
trans-Stilbene	480
Benzo(*e*)pyrene	485
Pyrene	490
1,4-Diphenylbutadiene	500
Anthracene	515
Benzo(*ghi*)perylene	520
Benzo(*a*)pyrene	520
1,6-Diphenylhexatriene	530
Benzo(*k*)fluoranthene	535
1,8-Diphenyl-1,3,5,7-octatetraene	535
Benzo(*b*)fluoranthene	540
Fluoranthene	550
Perylene	550
Naphtho(2,3-*a*)pyrene	565
Anthanthrene	570

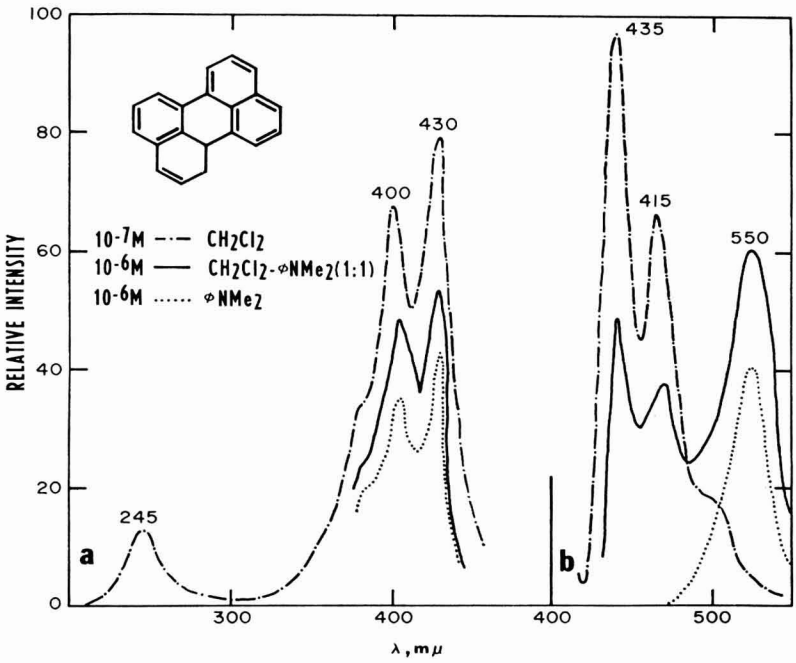


FIG. 8. Fluorescence excitation and emission spectra of perylene: 10^{-7} *M* in methylene chloride (—— .); 10^{-6} *M* in methylene chloride–dimethylaniline (1:1) (——); and 10^{-6} M in dimethylaniline (. . . .).

fluorescent in heptane than in heptane–dimethylaniline (1:1). In the latter solvent the intensities of the monomer band at 405 nm and the CTF band at 540 nm are approximately equal.

Ether and dioxane are strongly protophilic solvents, Table 8. *Zn* zwitterionic resonance structures containing a nitro group as the negative resonance terminal are usually fluorescent in dioxane and nonfluorescent in alcohol, e.g., 4-nitrophenylhydrazones of aromatic carbonyl compounds, 2-nitro-9-methylcarbazole, and 2-amino-7-nitrofluorene (*24*). Many of these compounds are fluorescent in both solvents at liquid nitrogen temperatures. The fluorene compound shows green fluorescence in dioxane, is at most weakly fluorescent in alcohol at room temperature, and is phosphorescent in EPA at liquid nitrogen temperatures. Compounds such as 2-amino-7-nitrofluorene, 2-amino-9-methylcarbazole, and 4-dimethylaminobenzal malononitrile are at least 1 thousand times more fluorescent in dioxane than in alcohol.

The third class is the dipolar aprotic solvents. These are protophilic solvents with dielectric constants greater than 16. Common dipolar aprotic solvents are hexamethylphosphoramide, dimethylformamide,

dimethylacetamide, *N*-methyl-2-pyrrolidone, and dimethylsulfoxide. Many of the properties of these solvents have been reviewed (*113*), as have the solvent properties of dimethylsulfoxide (*114*) and dimethylformamide (*115–118*). These solvents solvate cations strongly and anions weakly. Since there is a high electron density on the bare oxygen atom, they will form hydrogen bonds with compounds containing active hydrogen. Whereas water is a very weak hydrogen bond donor in these solvents, phenol is a strong donor. Picric acid is 500 times as strong an acid in dimethylsulfoxide as in water; acetic and benzoic acids are approximately 1 million times stronger acids in water than in dimethylsulfoxide (*114*). In the same way trifluoroacetic acid appears to be a weak acid in dimethylsulfoxide since the aza arenes give the fluorescence spectra of the base in dimethylsulfoxide containing $\sim$2% trifluoroacetic acid. Acridine shows no fluorescence under these conditions. The explanation of the unusual strength of the carboxylic acids in water is that their anions are highly stabilized in water relative to dimethylsulfoxide by general hydrogen bonding to the negative charge localized on the oxygen atoms, whereas picrate ion may be similarly solvated in both solvents, because its negative charge is strongly dispersed over the whole aromatic system so that hydrogen interactions with water are small (*113*).

Another property that will be discussed is that alcoholates are very much stronger bases (up to 10^{12} times) in these solvents than in water or the alcohols.

The various subclasses of the dipolar aprotic solvents can be readily differentiated spectrally. They show widely differing values of AB factor, Table 15, and of solvent polarity, Table 4. This class of solvents is readily differentiated spectrally from the other classes by these and other characteristics. Thus, 3-nitro-9-methylcarbazole is fluorescent in dimethylformamide, acetone, nitromethane, and pyridine; and nonfluorescent in dioxane, alcohol, dimethylaniline, and aqueous dimethylformamide. Similarly, 4-dimethylaminocinnamaldehyde is fluorescent in dimethylformamide or acetone but is nonfluorescent in methanol or methylcyclohexane. On the other hand, sulfanilamide is fluorescent in hydroxylic solvents and in dimethylformamide and formamide but nonfluorescent in acetone, 2-butanone, and nitromethane, Table 19 (*119*). The usual wide variation of fluorescence intensity with solvent changes is apparent in these data.

The quenching effect of acetone on the fluorescence of 2-naphthol has been used in the determination of acetone (*120*).

The nitroalkanes, the nitrobenzenes, and nitrogen dioxide–trifluoroacetic acid mixtures are solvents that form nonfluorescent excited-state

TABLE 19

FLUORESCENCE INTENSITY OF SULFANILAMIDE IN VARIOUS SOLVENTS (*119*)

Solvent	Relative intensity	Solvent	Relative intensity
Ethanol	107	Ethyl acetate	33
Water	100	Dioxane	24
Isopropanol	94	Light petroleum (bp 60–80°)	24
Propanol	89	Benzene	17
Ether	78	Toluene	9
Methanol	72	Isobutanol	7
Formamide	70	Chloroform	0
n-Butanol	69	Carbon tetrachloride	0
1,2-Dichloroethane	69	Acetone	0
Dimethylformamide	44	2-Butanone	0
n-Hexane	42	*p*-Xylene	0
		Nitromethane	0

complexes with many types of conjugated molecules and thus are useful in quenchofluorimetric (*9, 41*) and quenchophosphorimetric (*121*) analysis. Although most polynuclear arenes are nonfluorescent in nitromethane solution, compounds containing the fluoranthene ring structure are fluorescent in this solvent (*122*). Benzo(*k*)fluoranthene can be determined quenchofluorimetrically by analysis of the benzopyrene fraction [benzo(*a*)pyrene, benzo(*e*)pyrene, benzo(*k*)fluoranthene, and perylene] obtained through column chromatography of organic air particulates. Benz(*a*)acridine and benz(*c*)acridine can be characterized quenchofluorimetrically after column and thin-layer chromatographic separation of complex air pollution mixtures (*123*). Since many aza arenes and few arenes are fluorescent in acidified nitromethane, this solvent has been used in the filter fluorimetric analysis of benzo(*h*) quinoline and benz(*c*)-acridine isolated after thin-layer chromatographic separation of organic particulates obtained from urban atmospheres (*124*). Atmospheric benzanthrone and phenalen-1-one have been determined quenchofluorimetrically in solvent systems containing 2-nitrothiophene or *m*-dinitrobenzene in trifluoroacetic acid (*125*). The nitro solvents have also been used on thin-layer plates in a quenchofluorimetric manner in examining separated spots (*126, 127*).

Quenchophosphorimetry, like quenchofluorimetry, is a highly selective method of functional group analysis (*121*). For example, 2-nitrofluorene, carbazole, 4-hydroxyacetophenone, triphenylamine, and triphenylene are all intensely phosphorescent in EPA; in *o*-nitrotoluene-toluene (1:9) 2-nitrofluorene can be determined phosphorimetrically in the presence of all the other compounds since it is the only one that

phosphoresces in the nitrotoluene solvent. However, in 1,1-dimethylhydrazine solution 2-nitrofluorene is nonphosphorescent.

The data in Tables 6, 7, and 10 indicate that the dipolar aprotic, dipolar amphiprotic, and weakly dipolar aprotic solvents can be readily differentiated by spectral methods. The subgroups in the latter group of solvents can be differentiated readily. Thus, carbon disulfide with its high refractive index of 1.6276 is a good solvent for shifting the absorption wavelength maxima of hydrocarbons to longer wavelengths as compared to solvents like the alkanes, benzene, or the alcohols. Carbon disulfide is also useful in quenchofluorimetric analysis, since addition of the disulfide quenches the fluorescence of many fluorescent compounds (*9, 41*). Such compounds, nonfluorescent in carbon disulfide, include polynuclear aromatic amines, polynuclear carbazoles, and arenes. Ring carbonyl compounds, fluoranthene, perylene, and 1,4-dihydroxyanthraquinone are fluorescent in this solvent.

The aza arene solvents form strong hydrogen bonds with protogenic solutes. This property can strongly affect the spectra as shown in the section concerned with intermolecular hydrogen bonding. Thus, 1-anilinonaphthalene, 1-hydroxy-7-anilino-3-naphthalenesulfonic acid, and *N*-1-naphthyl *N*-2-naphthylamine are nonfluorescent in pyridine and fluorescent in dimethylformamide and acetone. On the other hand 1-anilino-8-napthalenesulfonic acid and 1-dimethylamino-5-napthalene sulfonate are fluorescent in pyridine. The latter is nonfluorescent in acetone and the former is fluorescent in acetone. Obviously, these widely differing solvent effects should be capable of much wider use in environmental trace analysis.

Amphiprotic solvents are probably the most popular solvents in organic trace analysis. Many aromatic carbonyl compounds are fluorescent in the hydroxylic solvents and are nonfluorescent in dioxane (*24*) and other nonhydroxylic solvents, Tables 9 and 10. Even a compound such as dipiperonal acetone is several hundred times more fluorescent in 95% ethanol than in dioxane and is fluorescent as a dry spot on paper. Cellulose could be considered amphiprotic.

On the other hand, the absorption spectrum of 4′-nitro-4-dimethylaminostilbene correlates well with Brooker's solvent polarity X_R values except for the hydroxylic solvents, Table 7. The various members of the subgroups can be readily differentiated. Even the various alcohols can be differentiated, as shown in the section on solvent polarity and in Table 12 (*18*). Note that the short-wavelength maximum is relatively unaffected by the solvent whereas the position of the long-wavelength band changes and its intensity increases with decreasing solvent polarity.

