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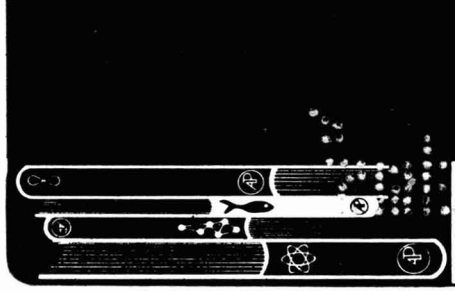

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Microchemical Journal, Vol. 14, No. 1

Briefs

Back Titration with Mercury(II) in Urotropine-Buffered Media; Estimation of Small Amounts of Aluminum and Tervalent Vanadium; Analysis of Mixtures. H. KHALIFA AND I. A. ISMAIL, *Chemical Department, Ministry of Industry, Cairo, U.A.R.*

The potentiometric back titration of EDTA in hexamine-buffered media is successfully applied to the determination of milli- and microgram amounts of aluminum and trivalent vanadium or the analysis of binary and ternary mixtures involving them.

Microchem. J. **14,** 353 (1969).

Use of Microcosmic Salt as a New Titrant for the Microdetermination of Aspartic and Glutamic Acids. A. K. SAXENA, M. N. SRIVASTAVA, AND B. B. L. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Aspartic and glutamic acids were determined in micro quantities with a new titrant, i.e., microcosmic salt solution, using bromocresol purple as indicator. Estimations were carried out in the range of 0.33–1.47 mg with a maximum error of $\pm 1.2\%$. Glycine, alanine, and leucine do not interfere.

Microchem. J. **14,** 361 (1969).

The Simultaneous Microdetermination of Carbon, Hydrogen, and Halogen in Highly Chlorinated or Brominated Organic Compounds. Y. A. GAWARGIOUS AND A. B. FARAG, *National Research Centre, Dokki, Cairo, U.A.R.*

The simultaneous determination of chlorine or bromine together with carbon and hydrogen in highly halogenated organic compounds was achieved by dry-oxidation of the sample with oxygen either in an empty or packed combustion tube. The carbon dioxide and water produced were absorbed in the usual way in soda-asbestos and anhydrone. The halogen was absorbed on a silver gauze roll kept at 550°C, placed in a specially-designed silica absorption tube, connected externally between the combustion and water absorption tubes, and determined gravimetrically.

Microchem. J. **14,** 363 (1969).

Direct Microdetermination of L-Lysine Separately and in a Mixture of Amino Acids. M. L. VERMA AND O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad-2, India.*

L-Lysine was determined by directly titrating against platinic chloride separately as well as in the presence of certain amino acids.

Microchem. J. **14,** 373 (1969).

On the Mechanism of Detecting Organic Substances by Means of Fuchsine Dyes and the Method of Thin-Layer Chromatography. JÓZEF ŚLIWIOK, *Department of Organic Chemistry, Silesian University, Katowice, Poland.*

This paper deals with the mechanism of detecting various compounds by means of thin-layer chromatography.

Microchem. J. **14,** 376 (1969).

Analytical Applications of Isonicotinyol Hydrazones. II. Spectrophotometric Determination of Aluminum with 1-Isonicontinoyl-2-Salicylidene Hydrazine as Chromogenic Reagent. G. S. VASILIKIOTIS AND J. A. TOSSIDIS, *Laboratories of Analytical and Inorganic Chemistry, University of Thessaloniki, Thessaloniki, Greece.*

A spectrophometric method is described for the microdetermination of aluminum in acid medium. Studies show the formation of a water-soluble complex. The range of sensitivity is 0.5–3.5 ppm of aluminum.

Microchem. J. **14,** 380 (1969).

Direct Titrimetric Microdetermination of L-Asparagine and DL-Valine. I. Determination of L-Asparagine and DL-Valine in Presence of Each Other without Separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad-2, India.*

The two amine acids are determined (without separation) and by titration in one solution using xylenol orange as indicator.