The fluorescence spectra in terms of emission-wavelength maxima and

quantum yields of some of the protein probes have been investigated in alcoholic and aqueous solutions, e.g., ε-dansyl lysine (*72*) and 2-*p*-toluidinylnaphthalene-6-sulfonate (*66*). The dielectric constants, solvent polarity *Z* values (*4*), fluorescence-wavelength maxima and quantum yields of three anilinonaphthalenesulfonic acids dissolved in a variety of solvents are compared in Table 20 (*38*). A good correlation is obtained between the solvent polarity of a hydroxylic solvent and the emission wavelength or quantum yield of the probe in the same solvent. For the nonhydroxylic solvents this correlation does not hold.

Clear solvent ices at liquid nitrogen temperatures are used in phosphorimetric examinations. Ethanol has been used for this purpose in obtaining phosphorescence excitation and emission and lifetimes for phosphorescent compounds (*128*). However, any nonphosphorescent solvent can be used in direct phosphorimetric analysis of chromatograms and pherograms (*129*).

The dipolar conjugated amphiprotic solvents are strongly protogenic. They are useful in quenchofluorimetric analysis because they can form an intermolecular hydrogen bond between two conjugated systems and in so doing partially or completely quench the fluorescence of the fluorogen. Many examples are given in the section on intermolecular hydrogen bonding. Phenol–water (9:1) radically decreases the fluorescence of 5-dimethylamino-1-naphthalenesulfonic acid, benzo(*a*)pyrene, and 3-nitro-9-methylcarbazole in dimethylformamide, methylene chloride, and pyridine. The quenchofluorimetric properties of *o*-cresol (*41*) and 3-methylthioaniline (*9*) have been reported. The former quenched the fluorescence of aza arenes; the latter quenched the fluorescence of carbazoles, aza arenes, ring-carbonyl compounds and 1,4-dihydroxyanthraquinone.

Water-insoluble chromogens containing a *Zn* resonance structure absorb at longest wavelength in phenols, Table 4. This property can be useful in trace analysis.

The various acids are classified according to their acid strength, Table 17. Acidic solvents are required for formation of many types of cationic resonance structures of use in organic trace analysis. Aqueous acids and concentrated sulfuric acid have seen the most use in organic analysis. For example, total aldehydes in the atmosphere are determined with 3-methyl-2-benzothiazolinone hydrazone in aqueous acid solution (*130*), atmospheric formaldehyde is determined with chromotropic acid in strong sulfuric acid solution (*131*), and atmospheric acrolein with 4-hexylresorcinol in aqueous acid solution (*132*).

Anhydrous aluminum chloride in nitromethane forms brilliantly colored salts with many weakly basic conjugated compounds, e.g., polynuclear arenes, aromatic carbonyl compounds, and quinones (*41*). This

TABLE 20

SOLVENT EFFECT ON SPECTRAL PROPERTIES OF SOME ANILINONAPHTHALENESULFONIC ACIDS (*38*)

Solvent (% water)	Dielectric constant D	Solvent polarity Z (*4*)	1.7-		1.5-		1.8-	
			λ_{em} (nm)[a]	Q[b]	λ_{em} (nm)	Q	λ_{em} (nm)	Q
Water	80	94.6	515	0.0091	529	0.0099	555	0.0032
Ethanol (80)	69	92.6	496	0.027	515	0.027	533	0.0072
Dioxane (80)	64	91.4	481	0.062	513	0.05	524	0.012
Methanol (50)	58	90.9	476	0.099	504	0.084	517	0.029
Ethanol (60)	58	90.5	477	0.11	500	0.083	518	0.020
Dioxane (60)	42	88.4	463	0.21	490	0.15	517	0.038
Ethanol (40)	47	87.9	465	0.26	485	0.20	512	0.050
Methanol (20)	42	87.3	463	0.28	482	0.27	509	0.077
Ethylene glycol	38	85.1	465	0.36	484	0.38	513	0.12
Dioxane (40)	25	85.0	457	0.42	472	0.33	505	0.099
Ethanol (20)	35	84.8	459	0.43	474	0.38	501	0.11
Methanol	33	83.6	455	0.39	470	0.63	492	0.17
Dioxane (20)	10	80.2	450	0.57	461	0.54	493	0.23
Ethanol	24	79.6	443	0.56	452	0.69	480	0.40
Acetic acid	6	79.2	454	0.025				
1-Propanol	20	78.3	439	0.70				
2-Propanol	18	76.3	433	0.65				
Acetonitrile	38	71.3	431	0.43				
Dimethylsulfoxide	49	71.1	447	0.46				
Dimethylformamide	37	68.5	436	0.67	434	0.70	473	0.39
Acetone	21	65.7	415	0.30			468	0.39
Pyridine	12	64.0	429	0.0046				
Dioxane	2		432	0.68				

[a] Fluorescence wavelength maximum.

[b] Q = Quantum yield.

solvent system is useful in the analysis of aza arenes since arenes, imino arenes, and aromatic amines are nonfluorescent while the aza arenes are fluorescent.

Dilute solutions of sulfuric acid do not sulfonate or oxidize the test substance or reagent as readily as the concentrated acid does; however, their acidity is much less. It is possible to change the solvent from water to a dipolar aprotic solvent and increase the acidity considerably. For example, a 0.01 *M* solution of sulfuric acid in sulfolane is a much stronger acid than is the same concentration of sulfuric acid in water (*133*). In the same way, the proton is extremely active in nitromethane (*134*).

A variety of super acids have been introduced (*135*). Protonated acetonitrile is regarded as a super acid (*136*). The super acids have not been used in analytical investigations as yet, but should certainly open up new fields.

Basic solvents are of especial importance in analysis of chromogens, fluorogens, and phosphorogens containing anionic resonance structures. With an increase in the basicity of a solvent the long-wavelength maximum shifts toward the red with an intensity increase, Fig. 9 (see also Tables 14 and 15). Definite knowledge of the basicity of a solvent is necessary to ensure that a weak acid chromogen is completely present as the anion for optimum measurement.

Figure 10 shows what happens when an analytically formed dye is not completely ionized. The dye, formed in the analysis of malonaldehyde and its precursors, 2-deoxyribose and DNA, is present as the nonfluorescent neutral acid in water and methanol and in increasing amounts as the fluorescent anion in hydroxylic solvents with increasing H_- values.

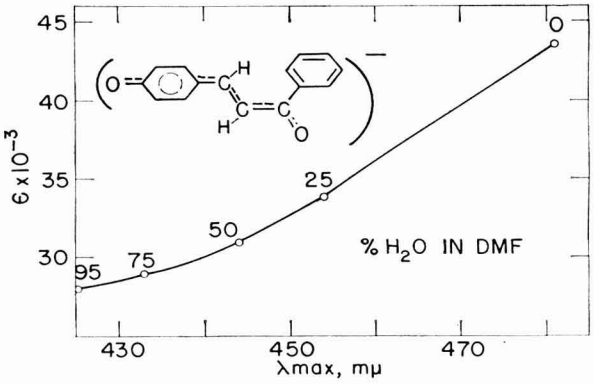


FIG. 9. Solvent basicity effect: change in wavelength maximum and molar absorptivity of 4′-hydroxychalcone with decreasing water in an aqueous dimethylformamide solution.

The acidity function, H_- is used to measure the basicity of strongly basic solutions. H_- describes the ability of a solvent to remove a proton from a neutral acid and is defined as (137).

$$H_- = -\log \frac{{}^{a}H + f_A}{f\,HA} = pK_a + \log \frac{(A^-)}{(HA)}.$$

H_- becomes identical with pH in aqueous solutions.

The enhancement in the basicity of the hydroxide ion in the dipolar aprotic solvents (poor solvators of anions) is due to these solvents driving the equilibrium,

$$OH_{aq}^- + HA \rightleftharpoons A^- + (n + 1)\,H_2O,$$

to the right (*137*). The activity of the hydroxide ion increases while the water activity decreases. The latter decrease is due to the dilution effect and to the ability of the dipolar aprotic solvents to complex with water through the formation of strong hydrogen bonds. The number n in the equation can be considered either as the hydration number of the hydroxide ion or, if the indicator or its anion is also intimately solvated, as the difference in the solvation numbers between the left and right sides of the equation.

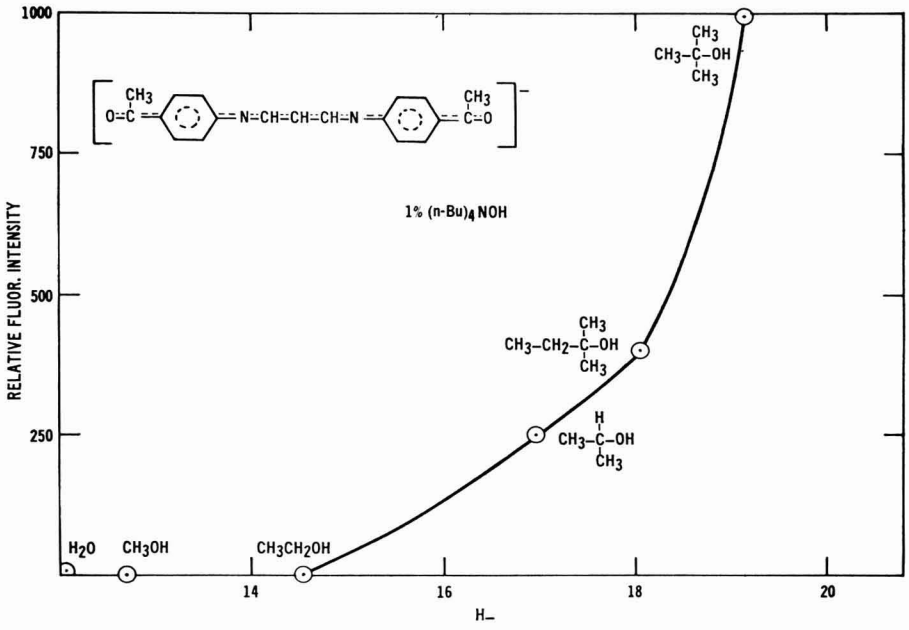


FIG. 10. Effect of solvent basicity on the fluorescence intensity of the chromogen obtained in the determination of malonaldehyde precursors with 4′-aminoacetophenone.

Basic solutions used in organic analysis include aqueous solutions of sodium hydroxide, potassium hydroxide, and ammonia; sodium or potassium alkoxides in alcohols; aliphatic amines; and pyridines. Some of the stronger basic solvents are shown in Table 17 (*138–140*). Many more have been reported by Stewart and O'Donnell (*141*). The strong bases soluble in organic solvents that have been used in organic analysis include the following hydroxides: tetramethylammonium (*142, 143*), tetraethylammonium (*6, 7, 9, 144–146*), tetra-*n*-butylammonium, tetra-*n*-propylammonium (*58*), benzyltrimethylammonium (*147, 148*), *n*-butyltrimethylammonium and benzethonium (*149*), and hyamine (*150, 151*) and benzyltrimethylammonium methoxide (*58, 60*). These bases are usually in an alcoholic or aqueous solution. A 1 *M* solution of tetra-*n*-butylammonium hydroxide in methanol is soluble ($> 1\%$) in organic solvents as weakly polar as benzene and *o*-dichlorobenzene, is slightly soluble in toluene or mesitylene, insoluble in pentane or cyclohexane, and very soluble in a large variety of dipolar, hydroxylic and miscellaneous solvents, as shown in Table 15. Addition of 1% amounts of the 1 *M* solution to triethylamine, carbon tetrachloride, isobutylvinyl ether, or isopropyl ether results in turbid solutions.

V. BAND TYPES

Since the solvent effect depends primarily on the nature of an electronic transition, the influence of any solvent varies according to whether the electron transition of a chromogen, fluorogen, or phosphorogen is some type of $n \leftrightarrow \pi^*$, $n \leftrightarrow \sigma^*$, *or* $\pi \leftrightarrow \pi^*$ transition.

A. $n \rightarrow \pi^*$ Transition

Many compounds that are not too highly conjugated and that contain a heteroatom connected by a double bond to a C or N atom, e.g., $-C{=}O$, $-C{=}S$, $-N{=}O$, $-N^+(=O)O^-$, $-N{=}N-$, $-C{=}N-$, show the presence of a low-intensity long-wavelength band derived from an $n{\rightarrow}\pi^*$ transition. The effect of solvents on the $n{\rightarrow}\pi^*$ bands was investigated by Burawoy in the 1930's through the 1950's (*152*) and by many other investigators since then. Burawoy called them *R* bands. With increasing solvent polarity the bands shifted toward shorter wavelength. In hydrogen-bonding solvents, a hydrogen bond formed between the appropriate heteroatom of the solute and the active hydrogen of the protogenic solvent results in a strong shift to blue in the $n{\rightarrow}\pi^*$ absorption transition, but it has practically no effect upon the $n{\leftarrow}\pi^*$ fluorescence transition. It is concluded that the strong hydrogen bond formed in the ground state is broken in the (n, π^*) singlet excited state (*153*). Because of the long lifetime of the n, π^* excited state, fluorescence is

usually quenched for molecules whose lowest singlet excited state is of this type. The dipole moment of the n, π^* singlet excited state is usually lower than the ground state, e.g., pyridazine has $\mu_g = 3.94$ D and $\mu_e = 1.10$ D (*153*).

These absorption bands have seen little use in organic trace analysis primarily because of their low intensity, ranging from about $m\varepsilon = 0.018$ at 265 nm for acetone in water to $m\varepsilon = 0.44$ at 440 nm for azobenzene in alcohol.

B. $n \rightarrow \sigma^*$ Transition

The ground state of this transition is far more strongly influenced by solvents which form hydrogen bonds than is the excited state. Many saturated molecules containing heteroatoms show this transition. Examples of compounds showing this transition are alkyl iodides and dialkyl sulfides. Since the band derived from this transition has low intensity, it is rarely used in organic trace analysis.

C. $\pi \leftrightarrow \pi^*$ Singlet and Triplet Transitions

The bands derived from these transitions, and especially those derived from the singlet transitions, are most used in photometric analysis. Unsaturated compounds with these transitions can be classified as zwitterionic (of the *Zn, Zb, and Zz* types), cationic, or anionic resonance structures. This classification is based on the widely differing spectral effects produced by the interaction of solvents with these various structures. These various resonance structures can be further subdivided into polyene, arene, and polymethine types. Again, the interaction of these subtypes with solvent molecules results in widely differing spectral effects.

1. Zn zwitterionic resonance structures. Since the excited state of a molecule can have entirely different chemical and physical properties from those of the ground state, solvent effects can be radically different for each state. The *Zn* zwitterionic resonance structures have a greater dipole moment in the excited state than in the ground state.

For the weakly polar polyenes and arenes, dipole moment changes and hydrogen bonding effects are weaker so that solvent polarity does not affect the spectra as it does with the more strongly polar compounds. For these compounds the refractive index of the solvent seems to be of prime importance. Even for compounds as polar as phenol blue and *p*-nitrosodimethylaniline the position of the long-wavelength maximum correlates with the refractive index if solvents are restricted to hydrocarbons and arvl halides (*153a*).

Carotenoids with a lutein-like structure have been found in airborne ragweed pollen (*154*). This type of chromophore shows a red shift with

increasing refractive index of the solvent, Table 21. It is obvious that other factors are involved, but the refractive index appears to be of main importance here. The correlation of spectra with refractive index has been demonstrated with lycopene also (*153a*).

Arenes show a similar phenomenon. The spectra of benzene, toluene, and chlorobenzene have been measured in carbon tetrachloride, chloroform, *n*-hexane, ethanol, and water (*155*). Compared with the gases the solution spectra are all displaced to the red by amounts that agree qualitatively with the predicted effect of the solvent refractive index and the transition intensity according to the theory of Bayliss (*156*). Positions of the absorption bands of rubrene in 19 solvents have been shown to be approximately dependent on the refractive index of the solvent (*157*). With one of the solvents as a standard there is an approximate linear relationship between $\Delta\gamma$ and $[(n^2 - 1)/(2n^2 + 1)]$. The long-wavelength maxima ranged from 522 nm in diethyl ether ($n_D^{20} = 1.3519$) to 541 nm in methylene iodide ($n_D^{20} = 1.742$). The linear relationship

TABLE 21

SOLVENT REFRACTIVE INDEX AND THE LONG-WAVELENGTH MAXIMA OF β-CAROTENE

Solvent	λ_{max} (nm)[a]	n_D^{20}
2,2,2-Trifluoroethanol–acetone (5:1)	425i, 446, 467	—
Methanol	424i, 446, 474	1.3288
Pentane	431i, 447, 475	1.3579
Ethyl ether	424i, 449, 477	1.3526
Ethanol	431i, 450, 478	1.3611
t-Butanol	435i, 451, 478	1.3838
Ethyl acetate	431i, 452, 480	1.3701[b]
Triethylamine	431i, 452, 481	1.4003
Acetone	433i, 452, 481	1.3588
Cyclohexane	433i, 452, 482	1.4266
Dipropylamine	434i, 455, 484	1.4046
Nitromethane	429i, 457, 482	1.3935
N-methylformamide	434i, 458, 483	
t-Butylformamide	437i, 458, 487	1.4312
p-Dioxane	441i, 458, 488	1.4224
Ethylene glycol–acetone (3:1)	432i, 459, 490	
Benzene	442i, 463, 491	1.5011
Phenol–water (9:1)	447i, 466, 487	
Dimethylsulfoxide	448i, 467, 494	1.4795
N,N-Dimethylaniline	449i, 468, 499	1.5587
Aniline	453i, 474, 503	1.5863
Quinoline	453i, 476, 506	1.6230
Carbon disulfide	460i, 481, 510	1.6255

[a] i = inflection.

[b] At 25°C.

actually relates the shift to red in solution ($\Delta\gamma$) with the oscillator strength (f), the size of the solute molecule and the index of refraction (*156*). Thus:

$$\Delta\gamma = \text{const.}\left(\frac{f}{\gamma a^3}\right)\left(\frac{n^2 - 1}{2n^2 + 1}\right),$$

where a is the radius of the solute molecule, and n is the refractive index of the solvent at the wavelength of the absorption band.

The long-wavelength band of naphthacene near 444 nm has also been correlated with the index of refraction of the solvent, for which values of n_D were varied between 1.3915 and 1.6219 (*158*). Except for the ends of the curve there was a linear relation between frequency and $(n^2 - 1)/(2n^2 + 1)$. The $'L_a$ transition of naphthacene near 470 nm has been observed in some 64 solvents with wavelength maxima ranging from 468 nm in pentane ($n = 1.3601$) to 478 nm in iodomethane ($n = 1.5293$) (*159*). In this study correlation with the refractive index is not quite as satisfactory. Other factors play a role also. The atmospheric carcinogen, benzo(*a*)pyrene, shows the refractive index effect since it absorbs at 382 nm in pentane and at 395 nm in carbon disulfide. Thus, for efficient organic trace analysis of hydrocarbons, the analyst must be cognizant of the refractive index of the test solvent.

The absorption spectra of the arenes contain three main types of $\pi \rightarrow \pi^*$ bands used extensively in organic trace analysis of atmospheric polynuclear aromatic hydrocarbons (*160*). These are the α, β, and para bands, extensively investigated by Clar (*161*). The α bands have a millimolar absorptivity of about 0.1 to 1.0 and are the long-wavelength bands in benzene, naphthalene, and phenanthrene. The β bands have a millimolar absorptivity of about 100 and are at shortest wavelength. The para bands have a millimolar absorptivity of about 3 to 32 and are the long-wavelength bands in anthracene, naphthacene, and the other acenes.

The somewhat polar monosubstituted benzene derivatives show solvent shifts that are dependent on the particular $\pi \rightarrow \pi^*$ transition under study. The para bands are usually displaced to longer wavelengths with increasing polarity of the solvent (ethanol $\rightarrow$ water), the effect being greater as the electron-acceptor strength of the substituent increases, e.g., nitrobenzene $>$ benzaldehyde $>$ acetophenone $>$ benzoic acid (*162*). The bands are shifted to shorter wavelength with increasing solvent polarity in benzene derivatives containing an electron-donor substituent.

The polymethine type of *Zn* structure contains definite negative and positive resonance terminals and thus has a much greater dipole moment

TABLE 22

GROUND AND EXCITED STATE DIPOLE MOMENTS OF SOME *Zn* STRUCTURES

Compound	Dipole moment, μ_g	μ_e (D)
4′-Nitro-4-dimethylaminostilbene	7.6	32
4′-Cyano-4-dimethylaminostilbene	6.1	29
7-Dimethylaminobenzo[α]phenazine	~2.	12
4-Aminobenzophenone	5.2	14[a]
4-Nitroaniline	6.3	14
4-Dimethylaminobenzaldehyde	5.6	13

[a] μ_e for the $n \rightarrow \pi^*$ excited state is about 3

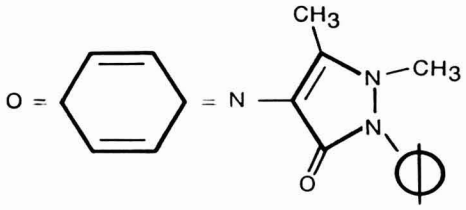
in the excited state than in the ground state, Table 22 (*163, 164*). Dipole moments of the ground and excited states of over 30 compounds with this type of structure have been reported and discussed (*164a*). Thus, a molecule like 4-nitroaniline shows a much greater change in dipole moment in going from the ground state to the first excited-singlet state than do less polar molecules such as aniline, nitrobenzene, or benzophenone (*165*). The dipole moment in the first excited-singlet state is so large that this state is often called a charge-transfer state.

In this type of *Zn* resonance structure any increase in solvent polarity results in a red shift as shown for the long-wavelength band of 4-nitroaniline found at 325 nm in cyclohexane, 351 nm in diethyl ether, 361 nm in dioxane, and 383 nm in ethylene glycol (*165*); for the next-to-longest wavelength band of lumiflavin found at 332 nm in cyclohexane–dioxane (1:1), 334 nm in dioxane, 353 nm in ethanol, and 369 nm in water (*166*); and for the long-wavelength band of Nile blue A oxazone found at 487 and 507 nm in 2,2,4-trimethylpentane, 507 nm in diethyl ether, 533 nm in acetone, and 564 nm in formamide (*105*).

It must be emphasized that solvent polarity is an empirical factor that is influenced by the interplay of many forces with the test solute. Thus, the long-wavelength band of 4′-nitro-4-dimethylaminostilbene shifts to the red with an increase in solvent polarity, Table 7. Hydroxylic solvents, and especially water, are exceptions in this correlation, the compound absorbing at the lowest wavelength in the aqueous solvent. 4-Methoxy-4′-nitrostilbene shows a similar phenomenon.

An example of this red shift effect can be seen in comparing two analytical procedures for the determination of phenols in auto exhaust (*168*). With 4-aminoantipyrine as the reagent, the final chromogen, I, absorbs at 455 nm in the chloroform solution of one procedure and at 507 nm in the aqueous solution of the second procedure. The pure

chromogen, I, shows the typical red shift, absorbing at 460 nm in chloroform and at 510 nm in water (*168a*).