Microchem. J. **14,** 385 (1969).

Purification of Labeled ^{203}Hg-Neohydrin. E. HALLABA, H. EL-ASRAG, AND Y. ABOU ZEID, *Nuclear Chemistry Department, Atomic Energy Establishment; and Faculty of Pharmacy, Cairo University, Cairo, U.A.R.*

An easy method for the purification of labeled Neohydrin was studied by the use of the weak cation exchange resin, Amberlite IRC-50, in a small chromatographic column. The resin shows a high selectivity for the removal of mercury ions from a neutral or alkaline solution.

Microchem. J. **14,** 391 (1969).

Titrimetric Microdetermination of Hippuric Acid. M. L. VERMA AND R. K. SRIVASTAVA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

Hippuric acid was determined with indium sulfate, using catechol violet as indicator. A complex between indium and hippuric acid is formed in the ratio of 1:3.

Microchem. J. **14,** 396 (1969).

Application of Back Titration of EDTA with Mercury(II) to the Analysis of Inorganic Pigments. II. Analysis of Zinc Pigments. H. KHALIFA AND A. M. ABDALLAH, *Chemical Department, Ministry of Industry, Petroleum and Mineral Resources, Cairo, U.A.R.*

The potentiometric back titration of excess EDTA with mercury(II) was used very successfully for the analysis of zinc pigments and mixtures of zinc oxide with copper phthalocyanine, especially when adulterated. This new method eliminates the use of hydrogen sulfide separation and is thus characterized by simplicity and rapidity; 28 different industrial samples were analyzed, with many advantages over the classical procedures used for analysis of such materials.

Microchem. J. **14,** 399 (1969).

Chemical Microscopy of 1,5-Naphthyridine: Reactions with Platinum Metals and Gold. HAROLD F. SCHAEFFER, *Department of Chemistry, Westminster College, Fulton, Missouri 65251.*

The reaction between 1,5-naphthyridine and platinum(IV), gold(III), and palladium(II) in acid solutions of the respective chlorides yields characteristic crystalline derivatives which can be easily identified under the microscope.

Microchem. J. **14,** 415 (1969).

On the Preparation of Alkali Bicarbonates Labeled with Carbon-14. M. F. BARAKAT AND A. N. FARAG, *Nuclear Chemistry Department, Atomic Energy Establishment, Cairo, U.A.R.*

Carbon dioxide, liberated by the acid decomposition of labeled barium carbonate, is absorbed in alkali hydroxide or carbonate solutions. Kinetics of the processes and mechanisms leading to the formation of labeled bicarbonates are discussed. A procedure is described for the isolation of solid bicarbonates from aqueous solutions to overcome the radioactivity loss of bicarbonate solutions on evaporation.

Microchem. J. **14,** 422 (1969).

Use of Microcosmic Salt as a New Titrant for the Microdetermination of Sulfanilic Acid. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Sulfanilic acid was determined in micro quantities with a new titrant, i.e., microcosmic salt solution using bromcresol purple as indicator. Estimations were carried out in the range of 0.1016–0.61 mg with a maximum error of ±0.0037 mg.

Microchem. J. **14,** 430 (1969).

An Improved Absorption System for Carbon and Hydrogen Determination. HOWARD J. FRANCIS, JR. *Pennwalt Corporation, King of Prussia, Pennsylvania 19406.*

The determination of carbon and hydrogen was improved by cooling the absorption tubes to eliminate weighing errors caused by temperature fluctuation. Absorption tubes were simplified to allow efficient rapid combustion.

Microchem. J. **14** 432 (1969).