I

Solvent	λ max (nm)
Chloroform	460
Acetone	465
Ethanol	480
Water	510

Proton addition to the negative resonance terminal of *Zn* resonance structures results in a red shift; proton addition to the positive resonance terminal results in a blue shift. The position of addition in acid solution depends on which terminal is more basic.

The fluorescence spectra of *Zn* resonance structures have not been explored as much as the absorption spectra. However, one could expect differing solvent effects on the position, intensity, bandwidth, and fine structure of the absorption and fluorescence wavelength maxima of a molecule, since the ground and excited-singlet states of a molecule can display widely differing physical properties. The intensity of the fluorescence emission wavelength maximum can be changed drastically with solvent changes, as shown in Table 18, and for a large number and variety of polynuclear compounds (*41*).

The polynuclear arenes tend to give sharper bands in nonpolar solvents. At low temperatures in heptane, quasilinear fluorescence spectra of many polynuclear arenes are obtained (*169*). These spectra consist of very fine "lines," which permit easy identification and sometimes direct determination of these hydrocarbons in mixtures. This has been done with benzo(*a*)pyrene.

The fluorescence emission wavelength maximum of 1,3-dimethylindole is at 322 nm in hexane and at 380 nm in water. The dielectric constant of the solvent is believed to play a major role in this shift (*170*).

Many factors contribute to the wide range of fluorescence intensities found for a fluorophore in various solvents. For example, consider 2-hydroxydibenzofuran, a constituent of some air samples, Table 23. Intermolecular hydrogen bonding with hydroxylic solvents and with pyridine

TABLE 23

SOLVENT EFFECT ON THE FLUORESCENCE INTENSITY OF 2-HYDROXYDIBENZOFURAN

Solvent	Relative fluorescence intensity
Methylene chloride	1000
Dimethylformamide	400
95% Ethanol	200
Water	70
Pyridine	30
N,N-Dimethylaniline	1
Nitromethane	0

decreases its intensity. Charge-transfer complex formation with dimethylaniline has an even more drastic effect. Complex formation with nitromethane in the excited state destroys the fluorescence completely.

The more polar *Zn* resonance structures are much less fluorescent in highly polar solvents such as alcohol than in nonpolar solvents such as dioxane (*24*). For example, 2-amino-7-nitrofluorene is about 10,000 times more fluorescent and 2-nitro-9-methylcarbazole about 1000 times more fluorescent in dioxane than in 95% ethanol.

Another factor of importance is the quenching property of solvents such as the nitroalkanes, carbon disulfide, pyridine, and phenol with families of compounds. This property is applied in a type of functional group analysis called quenchofluorimetry (*41*).

Several compounds with *Zn* resonance structures have been recommended as fluorescence standards. These include 9,10-diphenylanthracene (*171*), 3-aminophthalimide (*172, 173*), 2-naphthol (*172*), *m*-nitrodimethylaniline (*172, 173*), 4′-nitro-4-dimethylaminostilbene (*172, 173*), and 4-dimethylaminobenzylidene-5-oxo-2-phenyl-2-oxazoline (*174*). The last two compounds show strong wavelength shifts with solvent changes.

Solvent effects have been little studied in phosphorimetric analysis since a solvent that forms a nonphosphorescent clear rigid "glass" at low temperatures is usually necessary, and these have been difficult to obtain in profusion. To alleviate this problem, the behavior of 55 solvents and 27 solvent mixtures under rapid cooling with liquid nitrogen has been studied (*175*). Any nonphosphorescent solvent can be used, however, in direct phosphorimetric analysis of spots on chromatograms or pherograms (*129, 176*).

Cooling paper chromatograms to liquid nitrogen temperature permits detection of many polycyclic arenes by their phosphorescence. Phos-

phorimetric spectra of these compounds are shifted to the red by 2 to 4 nm as compared to their spectra in solutions (*177*).

Solvent effects can be more drastic. The emission spectrum of triphenylene on glass-fiber paper wet with chloroform shows four bands at 433, 460, 488, and 515s nm, but when the spot is moistened with EPA the 433-nm band disappears (*129*). Anthrone shows an even more drastic difference in phosphorescence spectra on glass-fiber paper when the dry spot is compared with the spot wet with EPA or alcohol (*129*).

Hydrogen bonding can play an important role in phosphorimetry. 2-Hydroxybenzophenone is nonphosphorescent in hydrocarbon glasses where it is intramolecularly hydrogen bonded, and is phosphorescent in hydroxylated solvent glasses where it is intermolecularly hydrogen bonded with the solvent (*178*).

We will now discuss some of the emission types of bands where excitation is at the more intense π, π^* band of the *Zn* type with the absorbed energy then being transferred through the *n*, π^* singlet state of lower energy to an *n*, π^* triplet state.

In conjugated molecules where the longer-lifed $n \rightarrow \pi^*$ transition is of lower energy than the $\pi \rightarrow \pi^*$ transition, fluorescence is decreased or quenched when intersystem transfer takes place to the excited triplet state. If the energy is not dissipated in nonradiative processes, phosphorescence takes place. This radiative process usually occurs at low temperatures with the emitting molecule rigidified in solid media. Under these conditions both S and T are *n*, π^* states.

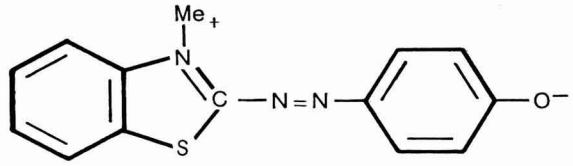
A nitro and sometimes a carbonyl or aza group can enhance intersystem crossing by means of vibrational coupling between the lowest excited-singlet state and the excited-triplet state. For example, quinoline in hydroxylic glasses shows both fluorescence and phosphorescence but in hydrocarbon glasses shows only phosphorescence. This difference is due to the fact that in the former glass the lowest excited-singlet state is of the π, π^* type whereas in the latter glass it is of the *n*, π^* type (*179*).

The external heavy-atom effect has been applied to the phosphorimetric analysis of heterocyclic nitrogen compounds (*180*). Thus, isoquinolines can be determined at much lower concentrations in mixtures of EPA and iodomethane than in EPA alone.

Probably the most selective photometric method of analysis is quenchophosphorimetry (*121*). This is a type of functional group analysis that depends on solvent effects. Some of the quenching solvents that have been investigated with a large variety of phosphorescent compounds include carbon disulfide, *N,N*-dimethylhydrazine, nitromethane, NO_2–trifluoroacetic acid, *o*-nitrotoluene, and trifluoroacetic acid. The possibilities of the method are shown by the fact that the pure phosphorimetric

spectra of 4-nitroaniline are obtained from a wet spot on glass-fiber paper containing the phosphorescent compounds 4-nitroaniline, carbazole, 4-hydroxyacetophenone, triphenylamine, and triphenylene in 10% *o*-nitrotoluene in toluene, all compounds in less than microgram amounts.

2. *Zz zwitterionic resonance structures.* The dipole moment of these structures is greater in the ground state than in the excited state. For this reason increased solvent polarity or the addition of a hydrogen ion to a negative terminal will give a blue shift and a decrease in intensity. An example is the chromogen, II, obtained in the determination of phenol with 3-methyl-2-benzothiazolinone hydrazone (*181*).

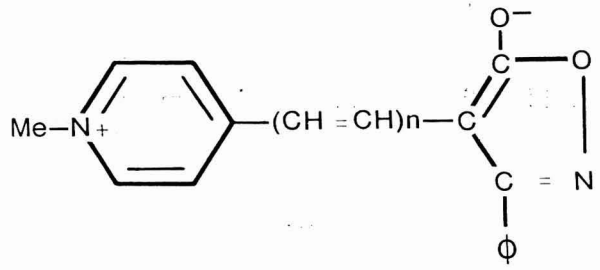


II

λ max	Solvent
525	Aqueous alkali
505	Aqueous acid

The method can be used in the determination of phenols in auto exhaust (*168*).

The compound, III, $n = 2$, has the typical *Zz* structure (*182*). Its



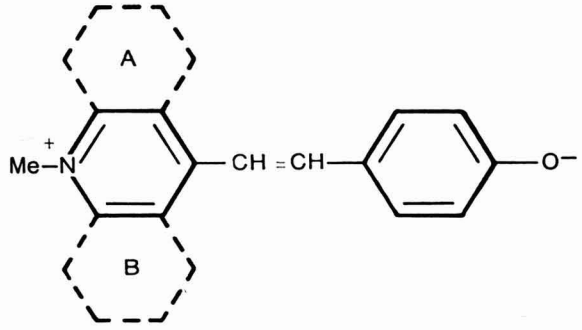
III

n		
0	364(28.0)	400(35.0)
3	488(30.0)	710(93.7)

dipole moment is 33 in the ground state and about 22 in the excited state. It absorbs at ~475 nm with mε 38 in water and in the less polar solvent, pyridine, at ~620 nm with mε 57 and in acid solution near 400 nm.

An increase in the length of conjugation of a *Zz* structure can result in an even more sensitive solvent polarity effect, as seen for III (*182*).

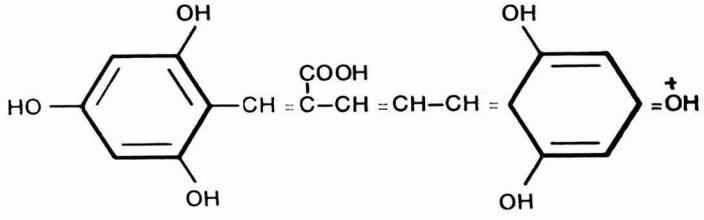
The literature describes compounds that have a *Zz* structure at one end of a solvent polarity scale and a *Zn* structure at the other end. Annulation can also cause a change of a *Zz* structure to a *Zn*, as shown for IV (*183*).



IV

Benzene rings	Structure	λ max(mε) Benzyl alcohol	Chloroform
None	*Zz*	523(41)	620
AB	*Zn*	700(55)	575(28)

3. Zb zwitterionic resonance structures. These molecules can be considered charged in both the ground and excited states. Increased solvent polarity or the addition of a hydrogen ion to the negative resonance terminal gives a blue shift. Some examples are given in Tables 4, 5, and 12. An analytical example is the chromogen, V, obtained in the determination of proline with isatin. Formation of the cationic salt results in a blue shift with a decrease in intensity. On the basis of these reactions proline has been identified in atmospheric samples (*184*).



V

Solvent	λ max	mε
Dimethylformamide	596	48
5 *N* HCl	484	2.9

4. Cationic resonance structures. Decreasing solvent polarity results in a red shift for these structures. An example is the chromogen obtained

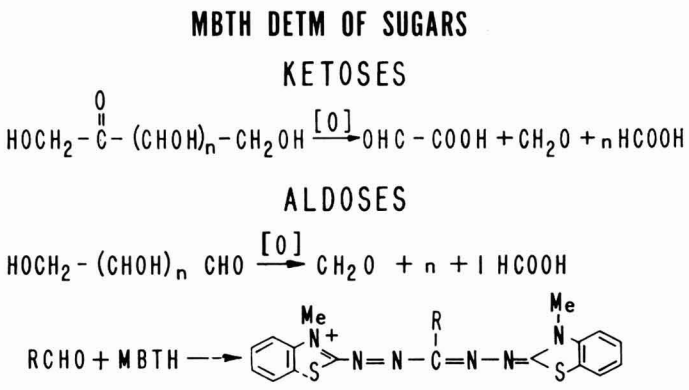
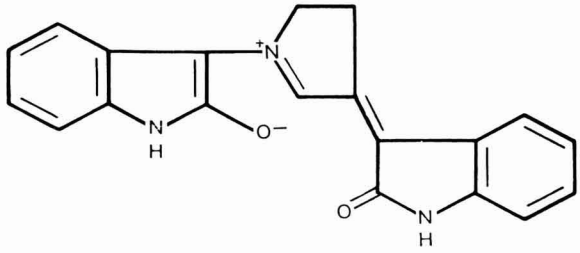


FIG. 11. 3-Methyl-2-benzothiazolinone oxidative determination of ketoses and aldoses.

in the determination of atmospheric aldehydes (*130*) and in the determination of carbohydrates, Fig. 11 (*185*). The long-wavelength maximum of this chromogen shifts dramatically to the red with decreasing solvent polarity, e.g., in water 655 nm, in chloroform 675 nm, and in *o*-dichlorobenzene 708 nm. A similar effect has been shown for the chromogen, VI, obtained in the determination of nicotinic acid with phloroglucinol (*186*).