TLC-Fluorimetric Analysis for Atmospheric Scopoletin. E. SAWICKI AND C. GOLDEN, *U.S. Department of Health, Education, and Welfare, Consumer Protection & Environmental Health Service, National Air Pollution Control Administration, Chemical and Physical Research and Development Program, Cincinnati, Ohio 45226.*

The evidence for the presence of scopoletin in airborne particulates, house dust, and coffee roast effluents consists of R_f values obtained with silica gel thin-layer chromatography and mobility values obtained with paper electrophoresis, as well as fluorescence spectra obtained from the chromatogram and from methanolic, alkaline methanolic, and sulfuric acid solutions. By a procedure involving TLC SPF, scopoletin has been assayed in these various samples. Recovery of scopoletin from enriched airborne particulates was 97%. These various separations and fluorimetric examinations have shown that a large number of unknowns was also present in these samples.

Microchem. J. **14,** 437 (1969).

A Rapid Colorimetric Method of Estimation of Micro-Quantities of Diphenylamine. K. VISVESWARIAH AND M. JAYARAM, *Central Food Technological Research Institute, Mysore-2, India.*

A rapid and convenient method for determining pure diphenylamine in microquantities was developed. The orange colored solution formed between diphenylamine and *p*-nitrobenzene-diazonium fluoborate is stable for sufficently long time. At 490 mμ, the region of maximum absorption. Beer's law is obeyed up to 4 μg/ml of the solution. With the above method diphenylamine can be estimated in pure form with an accuracy of $\pm$0.5 to 2%.

Microchem. J. **14,** 448 (1969).

Combined Micro Dry-Column Chromatography and Mass Spectrometry. A. J. BAUMAN AND HEINZ G. BOETTGER, *Jet Propulsion Laboratory, Pasadena, California 91103.*

The preparative scale method of dry-column chromatography has been adapted to the micro scale and combined with mass spectrometry (MS). The micro dry columns (MDC) are broken into resolved-peak segments after development and these are inserted directly into the instrument. The use of combined MDC/MS shortens the time of analysis of complex mixtures. The micro dry columns appear to be generally useful adjuncts to other forms of chromatography.

Microchem. J. **14,** 452 (1969).

Application of Back titration of EDTA with Mercury(II) to the Analysis of Illmenite. H. Khalifa and I. A. Ismail, Chemical Department, Ministry of Industry, Cairo, U.A.R.

Potentiometric back titration of excess EDTA with mercury(II) is used for the analysis of ilmenite. Quaternary mixtures can be analyzed by the technique. Potential breaks averaged 100 mv/0.1 ml of 0.01 *M* mercury(II).

Microchem. J. **14,** 464 (1969).

Vapor Sampling and GLC of Some Volatile Materials in Biological Solutions. BASSETTE AND GEORGE WARD, *Department of Dairy and Poultry Science, Kansas State University, Manhattan, Kansas 66502.*

This paper shows the reliability of head space gas sampling for GLC analysis.

Microchem. J. **14,** 471 (1969).

Simple Spectrophotometric Microassay Method for Cycloalkanol Compounds. A. JOSEPH KALB AND SARAH ERLICH-ROGOZINSKY, *Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel.*

The method is based on the observation that a cycloalkanol mixed with aqueous phenol gives a stable, intensely colored solution upon the addition of sulfuric acid.

Microchem. J. **14,** 478 (1969).

Labelling of I^{131}-Hippuran by Ultraviolet Excitation. E. HALLABA AND M. RAIEH, *Nuclear Chemistry Department, Isotope Division, Atomic Energy Establishment, Cairo, U.A.R.*

The spectrophotometric study of hippuran solutions before and after ultraviolet irradiation revealed that the two spectra are identical and show maximum absorption at 273 mμ.

Microchem. J. **14,** 481 (1969).

The Rapid and Direct Determination of Thiosulfate in the Presence of Sulfite Assayed as Barium Salts. JOSEPH F. ALICINO, *Squibb Institute for Medical Research, New Brunswick, New Jersey 08903.*

Peroxide oxidation is used to convert the repective ions to sulfate. This is followed by titration with standard sodium hydroxide or standard barium perchlorate.

Microchem. J. **14,** 486 (1969).