VI

Solvent	λ max
Methanol	632
Ethanol	639
n-Propanol	641

On the other hand, in the determination of hexoses by anthrone in strong sulfuric acid, the cationic salt of the 1,2-dianthronylidene ethane chromogen absorbs at greater wavelength and with greater intensity with increasing acidity of the sulfuric acid (*187*).

Fluorimetric effects are also important. An acid solvent is of value in determining atmospheric phenalen-1-one and 7*H*-benz(*de*)anthracen-7-one (*188*). With ITLC and trifluoroacetic acid these compounds can

be quickly and simply determined fluorimetrically. The acid seems to evaporate less rapidly on the glass-fiber chromatogram so that assay is possible.

Phosphorimetric spectra of cations have been reported (*129*). Thus, 4-hydroxyacetophenone gives the spectra of the cation, anion, or neutral compound dependent on the solvent used.

Solvent effects of fluorogen and phosphorogen cations have been little studied.

5. Anionic resonance structures. These structures are formed either through loss of a cation from a zwitterionic resonance structure or through addition of an activated anion to a molecule substituted with one or more electronegative groups. Formation of the anion from the neutral compound results in a red shift and an increase in intensity as shown for 4-(4′-nitrophenylazo)diphenylamine, Fig. 12. For most of these anions an increase in solvent basicity results in a red shift and an increase in intensity, Fig. 9 and Tables 14 and 15 [see also (*60*)], as shown by the wavelength maxima and millimolar absorptivities of 4-(4′-nitrophenylazo)phenol in the following solvents: water, 472,28.0; ethanol, 505, 35.6; benzene, 519, 36.4; methylene chloride, 551, 43.5; *p*-dioxane, 547, 44.3; and dimethylformamide, 591, 60.3.

Many methods of organic trace analysis depend on the formation of a chromogen anion. Individual phenols have been determined in auto

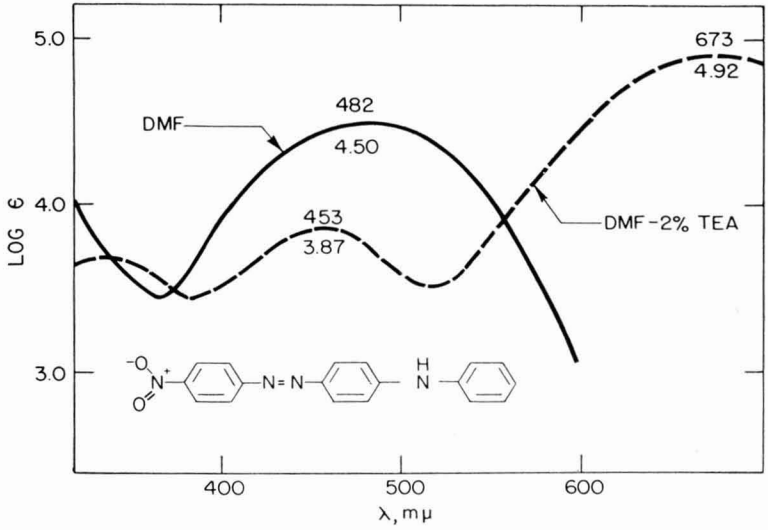


FIG. 12. Absorption spectra of 4-(4′-nitrophenylazo)diphenylamine in dimethylformamide (——); and in dimethylformamide containing 2% of 10% aqueous tetraethylammonium hydroxide (– – –).

exhaust photometrically after paper chromatography of their 2-nitrophenylazo derivatives (*189*). Total phenols in automotive exhaust (*181*) and in cigarette smoke condensate (*190*) have been determined colorimetrically as their 4-nitrophenylazo derivatives. In the former method phenol gave λ_{max} 485 and mε 27 in aqueous alkali; in the latter, λ_{max} 358 and mε 23.4 in carbon tetrachloride. With a much more basic solvent (see Table 15) the sensitivities of these methods could be improved considerably.

Anionic chromogens are obtained in determining nitrofurans with phenylhydrazine (*191*), 17-ketosteroids with *m*-dinitrobenzene (*192–195*), formaldehyde with 2-hydrazinobenzothiazole (*196, 197*), glyoxal with 4-nitrophenylhydrazine or 2,4-dinitrophenylhydrazine (*145*), nitrites and nitrosamines with *p*-phenylazoaniline (*198, 199*), and ammonia either directly or through precursor methods. In the ammonia methods indophenol is formed and measured in the determination of ammonia (*200, 201*), ornithine carbamoyltransferase (*202*), urea (*203*), and nitrogen in biological material (*204*). The long-wavelength band of the indophenol anion moves to the red as the solvent is changed from water to acetone (*205*).

The solvent basicity effect has been demonstrated in the determination of glyoxal (*145*). Most of these methods could benefit from use of a solvent of higher basicity at the measurement stage.

The fluorescence spectra of examined anions do not show as drastic solvent basicity effects. Thus, the anion from 1-tosylaminoanthracene and the anion obtained in the determination of malonaldehyde and its precursors show a much larger red shift in their excitation spectra than in their emission spectra on increasing solvent basicity. The emission spectra of polynuclear phenols, such as 2-fluorenol and 6-chrysenol, show a moderate red shift with increasing solvent basicity, e.g., about 40 nm from water to dimethylformamide.

Many solvent effects are described in this paper. Their use in the investigation of various types of environmental problems indicates their possible application to studies of air pollution. Some of the methods have been applied to analysis of polluted atmospheres. Many others could be applied with profit to the characterization and analysis of our environmental toxicants and thus provide greater understanding of our increasingly polluted environment and a greater possibility of more intelligent control.

SUMMARY

Solvents play an important role in photometric analytical methodology through ultraviolet absorptiometric, colorimetric, fluorimetric, low-temperature fluorimetric,

and phosphorimetric methods of measurement. Stress is placed on solvent-effected changes (occurring in the position and intensity of the long-wavelength maxima of chromogens, fluorogens, and phosphorogens) of definite or potential use in organic trace analysis of environmental pollutants. Many techniques useful in air pollution analysis are discussed. Also discussed are factors affecting the electronic spectra, including the physical properties of solvents and solutes and the various types of spectral bands useful in photometric analysis.

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Simultaneous Determination of Hydrogen and Nitrogen in Organic Compounds by a Gas-Volumetric Method Using a Newly Designed Pt–P_2O_5 Electrolytic Cell

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INTRODUCTION

In recent years, Monar (*1*) reported a new method for the simultaneous determination of hydrogen and nitrogen using a double tube automatic apparatus. According to this method, hydrogen gas which is quantitatively produced by the reaction of water with calcium hydride is measured by an automatic azotometer:

$$CaH_2 + 2H_2O \rightleftarrows Ca(OH)_2 + H_2.$$

This principle has been applied to other analytical instruments (*2–4*). Gouverneur and co-workers (*5*), however, pointed out a drawback in this principle, which is caused by the side reaction of carbon dioxide with calcium hydroxide:

$$Ca(OH)_2 + CO_2 \rightleftarrows CaCO_3 + H_2O.$$

Thus we sought to develop another way for the simultaneous determination of hydrogen and nitrogen.

Keidel (*6*) described a coulometric determination of moisture in gases based on Faraday's law of electrochemical equivalence using a Pt–P_2O_5 electrolytic cell. Czuha *et al.* (*7*) discussed in detail the principal reaction in the cell. Phosphorus pentoxide coated on the electrode is converted to metaphosphoric acid by absorption of water produced by combustion of a sample. On electrolysis metaphosphoric acid is reconverted to phosphorus pentoxide by generating hydrogen and oxygen:

$$(P_2O_5)_n \underset{-nH_2O}{\overset{+nH_2O}{\rightleftarrows}} (HPO_3)_{2n},$$

$$2HPO_3 \xrightarrow{\text{electrolysis}} H_2 + 1/2O_2 + P_2O_5.$$

Cathode: $2H^+ + 2e \rightleftarrows H_2,$

Anode: $2PO_3^- - 2e \rightleftarrows P_2O_5 + 1/2O_2.$

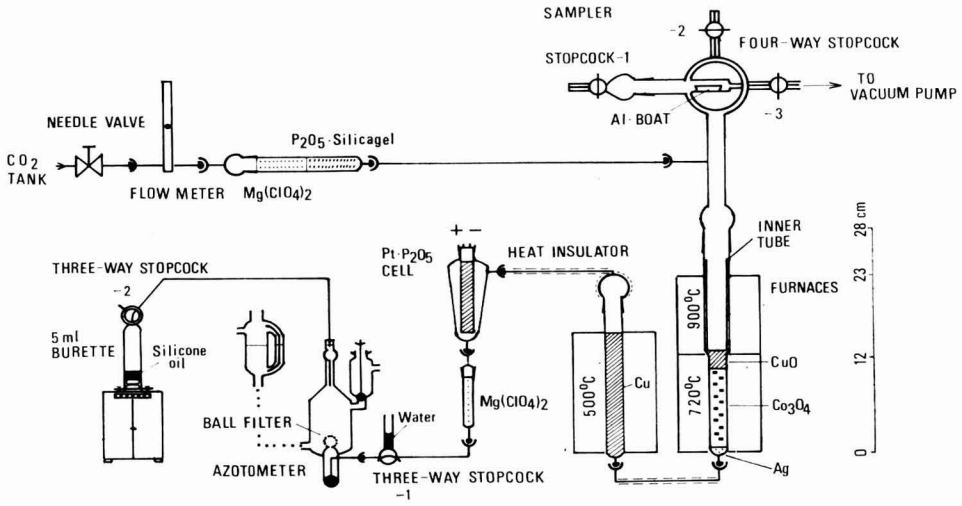

FIG. 1. Schematic diagram of apparatus.

The theoretical volume for 1 mole of water consisting of 2:1 hydrogen-oxygen mixture should be 0.1741 ml/C at standard conditions (*8*). Sufficient accuracy is to be expected for the gas-volumetric hydrogen analysis by this method. Several papers on coulometric determination of hydrogen in organic compounds have appeared using Keidel cell (*9–12*) and modified Keidel cells (*13,14*), but none of them used a gas-volumetric method.

In our apparatus, the cell coated by phosphoric anhydride plays double rolls for desiccant and electrolyte under high purity carbon dioxide. In case of nitrogen-containing compounds, nitrogen is measured by a dispersion type azotometer,[1] prior to electrolysis of the water absorbed in the cell. The evolved mixture of hydrogen and oxygen is also measured by the azotometer. Satisfactory analytical results are shown in Table 5. For hydrogen analysis only several decimilligrams of sample are available.