Determination of Trace Cobalt in High-Purity Nickel. H. FLASCHKA AND R. M. SPEIGHTS, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332; and J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865.*

Cobalt in trace amounts in nickel was determined photometrically. The metals were initially converted to the EDTA complexes. Cobalt was then displaced from its EDTA complex by bismuth and extracted into chloroform as the cobalt (III)–PAN complex. The nickel is masked kinetically as the EDTA complex. In the extract, small amounts of the 1-pyridylazo-2-naphtol (PAN) complexes of nickel and bismuth were destroyed by an acid treatment and cobalt was determined photometrically.

Microchem. J. **14,** 490 (1969).

MICROCHEMICAL JOURNAL 14, 353–360 (1969)

Back Titration with Mercury(II) in Urotropine-Buffered Media; Estimation of Small Amounts of Aluminum and Tervalent Vanadium; Analysis of Mixtures

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Chemical Department, Ministry of Industry, Cairo, U.A.R.

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INTRODUCTION

The development of chelatometry as a new branch in analytical chemistry has created renewed interest in methods for determination of a vast number of metal ions or analysis of their mixtures. The present work stems from the fact that the potentiometric procedure using mercury(II) as back titrant for excess EDTA or CDTA (*5, 3*) in urotropine-buffered media of varying pH, is rapid, simple and highly accurate. This led us to test the feasibility of applying it to the determination of aluminum and tervalent vanadium as well as to the analysis of their mixtures.

Aluminum is the consistuent of many industrially important products such as cements and ceramics. Vanadium is of great value to nuclear scientists because of its low neutron capture cross section. Furthermore it enters into several useful alloys and catalysts.

Přibil (*8*) determined aluminum potentiometrically by back titrating excess EDTA with Fe(III) at pH 5–6. Theis (*11*) directly titrated a hot solution of aluminum at pH 4 with EDTA and chrome azurol S. He also back titrated a small excess of EDTA with aluminum stating that in presence of Fe(III), Ca, Mg, Mn, and titanyl ions good results are obtained with 5.8–6.9 μg of aluminum. Velimir (*12*) back titrated aluminum and excess EDTA in alcoholic ammonium acetate with zinc and eriochrome black T (EBT). Klassova (*7*) reported a method for aluminum in minerals and rocks which seems to be complicated, tedious, time and reagent consuming, and further, it involves an error of at least 4%. Khalifa (*2*) determined milli- and microgram amounts of aluminum by potentiometric back titration of excess EDTA with mercury(II) in ammoniacal buffers.

Kinnunen (*6*) adopted a long procedure for vanadium involving a final back titration of EDTA with Mn(II) and EBT. Sajo (*9*) reported that the formation constant of V(V)–EDTA is a maximum at pH 4–7. He described a volumetric direct and indirect method for

V(IV) and V(V). Saxena and Sharma (*10*) determined vanadium by conductometric titration, using the reaction between $AgNO_3$ and Na_3VO_4. The permanganometric method is time consuming and gave somewhat higher results due to the presence of traces of SO_2. not expelled by CO_2. The silver vanadate method (*13*) is not suited for small amounts of vanadium. Khalifa (*4*) adopted a potentiometric procedure for V(IV) and analysis of its mixtures. Most of the published work refers only to V(IV) and V(V). This and the fact that V(III)–EDTA has an exceptionally high stability as shown by its log *K* value of 26 promoted the present work.

EXPERIMENTAL METHOD

The water used was always deionized. The chemicals were all of the requisite purity. They were titanium potassium oxalate; zirconium oxychloride; sulfates of Fe(III), Cd, and Cr(III); permanganate, iodide, and nitrate of potassium; vanadate and persulfate of ammonium; acetate and thiosulfate of sodium; zinc oxide; aluminum metal; elemental sulfur; hydrogen peroxide cupferron; mercuric nitrate; EDTA; hexamine; sulfuric, nitric, hydrofluoric, and sulfurous acids; starch and EBT indicators.

Solutions

The 0.05–0.005 *M* EDTA solutions were prepared in the usual way and standardized with standard zinc solutions prepared from zinc oxide and nitric acid.