MATERIALS AND METHODS

Apparatus

The complete assembly is illustrated in Fig. 1. All the joints used in the apparatus are glass ball joints (12 mmϕ), lubricated with silicone grease. Carbon dioxide, at a flow rate of 15 ml/min, is dried by passing through Anhydrone and P_2O_5–silica gel before entering the combustion tube.

[1] Japanese Utility Model No. 41–4718.

Combustion Tube

A quartz vertical combustion tube consists of two parts. In upper voluminous part (19-mm i.d.), a quartz inner tube (18-mm o.d.) having stainless steel, gauze positioned at the lower bottom end. In the lower part (15-mm o.d.) are packed copper oxide wire, granular cobaltosic oxide, and granular silver.

Reduction Tube

The reduction tube is a vertical quartz tube (15-mm o.d.) and the temperature of the copper layer should be kept at 490–500°C using a thermocontroller.

Sampler

A sampler (*15*) is constructed using a four-way stopcock and three additional stopcocks, as shown in Fig. 1. Stopcocks 1 and 3 are used for introducing a sample boat into the four-way stopcock with the aid of a vacuum pump. Stopcock 2 is used for expelling air when the inner tube is changed after about 50 analyses. Stopcock 3 is connected to a vacuum pump.

Azotometer

The dispersion type azotometer equipped with a glass ball filter is charged with 70 ml of 50% aqueous potassium hydroxide solution. Volumetric determinations of nitrogen and the gaseous mixture of hydrogen and oxygen are carried out using a 5-ml piston burette (Metrohm Ltd.) with a microliter scale. A fresh potassium hydroxide solution can be introduced from the reservoir and is replenished after every 10 analyses.

Pt–P_2O_5 Electrolytic Cell and Power Supply

Figure 2 shows a diffusion type Pt–P_2O_5 electrolytic cell. The cell is a Teflon rod, 10 mm in diameter and 70 mm in length, fitted into a glass tube. A two-way screw thread with a pitch of 0.75 mm and a depth of 0.15–0.20 mm was cut in the rod. Two electrodes from platinum wire, 0.25 mm in diameter and 320 cm in length, are wound tightly into the screw. The platinum electrodes are coated with a solution of phosphoric acid in acetone and are set in a glass tube. Then the cell is electrolyzed to dryness. The cell operates at 30 V with a transistorized power supply, Tokyo Electro. Dev. Co., Ltd. Model-SSS. The internal resistance of the cell is varied from 375 to 15,000 ohm with the ratio of phosphoric acid to phosphorous pentoxide, and the maximum electrolytic current is about 80 mA and the minimum is 2.0 mA at 30 V.

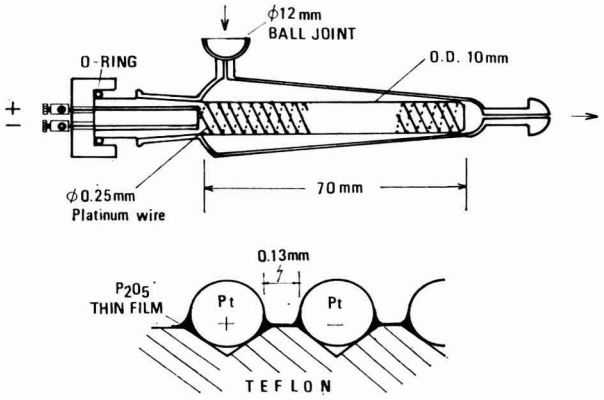

FIG. 2. Electrolytic cell and detail of electrode.

Procedure A. Hydrogen Determination

A sample containing no nitrogen is weighed accurately in an aluminum boat, and 300 mg of oxidant, finely powdered Co_3O_4, is added. The boat is placed on the inlet port of the sampler, and stopcocks 1 and 3 are opened. By means of the applied vacuum, the sample boat is introduced into the center of the four-way stopcock. Stopcock 1 is closed, air purging is completed in 15 seconds (5 mm Hg). Stopcock 3 is closed, the four-way stopcock is rotated to drop the boat into the inner vertical combustion tube. Prior to each determination, the cell is dried by electrolysis until the electrolytic current falls to 2.0 mA. The water vapor is introduced into the cell by CO_2 carrier gas flow. The electrolytic current increases to about 80 mA instantly. The carrier gas flow is vented to the water layer when the electrolytic current decreases to 2.0 mA. The generated hydrogen and oxygen gases are collected in the azotometer, and the volume is determined. To determine the blank value, the electrolysis time is measured with a stopwatch.

Procedure B. Hydrogen and Nitrogen Determination

A sample is weighed in an aluminum boat and, after addition of the oxidant, dropped into the combustion tube, as described in Procedure A. The cell, which has been dried to 2.0 mA, absorbs the water vapor carried into the cell prior to the electrolysis, while the nitrogen produced is swept into the azotometer by CO_2 gas flow. After 10 minutes, the carrier gas is vented to the water layer by the three-way stopcock 1, and the volume of nitrogen is determined. Then, the nitrogen gas is vented through the three-way stopcock 2 and the meniscus is adjusted roughly with the leveling vessel. The cock is turned to the azotometer again and the zero point value is determined. Three-way stopcock 1 is

turned to the azotometer again and the absorbed water is electrolyzed. The electrolysis is carried out until the current is minimized to 2.0 mA. The volume of the generated gases, the electrolysis time, and the blank value are also measured in the same way as described in Procedure A.

Calculation

The hydrogen content is calculated from the following equations:

$$H\% = F \cdot V/S, \qquad V = V' - (V_1 + V_2),$$

where F = weight of hydrogen derived from 1 ml of the mixture gas (mg);
V' = observed volume (ml);
V_1 = calibrated correction of the scale (ml);
V_2 = blank correction for the analysis time (ml); and
S = weight of the sample (mg).

The factor(F)-table was computed, taking into consideration of the reduction of the barometer reading and of the vapor pressure of the potassium hydroxide solution. Results are shown in Table 1.

RESULTS

Results of analysis on sucrose weighing from 100 to 900 μg are shown in Table 2, and those of 1.5-3 mg are shown in Table 3. Analytical data of other standard compounds containing C, H, O, Cl are shown in Table 4. Results of the simultaneous determination of some standard compounds are shown in Table 5.

DISCUSSION

The results from these experiments, the standard deviation was satisfactory with 100–900-μg samples of sucroses so that this method is promising for decimilligram hydrogen analysis. The blank values of hydrogen and nitrogen were 20 and μl/min. respectively. The electrolysis current efficiency attained 100%. There is no problem of the recombination of the hydrogen and oxygen (*12*) generated by electrolysis of water with this cell.

The analysis time required for hydrogen is 10 to 20 minutes, depending upon the quantity of the hydrogen contained in the samples. For the simultaneous determination of hydrogen and nitrogen, 20 to 30 minutes were required. Primarily solid standard compounds were analyzed. Liquid samples could be weighed in an aluminum capillary tube, which was sealed at both ends and placed in an aluminum boat with the oxidant. In the case of compounds which sublime easily, the sample boat is introduced with a pure oxygen gas flow without evacuation.

Table I CALIBRATED FACTOR OF HYDROGEN ANALYSIS

TEMP. °C \ PRESS. mmHg	750.0	751.0	752.0	753.0	754.0	755.0	756.0	757.0	758.0	759.0	760.0
20.0	5.4559	5.4632	5.4706	5.4779	5.4853	5.4927	5.5000	5.5074	5.5147	5.5221	5.5294
20.5	5.4449	5.4522	5.4596	5.4669	5.4743	5.4816	5.4890	5.4963	5.5036	5.5110	5.5183
21.0	5.4339	5.4412	5.4486	5.4559	5.4632	5.4706	5.4779	5.4852	5.4926	5.4999	5.5072
21.5	5.4229	5.4303	5.4376	5.4449	5.4522	5.4595	5.4668	5.4742	5.4815	5.4888	5.4961
22.0	5.4120	5.4193	5.4266	5.4339	5.4412	5.4485	5.4558	5.4631	5.4704	5.4777	5.4850
22.5	5.4010	5.4083	5.4156	5.4229	5.4302	5.4375	5.4448	5.4521	5.4594	5.4666	5.4739
23.0	5.3901	5.3973	5.4046	5.4119	5.4192	5.4265	5.4337	5.4410	5.4483	5.4556	5.4629
23.5	5.3791	5.3864	5.3936	5.4009	5.4082	5.4154	5.4227	5.4300	5.4372	5.4445	5.4518
24.0	5.3681	5.3754	5.3826	5.3899	5.3972	5.4044	5.4117	5.4189	5.4262	5.4334	5.4407
24.5	5.3572	5.3644	5.3717	5.3789	5.3862	5.3934	5.4006	5.4079	5.4151	5.4224	5.4296
25.0	5.3462	5.3535	5.3607	5.3679	5.3751	5.3824	5.3896	5.3968	5.4041	5.4113	5.4185
25.5	5.3353	5.3425	5.3497	5.3569	5.3641	5.3714	5.3786	5.3858	5.3930	5.4002	5.4075
26.0	5.3243	5.3315	5.3387	5.3459	5.3531	5.3603	5.3676	5.3748	5.3820	5.3892	5.3964
26.5	5.3134	5.3205	5.3277	5.3349	5.3421	5.3493	5.3565	5.3637	5.3709	5.3781	5.3853
27.0	5.3024	5.3096	5.3168	5.3239	5.3311	5.3383	5.3455	5.3527	5.3599	5.3670	5.3742
27.5	5.2914	5.2986	5.3058	5.3129	5.3201	5.3273	5.3345	5.3416	5.3488	5.3560	5.3631
28.0	5.2805	5.2876	5.2948	5.3019	5.3091	5.3162	5.3234	5.3306	5.3377	5.3449	5.3520
28.5	5.2695	5.2766	5.2838	5.2909	5.2981	5.3052	5.3124	5.3195	5.3267	5.3338	5.3410
29.0	5.2585	5.2656	5.2728	5.2799	5.2870	5.2942	5.3013	5.3084	5.3156	5.3227	5.3299
29.5	5.2475	5.2546	5.2618	5.2689	5.2760	5.2831	5.2902	5.2974	5.3045	5.3116	5.3187
30.0	5.2365	5.2436	5.2507	5.2578	5.2650	5.2721	5.2792	5.2863	5.2934	5.3005	5.3076