The 0.05 *M* aluminum solution was prepared by dissolving the calculated amount of the metal in few milliliters of conc H_2SO_4 acid sufficient to make the final solution 6% in H_2SO_4. The cool solution was made with water to the requisite volume. From this solution other molarities down to 0.005 were prepared by accurate dilution. These were standardized either gravimetrically or potentiometrically by the present method.

The 0.1 *M* tervalent vanadium solution was prepared by:

(a) Reducing a solution containing the calculated amount of NH_4VO_3 in conc H_2SO_4 acid with elemental sulfur; heating to 170–200°C with continuous stirring for 16 hours, to the formation of the lemon yellow vanadic sulfate powder which is insoluble in conc but soluble in dil H_2SO_4; dissolving the $V_2(SO_4)_3$ in 5% H_2SO_4 containing few grams of $(NH_4)_2SO_4$; filtering from sulfur and making with water up to the requisite volume.

(b) Reducing a solution of NH_4VO_3 in dil H_2SO_4 acid at a platinum cathode until the solution passes from lemon yellow color of V(V)

to blue color of V(IV) and finally to the green color of V(III) without any trace of blue. This method is much more rapid than the previous one.

Lower molarities down to 0.01 were prepared by accurate dilution. The resulting solutions were standardized gravimetrically by aid of cupferron [after oxidation with dil $KMnO_4$ to V(V)] or potentiometrically by the present method.

The 0.01 *M* solutions of all metal ions involved in mixtures or those used for volumetric determination were prepared and standardized following recommended procedures.

The 0.2% H_2O_2 solution was prepared by ordinary dilution from 100-vol sample.

The titration assembly consisted of a 150-ml beaker, a $\frac{1}{50}$ graded microburette; a mechanical stirrer, calomel and silver amalgam electrodes.

The pH and potentiometer were model PYE cat. No. 11085.

PROCEDURES

A. Aluminum alone is determined by back titrating excess EDTA with mercury(II) in slightly acid solution (pH 6.5–6.8) previously boiled for 2–3 minutes, cooling, and buffering with 40 ml of 2% urotropine solution.

B. With binary mixtures of Al + Fe(III), Cu(II), V(IV) or Cr(III), the total is determined following (A). In another identical mixture Fe, Cu, V, or Cr is determined volumetrically (Fe permanganometrically after reducing it with H_2S, Cu iodometrically, V by direct titration with $KMnO_4$ solution, and Cr by treating its solution—after oxidation with $(NH_4)_2S_2O_8$ in the presence of 2.5% $AgNO_3$ solution as a catalyst—with excess ferrous ammonium sulfate and titrating the excess with $KMnO_4$ solution. With Al + Zr the total is determined following (A) at pH 6.5. In another identical mixture, Zr is determined by aid of cupferron without interference from Al.

C. With ternary mixtures of Al + Cr(III) + V(IV) or Fe(III) the total is determined following (A). In another identical mixture Cr is determined following (B). In a third mixture Fe or V is determined following (B).

D. With Al + Zr + Fe(III) or Cu(II) the total is determined at pH 6.5 as under (A). In another identical mixture, Fe is determined as under (B), and Fe + Zr in the same solution are determined by aid of cupferron, after adjusting the acidity with H_2SO_4 acid to 10%. The Cu is separated by electrolysis and Zr in the same solution is determined by aid of cupferron as above. With Al + Fe + Ti the

total is determined by one back titration following (A) in the presence of H_2O_2 using 5 ml of 2% urotropine and sodium acetate sufficient to adjust the pH to 6.5–6.8. In another identical mixture treated with NaOH, Fe + Ti are determined in a 25-ml aliquot of the solution of their separated hydroxides in dilute H_2SO_4 acid (made with water up to 50 ml) by back titrating excess EDTA in the presence of H_2O_2. The Al is then computed by difference. In the remaining 25-ml aliquot Fe is determined after masking Ti with HF acid without using H_2O_2.

E. V(III) alone is determined by back titrating excess EDTA with mercury(II) in urotropine at pH 9.

F. With binary mixtures containing V(III) + Zn or Cd one back titration following (E) is carried out for total at pH 8. In another identical mixture V(III) is determined volumetrically by oxidizing it with dil $KMnO_4$ solution to V(V) and then reducing the latter with H_2SO_3 acid to V(IV) which is titrated with a standard $KMnO_4$ solution. With V(III) + Al the total is determined following (E) at pH 6.5. The V(III) is determined as above.

RESULTS AND DISCUSSION

Tables (1–5) list the results of determining Al or V(III) alone and those representing analysis of their various mixtures. The data in Tables (1–5) indicate that the methods described herein for determining aluminum and vanadium(III) or analysis of their mixtures are quite reliable.

The aluminum–EDTA complex forms only very slowly, and EDTA

TABLE 1

DETERMINATION OF ALUMINUM

No.[a]	Al (mg) Taken	Al (mg) Found	Error (± %)	Titrant (mV/0.1 ml)
1	3.3456	3.3658	0.60	126
2	2.2304	2.2439	0.60	125
3	1.1152	1.1163	0.14	139
4	0.6424	0.6348	1.18	111
5	0.4282	0.4325	1.07	125
6	0.3747	0.3700	1.26	134
7	0.2676	0.2663	0.53	141
8	0.2141	0.2127	0.63	113
9	0.1070	0.1070	0.00	128

[a] Nos. 1–9 are the mean results of 3 parallel experiments.

TABLE 2

ANALYSIS OF BINARY MIXTURES [a]

Al (mg)		Error	Metal (mg)		Error
Taken	Found	(± %)	Taken	Found	(± %)
0.4286	0.4272	0.32	0.6147 Cu	0.6147	0.0
0.2143	0.2144	0.05	1.2293 Cu	1.2293	0.0
0.4286	0.4293	0.16	0.6086 Fe	0.6086	0.0
0.2143	0.2134	0.43	1.2173 Fe	1.2173	0.0
0.4286	0.4286	0.00	0.5043 V^4	0.5036	0.14
0.2143	0.2152	0.43	1.0086 V^4	1.0072	0.14
0.2143	0.2102	1.91	1.2960 Cr	1.3033	0.56
0.4286	0.4264	0.50	0.6480 Cr	0.6516	0.55
0.4286	0.4253	0.78	1.0058 Zr	1.0106	0.48
0.2143	0.2126	0.80	2.0118 Zr	2.0041	0.38

[a] In all cases the analysis is run in duplicate.

TABLE 3

ANALYSIS OF TERNARY MIXTURES [a]

Al (mg)		Metal (mg)		Metal (mg)	
Taken	Found	Taken	Found	Taken	Found
0.2143	0.2168	0.5043 V^4	0.5036	1.2960 Cr	1.2920
0.2143	0.2161	1.0086 V^4	1.0073	0.6480 Cr	0.6460
0.4286	0.4289	0.6086 Fe	0.6086	0.6480 Cr	0.6460
0.2143	0.2170	0.6086 Fe	0.6086	1.2960 Cr	1.2920
0.4286	0.4307	0.6146 Cu	0.6109	1.0058 Zr	1.0049
0.2143	0.2188	1.2293 Cu	1.2219	2.0117 Zr	2.0099
0.2143	0.2123	0.6086 Fe	0.6086	2.0117 Zr	2.0172
0.4286	0.4273	1.2173 Fe	1.2173	1.0058 Zr	1.0086
0.2143	0.2151	1.2173 Fe	1.2156	0.2665 Ti	0.2670
0.1071	0.1072	1.2173 Fe	1.2156	0.2665 Ti	0.2670

[a] In all cases the analysis was run in duplicate.

is added in a large excess and the mixture is boiled for 2–3 minutes before buffering to avoid also hydrolysis, otherwise low results are obtained. Raising the pH value of a solution of Al–EDTA higher than 7 causes the release of Al from the complex. Potential end point-breaks ranged from 113 to 141 mV/0.1 ml of 0.005–0.05 *M* titrant which are very satisfactory with such metals as Al.