TEMP. \ PRESS.	760.0	761.0	762.0	763.0	764.0	765.0	766.0	767.0	768.0	769.0	770.0
20.0	5.5294	5.5368	5.5441	5.5515	5.5589	5.5662	5.5736	5.5809	5.5883	5.5956	5.6030
20.5	5.5183	5.5257	5.5330	5.5403	5.5477	5.5550	5.5624	5.5697	5.5771	5.5844	5.5917
21.0	5.5072	5.5145	5.5219	5.5292	5.5365	5.5439	5.5512	5.5585	5.5659	5.5732	5.5805
21.5	5.4961	5.5034	5.5108	5.5181	5.5254	5.5327	5.5400	5.5473	5.5547	5.5620	5.5693
22.0	5.4850	5.4923	5.4996	5.5069	5.5142	5.5216	5.5289	5.5362	5.5435	5.5508	5.5581
22.5	5.4739	5.4812	5.4885	5.4958	5.5031	5.5104	5.5177	5.5250	5.5323	5.5396	5.5469
23.0	5.4629	5.4701	5.4774	5.4847	5.4920	5.4993	5.5065	5.5138	5.5211	5.5284	5.5357
23.5	5.4518	5.4590	5.4663	5.4736	5.4808	5.4881	5.4954	5.5026	5.5099	5.5172	5.5245
24.0	5.4407	5.4479	5.4552	5.4625	5.4697	5.4770	5.4842	5.4915	5.4987	5.5060	5.5133
24.5	5.4296	5.4369	5.4441	5.4513	5.4586	5.4658	5.4731	5.4803	5.4876	5.4948	5.5021
25.0	5.4185	5.4258	5.4330	5.4402	5.4475	5.4547	5.4619	5.4692	5.4764	5.4836	5.4909
25.5	5.4075	5.4147	5.4219	5.4291	5.4363	5.4436	5.4508	5.4580	5.4652	5.4724	5.4797
26.0	5.3964	5.4036	5.4108	5.4180	5.4252	5.4324	5.4396	5.4468	5.4540	5.4612	5.4685
26.5	5.3853	5.3925	5.3997	5.4069	5.4141	5.4213	5.4285	5.4357	5.4429	5.4501	5.4573
27.0	5.3742	5.3814	5.3886	5.3958	5.4030	5.4101	5.4173	5.4245	5.4317	5.4389	5.4461
27.5	5.3631	5.3703	5.3775	5.3847	5.3918	5.3990	5.4062	5.4133	5.4205	5.4277	5.4348
28.0	5.3520	5.3592	5.3664	5.3735	5.3807	5.3878	5.3950	5.4022	5.4093	5.4165	5.4236
28.5	5.3410	5.3481	5.3552	5.3624	5.3695	5.3767	5.3838	5.3910	5.3981	5.4053	5.4124
29.0	5.3299	5.3370	5.3441	5.3513	5.3584	5.3655	5.3727	5.3798	5.3869	5.3941	5.4012
29.5	5.3187	5.3259	5.3330	5.3401	5.3472	5.3544	5.3615	5.3686	5.3757	5.3829	5.3900
30.0	5.3076	5.3147	5.3219	5.3290	5.3361	5.3432	5.3503	5.3574	5.3645	5.3716	5.3788

TABLE 2

RESULTS ON SUCROSE IN THE 100–900-μg RANGE
(Theory H% = 6.48)

No.	Sample wt (μg)	Vol (μl)	Temp (°C)	Pressure (mm Hg)	Factor	% Hydrogen Found	% Hydrogen Diff.
1	145	380	26.5	764	5.4141	6.35	−0.13
2	268	325	27.0	764	5.4030	6.55	+0.07
3	364	430	27.5	768	5.4205	6.40	−0.08
4	435	540	27.0	764	5.4030	6.71	+0.23
5	499	590	27.0	764	5.4030	6.39	−0.09
6	634	781	28.0	764	5.3807	6.67	+0.19
7	640	750	27.5	768	5.4205	6.35	−0.13
8	641	760	27.5	768	5.4205	6.43	−0.05
9	814	984	27.5	767	5.4133	6.54	+0.06
10	868	1017	27.5	767	5.4133	6.34	−0.14
					Mean	6.47	−0.01
					SD	0.14	

TABLE 3

RESULTS ON SUCROSE IN THE 1.5–3-mg RANGE
(Theory H% = 6.48)

No.	Sample wt (mg)	Vol (ml)	Temp (°C)	Pressure (mm Hg)	Factor	% Hydrogen Found	% Hydrogen Diff.
1	1.454	1.828	26.5	766	5.4285	6.82	+0.34
2	2.161	2.667	26.5	766	5.4285	6.70	+0.22
3	2.165	2.675	26.5	766	5.4285	6.71	+0.23
4	2.247	2.726	27.5	765	5.3990	6.54	+0.06
5	2.316	2.704	26.5	767	5.4357	6.35	−0.13
6	2.561	3.083	26.5	768	5.4429	6.55	+0.07
7	2.644	3.132	26.5	766	5.4285	6.43	−0.05
8	2.690	3.262	26.5	767	5.4357	6.59	+0.11
9	2.894	3.358	27.0	767	5.4245	6.29	−0.19
10	3.047	4.564	26.5	766	5.4285	6.35	−0.13
					Mean	6.53	+0.05
					SD	0.18	

TABLE 4

RESULTS OF DUPLICATE ANALYSIS ON STANDARD COMPOUNDS

Compound	Sample wt (mg)	Vol (ml)	Temp (°C)	Pressure (mm Hg)	Factor	% Hydrogen		
						Theory	Found	Diff.
Cholesterol	1.791	4.044	26.5	768	5.4429	11.99	12.23	+0.24
	1.467	3.252	27.5	767	5.4133		12.00	+0.01
Naphthalene	2.220	2.588	27.0	767	5.4245	6.29	6.32	+0.03
	2.789	3.290	27.5	767	5.4133		6.39	+0.10
Benzoin	3.379	3.503	26.5	768	5.4429	5.70	5.65	−0.05
	2.846	3.014	27.5	767	5.4133		5.73	+0.03
2,4-Dichloro phenoxyacetic acid	3.168	1.614	27.0	767	5.4245	2.74	2.76	+0.02
	3.332	1.640	27.5	767	5.4133		2.66	−0.08

TABLE 5

RESULTS OF DUPLICATE ANALYSIS ON STANDARD COMPOUNDS

Compound	Sample wt (mg)	Nitrogen (%)			Hydrogen (%)		
		Theory	Found	Diff.	Theory	Found	Diff.
Acetanilide	2.423	10.36	10.15	−0.21	6.71	6.43	−0.28
	2.425		10.34	−0.02		6.61	−0.10
Sulfathiazole	2.695	16.46	16.39	−0.07	3.55	3.42	−0.13
	2.541		16.32	−0.14		3.37	−0.18
m-Dinitrobenzene	2.617	16.66	16.65	−0.01	2.40	2.18	−0.22
	3.266		16.60	−0.06		2.17	−0.23
Hippuric acid	2.497	7.82	7.72	−0.10	5.06	4.81	−0.25
	2.645		7.92	+0.10		4.92	−0.14

ACKNOWLEDGMENT

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SUMMARY

The simultaneous gas-volumetric determination of hydrogen and nitrogen with a newly designed Pt–P_2O_5 electrolytic cell has been investigated. The combustion was carried out in a vertical combustion tube and about fifty samples could be analyzed in sequence. The gaseous products were passed through copper oxide, active cobalt oxide, silver and copper fillings by pure carbon dioxide carrier gas. First the water is absorbed in the Pt–P_2O_5 cell, and nitrogen produced was determined with a dispersion type azotometer. Then by electrolysis of the absorbed water, 2:1 hydrogen–oxygen mixtures were evolved and the volume was determined with the same azotometer.

From 1 mole of water, 1 mole of hydrogen and 0.5 mole of oxygen are produced. From this chemical relation, it is practical to determine hydrogen in decimilligram quantities.

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Book Reviews

Molecular Processes on Solid Surfaces. Edited by EDMUND DRAUGLIS, RONALD D. GRETZ, AND ROBERT I. JAFFEE. McGraw-Hill, New York, 1969. xvii + 651 pp. $37.50.

This book presents the proceedings of the third Battelle Institute Materials Science Colloquium, held in Kronberg, Germany in May, 1968. Included are a preface, in which I. N. Stranski summarizes nearly half a century of work on crystal growth and shapes, and 28 articles on assorted experimental and theoretical studies of surfaces.

The articles are divided into six groups, under the headings Introductory Lectures, Characterization and Structure, Electronic Interactions, Adsorption, Nucleation and Growth, and Macroscopic Effects. The four introductory lectures establish the foundation for many of the research papers that follow: Mobility of Molecules along Adsorbing Surfaces, by J. H. de Boer; Methods for Investigating the Mechanisms of Catalytic Hydrogen and Oxygen Transfer Reactions, by C. Wagner; Some Applications of Low-Energy Electron Diffraction to Surface Problems, by H. E. Farnsworth and M. Onchi; and A Theory of Chemisorption on *d*-electron Metals, by L. Jansen.

The diversity of topics covered makes this volume a valuable survey of research on the surface state. The uniform quality of the writing and the low frequency of misprints suggest careful editing. The articles are authoritative while being sufficiently general to be intelligible to the nonspecialist.

An especially valuable feature is the inclusion of discussion sections after most of the papers. In addition, four of the six sections conclude with agenda discussions in which the chairman analyzes the important aspects of the proceedings of his session. These discussions constitute a concise summary of the colloquium, and they show which topics are controversial.

The editors have produced more than just a collection of papers, and this book is highly recommended to all students of surfaces. It is unfortunate that the high costs of book publishing may keep it out of many personal libraries.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506*

Trace Elements in Agriculture. By VINCENT SAUCHELLI. Van Nostrand Reinhold, New York, 1969. viii + 248 pp. $15.00

The late Vincent Sauchelli felt that the role of small quantities of chemicals had not received the same emphasis in agriculture that it has had in animal and human nutrition. Accordingly, he compiled an amazing amount of information from a large variety of sources and presented it in this book. Just which of the chemicals found in plants are required for their nutrition and which are merely picked up from the soil, is moot, but the essential nature of many of them in extremely small amounts is definite.

Trace elements, minor elements, and oligoelements are terms which are found in the literature and mean the same thing to the nutritionist, but they can lead to confusion. Micronutrients would seem to be more specifically descriptive but trace elements is widely understood and is used throughout this book.

After introductory chapters on the historical background, biophysicochemical relationships, soil–plant relationship, trace elements in nutrition, and deficiency symptoms, Sauchelli devotes a chapter each to iron, manganese, boron, zinc, molybdenum, copper, chlorine, sodium, selenium, cobalt, and fluorine with nickel, lithium, vanadium, silicon, and aluminum treated together in a single chapter. The next section is concerned with fertilizer and market problems. The final chapter, very short, discusses analysis by atomic absorption.

To the analyst the book will be a disappointment, yet presents a challenge. Except for the chapter on atomic absorption, no mention is made of analytical procedures except in the chapters on manganese and selenium. The challenge lies in the difficulty of determining micronutrients by methods that could be made adaptable to field conditions (or at least, nearly so). The mention of the bioassay of manganese using growth of *Aspergillus niger* may point the way to a possible solution. Agriculture's problem is to maintain a balance of all nutrients in the soil. Deficiencies vary from area to area and within locations in a given area, making the formulation of standard fertilizers almost impossible. Distribution of trace elements in a way to prevent local concentrations that may be toxic is another problem.

The book is well written, amply documented, and has a good index. The photographs are descriptive of the points to be illustrated. It is fascinating to read, but of limited professional interest to the microanalyst, unless hydroponics is his hobby.

DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York 10705*

Applied Spectroscopy Reviews. Volume 2. Edited by EDWARD G. BRAME, JR. Dekker, New York, 1969. viii + 376 pp. $17.50.

As the title suggests, this is the second book in the series "Applied Spectroscopy Reviews," and like its predecessor, is a review of the recent literature. Being an international publication dealing with the principles, methods, and applications of spectroscopy in the various fields of science, the volume under review has contributors from the U.S., India, and Italy. The book comprises four reviews, two of which are devoted to recent advances in the field of infrared spectroscopy (IR) one is concerned with the various spectroscopic studies that can be used in investigations. Of these studies, IR probably has played the chief role in hydrogen bond research. The fourth review is concerned with an important type of analysis in nuclear magnetic resonance (NMR) spectroscopy.

Each review begins with a well-written introduction to that particular field and concludes with a complete list of references. It provides the latest information on principles, methods, and applications of spectroscopy for the researcher with discussions that relate physical concepts to chemical applications. Recent advances in the field through 1967 are reviewed, assessed, and critically evaluated.

The most valuable and comprehensive chapter in the entire book is the review by Dr. Whetsel on "Near-Infrared Spectrophotometry" which contains the most recent information in this rather neglected field of IR spectroscopy. This chapter

does an important service by calling attention to the usefulness of this region of the IR for both organic and inorganic analysis. The review by Dr. Zerbi on "Molecular Vibrations of High Polymers" constitutes a general review of the usefulness of IR spectroscopy in the structure elucidation studies of polymers. In the review on "Spectroscopic Studies of the Hydrogen Bond" by Drs. Murthy and Rao, the latest information encompassing IR, Raman, NMR, and electronic spectroscopy, along with excellent and comprehensive tables giving thermodynamic data on a large number of compounds is presented. The last review entitled "Analysis of ABX Spectra in NMR Spectroscopy" by Dr. Slomp should be extremely valuable to anyone doing structure analysis in NMR.

Typographically, the book is excellent and well produced. The print is clear, and only a few minor errors of fact or transcription are apparent. Being moderately priced and containing a valuable review of the recent literature, the book should appeal to a broad cross section of scientists engaged in specific spectroscopic studies. It is a book which is hard to fault and on which the authors are to be congratulated. As such its acquisition is strongly recommended.

GEORGE WIENER, *Chas. Pfizer Co., Inc., Brooklyn, New York 11200*

The Laboratory Handbook of Methods of Food Analysis. By R. LEES. Hill, London; *Chem. Rubber Pub. Co.,* Cleveland, Ohio, 1968. XVII + 181 pp. $18.50.

In general this is a poor manual for those concerned with the analysis of food in the United States. The book was apparently written for use in England in British laboratories and factories. Therefore, many of the procedures and descriptive terms used are European in origin and will be strange to workers in the United States. Additionally, many of the methods differ more or less significantly from those of the Association of Official Analytical Chemists, and these latter are considered standard in the United States.

The general descriptions of complicated procedures in this Handbook are poor, and some are incomplete. The indexing is such that the applicability of a method to a specific food, especially if two variations of a method are given is impossible to determine if the method is not listed under the food in question. Some of the general remarks and Tables in the first part of the book provide useful information for the beginning analyst but a statement such as "a true moisture determination is given by the Karl Fischer method" should be qualified because of the many problems encountered in applying this procedure to some food products. In addition, the description of this method is poor.

The cost of the book appears to be exorbitant for the size, information supplied, and type of paper used.

K. G. SLOMAN, *General Foods Corp., Technical Center, White Plains, New York 10602*

Modern Microcrystal Tests for Drugs. By CHARLES C. FULTON. Wiley (Interscience), New York, 1969 vxiii + 466p. $29.95,

This is a remarkable book, bringing together in one volume an heterogeneous array of tests for a multitude of substances. Tabulating and correlating materials,

tests, methods, and results would be, by itself, a tremendous undertaking. Mr. Fulton is to be congratulated on a magnificant job well done. His preface is altogether too modest.

The introduction outlines the purpose of the book and discusses optical crystallography, and chemical identification tests with their forensic uses. There is also a list of abbreviations. This latter is quite necessary as the abbreviations are contrived and would be unintelligible otherwise.

The chapter on procedures and techniques is clear, well-written and the best this reviewer has ever read. In the discussion of reagents, Mr. Fulton not only tabulates them but classifies them in relation to reliability, satisfaction, etc., along with sensitivities and results. The remainder of the book is devoted to the tests themselves and their applicability to practically the entire range of drugs—alkaloids and other botanicals, the modern synthetics with particular attention to the tranquilizers and amphetamine-type groups, and putrefactive bases—so important forensically.

The classical methods of the past are presented along with several developed by Mr. Fulton in his 40-years experience. The most important of these is his use of nonaqueous media such as phosphoric and sulfuric acids. Gradual evaporation, allowing the growth of crystals that are slow in making their appearance is the key to this procedure and is admirably successful in many instances.

There is a profusion of lists and tables, both numbered serially in Roman numerals, but in separate sequences. This reviewer found it confusing. Perhaps it would have been wrong semantically to have called them all tables and numbered them in one sequence, but it would have made it much easier for the user. However, it is no problem once it is worked out.

Tests are grouped according to type, and substances by composition or physiological effect, with examples for each. Formulas are given for reagents and specific tests are given for a large number of compounds. The chapter describing and classifying the forms of microcrystals is exemplary. In this field, words used by one person to describe a certain formation may not mean the same to another. Mr. Fulton explains his terms explicitly. His descriptions are so vivid that the reader is in no doubt about what is meant and can easily relate his own observations to the description in the text and tables.

At the end of the book are 35 pages containing 175 black and white photomicrographs. These are clear and well reproduced and serve to classify types. They are grouped, in a separate list, into eight classes with from 12 to 28 derivatives in each class. There are two indexes—one of substances for which tests are given in the text and the other for substances mentioned but for which no tests are directly given. Chapter bibliographies are adequate but not comprehensive.

According to the foreword by Dr. E. C. G. Clarke "This book is for the toxicologist. . ." but it goes beyond forensic applications of toxicology. Everybody needs it in these days of stress about food additives, new medicinals, and environmental pollution. It is a must for everyone seriously concerned with these problems. The price is high but not unreasonable in relation to the value of the information provided.

DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York 10705*

Encyclopedia of Industrial Analysis. Vol 8. Edited by FOSTER DEE SNELL AND L. S. ETTRE. Wiley (Interscience). New York, 1969. xvi + 737 pp. $45.00 ($35.00 by subscription).

Frequent frustration encountered in waiting for the latter volumes of a compendium to appear is an overt symptom that the work is important. The reviewer recognizes this frustration syndrome with the *Encyclopedia of Industrial Analysis.* Any technical library with a clientele actively involved in applied chemical analysis should have this encyclopedia on its shelves.

Some earlier volumes of this work (see *Microchem. J.* **12,** 282 (1967); **13,** 165 (1968) have been reviewed in this journal. Volume 8 continues the high standards and objectives.

Volumes 1 through 3 considered general techniques and methods. The trek through the chemical alphabet for products and groups of products began with Vol. 4. With Vol. 8, the journey has reached Bromine (and its compounds) and continues as far as Carrier Gas and Vacuum Fusion Methods. Some intermediate stops include the elements calcium, cadmium, and cobalt (and their alloys and compounds), carbon, carbonyl compounds, and carboxylic acids (unsubstituted, amino, and derivatives). The topic Carboxylic Acids, Derivatives is covered well by L. S. Ettre, who with this volume joins the work as Executive Editor.

Chemists oriented to microanalytical techniques and trace analysis will find much of practical interest in this volume. The 19-page article Carbon, Hydrogen, and Nitrogen Microanalysis considers commercial, automated instruments for the determination of these elements in organic samples (nonautomated approaches were treated in Vol. 2.)

In most articles, a satisfactory balance is achieved between the statement of established procedures for the control of commercial products and the discussion of methods and approaches of broader interest. Some reviewers might hold that to reduce the cost of the entire work some discussions could be compressed and that some procedures might be either omitted or abbreviated. However, this reviewer maintains that the corporate and institutional subscribers, that must be the mainstay of any multivolume technical compendium, will support a fuller treatment rather than excessive economy that might reduce the usefulness of the work.

This volume has a 42-page, 2-column subject index of adequate depth. A cumulative index is planned for the final volume. With the number of volumes and the elapsed time involved, this reviewer wonders whether the subscribers would be well served by an interim cumulative index, possibly set in cold type and paper bound, at the midpoint of the entire work.

A. J. BARNARD JR., *J. T. Baker Chemical Company, Phillipsburg, New Jersey, 08865*

Organic Structure Determination. By DANIEL PASTO AND CARL JOHNSON, Prentice-Hall, Englewood Cliffs, New Jersey, 1969. 513 pp. $11.95.

This is an excellent book for the student and should also be required reading for beginning organic chemists. It contains some of the best descriptions of how to solve the problem of separation, isolation and purification of an unknown organic compound. The introduction ot Part I (pp. 3–5) outlines the method of attack on this problem and is alone worth the modest price of this book.

The authors have separated the discussion into three parts. Part I, "Physical Methods of Separation, Purification and Characterization," introduces the various procedures which are needed to accomplish the above goals. The sections on crystallization and separation are particularly useful. Part II entitled "Absorption Spectroscopy", covers UV, IR, NMR, EPR, mass spectroscopy and determination of absolute stereochemistry. These topics are covered of necessity only briefly; however, enough information is given to enable the student to interpret information obtained through the use of these physical methods. Part III, "Identification of Organic Compounds", covers such topics as notebook keeping, laboratory safety and first aid, solubility, acid–base properties, elemental analysis, functional group characterization and a final chapter on literature searching. The longest chapter in this part deals with functional group classification and characterization. Most of this material is similar to that found in Shriner and Fuson with the added feature that many IR curves are included as an aid to the identification of the various functional groups. A final chapter has several structural problems.

With the exception of a few commercial charts (pp. 133–136) which have been reduced to page size and are almost illegible and reproductions of some NMR spectra, the book is very clearly printed and remarkably free of typographical errors.

E. MAGNIEN, *USV Pharamaceutical Corp., Yonkers, New York 10701*

Advances in Optical and Electron Microscopy. Vol. 3. *Edited by* R. BARER AND V. E. COSSLETT. Academic Press, London/New York, 1969 xii + 286 pp. $15.00; 100 s.

This series presents articles on a wide assortment of topics in optical and electron microscopy. The current nature of many of the subjects and the extensive lists of publications characterize these as review articles. However, the thoroughness of the treatments gives them permanent value, and this series may well evolve into a multivolume treatise on microscopy.

In the first of five articles in this third volume, J. R. Benford and H. E. Rosenberger describe zoom systems in microscopy. After presenting the historical background, the authors discuss the design of systems, some actual zoom microscopes, and the prospects for future developments. In the next article D. W. Humphries summarizes mensuration methods in optical microscopy. Topics discussed include the specification of the size of an irregular particle, the techniques and instrumentation of size measurement, and the interpretation of the results.

In the first of three articles pertaining to electron microscopy, R. S. Thomas describes microincineration techniques for localization of the mineral content in biological materials. Included are experimental methods, examples of results, and applications. Next, R. P. Ferrier treats small angle electron diffraction in the electron microscope. The author describes the use of an electron microscope as a diffraction camera for studying crystalline specimens with periodicities exceeding 20 angstroms, he discusses applications in physics, biology, and polymer studies, and he outlines the theory of predicting elastic and inelastic electron scattering cross sections. Finally, M. A. Williams describes the preparation and study of electron microscopic autoradiographs. This is a valuable method for observing the locations of radioactive atoms in biological specimens, and the author discusses the technique and some of the results it has produced.

The writing throughout this volume is of high quality. The book is profusely and clearly illustrated with drawings and photographs. All of the articles except the first have extensive bibliographies; the literature coverage extends into 1968.

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Hydrophobic Surfaces

Edited by **Frederick M. Fowkes**
Department of Chemistry
Lehigh University
Bethlehem, Pennsylvania

This volume presents the proceedings of the Kendall Award Symposium on "Hydrophobic Surfaces" honoring Professor Albert C. Zettlemoyer, held in conjunction with the 155th meeting of the American Chemical Society.

Most of the papers included are concerned with the solid/liquid interface, especially hydrophobic solid/liquid interfaces. The contributors are among the leading international authorities in colloid and surface chemical research, and include some former students and co-workers of Professor Zettlemoyer.

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