TABLE 4

DETERMINATION OF TERVALENT VANADIUM [a]

No.	V (mg) Taken	V (mg) Found	Error (± %)	Titrant (mV/0.1 ml)
1	5.2386	5.1836	1.05	158
2	5.2081	5.2539	0.88	237
3	2.6193	2.5989	0.77	170
4	1.6511	1.6521	0.06	203
5	1.3096	1.3045	0.38	215
6	1.1007	1.1027	0.18	108
7	0.5503	0.5554	0.92	103
8	0.2751	0.2753	0.07	106
9	0.0550	0.0556	1.09	70

[a] In all cases the analysis was run in triplicate.

TABLE 5

ANALYSIS OF BINARY MIXTURES [a]

V(III) (mg) Taken	V(III) (mg) Found	Error (± %)	Metal (mg) Taken	Metal (mg) Found	Error (± %)
1.0964	1.0957	0.06	0.6538 Zn	0.6544	0.09
0.5482	0.5478	0.07	1.3078 Zn	1.3082	0.03
0.5482	0.5478	0.07	2.0996 Cd	2.0984	0.06
1.0964	1.0957	0.06	1.0483 Cd	1.0514	0.29
1.0946	1.0957	0.06	0.2143 Al	0.2143	0.00
0.5483	0.5478	0.07	0.4286 Al	0.4287	0.02

[a] In all cases the analysis was run in duplicate.

In determining Al alone we observed, with difficulty, slight fading of potential in the vicinity of the end point, which had no influence on the accuracy of the method. This is in harmony with the fair stability of Al–EDTA as shown by its log K value of 16.13 (*1*). However, in determining the total of Al plus any metal ion whose EDTA complex is stronger than Al–EDTA, no fading of potential was observed.

Trials to mask Al as aluminate and to determine copper or vanadium potentiometrically were not successful.

In determining vanadium(III) alone, we obtained larger potential end point-breaks (Table 4) which is attributed to the very high stability of V(III)–EDTA as shown by its log K value of 26. This complex

is yellow in acidic and rose red in alkaline media, thus distinguished from the blue V(IV)–EDTA complex.

The V(III) is a strong reducing agent, a reason why the back titration of its mixtures with metal ions of variable valency as copper and iron gave erroneous results.

In analysis of binary mixtures it was thought advantageous to oxidize V(III) by boiling the mixture with few drops of dilute ammonium persulfate, as the resulting V(V) does not react with EDTA. The mixture is thus analyzed by carrying out two back titrations with and without the above treatment. However, it was less time consuming to determine the total potentiometrically and V(III) volumetrically.

SUMMARY

The potentiometric back titration of EDTA in hexamine-buffered media is successfully applied to the determination of milli- and microgram amounts of aluminum and trivalent vanadium or the analysis of binary and ternary mixtures involving them. The end points are accurately determined with very satisfactory potential breaks. The present methods can be applied with advantage to the analysis of aluminum-containing materials of industrial importance, such as cements and ceramics.

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Use of Microcosmic Salt as a New Titrant for the Microdetermination of Aspartic and Glutamic Acids

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Amino acids are readily titrated by Sørenson's formol *(1)* titration method. In the case of dicarboxylic acids, such as aspartic and glutamic acids, both carboxylic groups are titratable under such conditions. The present paper describes a new method for their determination in which these dicarboxylic amino acids have been titrated by microcosmic salt ($NaNH_4HPO_4$, $4H_2O$) solution using bromcresol purple as indicator. The end point occurs at one equivalence (i.e., only one –COOH group is titrated), when the color changes from yellow to light purple. The results also were confirmed potentiometrically, in which inflexions were observed in the pH range 4.5–6.0. Glycine, alanine, and leucine do not interfere.

EXPERIMENTAL METHOD

Reagents used:—Aspartic Acid (E.Merck) Glutamic Acid (B.D.H.) Microcosmic salt (E.Merck) and Brom-cresol Purple (B.D.H.).

Stock 0.01 M solutions of aspartic and glutamic acids were prepared and their strengths were checked with Sørenson's method.

PROCEDURE

To 5 ml of a solution of aspartic and glutamic acids add some distilled water to raise its volume to about 20 ml; followed by 1 or 2 drops of a 0.1% solution of bromcresol purple indicator. The solution is yellow at this point. Now titrate it with a standard microcosmic salt *(2)* solution till the yellow color is completely discharged and the solution acquires a faint purple color.

RESULTS

The results are given in Tables 1 and 2; the two amino acids were estimated over a concentration range of 0.0005–0.002 *M*.

SUMMARY

Aspartic and glutamic acids were determined in micro quantities with a new titrant, i.e., microcosmic salt solution, using bromcresol purple as indicator. Estimations were carried out in the range of 0.33–1.47 mg with a maximum error of ±1.2%. Glycine, alanine, and leucine do not interfere.

TABLE 1

DETERMINATION OF ASPARTIC ACID

S.N.	Aspartic acid taken (*M*)	Micro-cosmic salt soln (*M*)	Titer value (ml)	Aspartic acid (mg) Found	Theo-retical value	Error (mg)
1	0.002	0.002	5.04	1.342	1.331	0.011
			5.00	1.331	1.331	0.000
2	0.001	0.001	5.06	0.674	0.666	0.008
			5.02	0.668	0.666	0.002
3	0.0005	0.001	2.52	0.336	0.333	0.003
			2.54	0.338	0.333	0.005

TABLE 2

DETERMINATION OF GLUTAMIC ACID

S.N.	Glutamic acid taken (*M*)	Micro-cosmic salt soln (*M*)	Titer value (ml)	Glutamic acid (mg) Found	Theo-retical value	Error (mg)
1	0.002	0.004	2.50	1.471	1.471	0.000
			2.48	1.459	1.471	0.012
2	0.001	0.001	5.02	0.739	0.736	0.003
			5.04	0.742	0.736	0.006
3	0.0005	0.001	2.50	0.368	0.368	0.000
			2.52	0.371	0.368	0.003

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The Simultaneous Microdetermination of Carbon, Hydrogen and Halogen in Highly Chlorinated or Brominated Organic Compounds

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INTRODUCTION

Highly halogenated organic compounds can be considered as those containing more than 50% halogen and not less than three halogen atoms per mole. These compounds are known to be thermally stable *(1, 7)* owing to complete absence or low content of hydrogen. Under fast conditions of ignition they sublime along the tube and travel in solid rings ahead of the flame and are eventually driven in high concentration into the combustion chamber *(7)*. Ingram *(7)* suggested a higher combustion temperature of 1000°C and the use of a layer of platinised asbestos in the tube instead of the quartz wool plug. This form of catalyst is not recommended for the ordinary classes of chlorine-containing organic compounds. Recently *(1)*, the rapid straight empty tube method was applied for the microdetermination of carbon and hydrogen in highly chlorinated and brominated organic compounds. The present work is a further extension of the subject but also with the purpose of determining the halogen simultaneously with carbon and hydrogen in such organic materials. If possible, this is of course more desirable for economy both in sample and time. A detailed study was thus initiated of the various factors influencing the complete decomposition and combustion of these samples using the following three combustion methods of general use *(3, 4)*.

(i) The rapid straight empty tube method of Korshun–Klimova *(9)*.

(ii) The rapid cobalto–cobaltic oxide method of Večeřa as modified by Gawargious and Macdonald *(5)*.

(iii) The rapid empty tube method of Belcher–Ingram *(2)*.

EXPERIMENTAL METHODS

Reagents and Materials

All the reagents were M.A.R. grade.

Anhydrone and soda-asbestos 14–22 mesh granules.

Silver gauze 30–60 mesh. Manganese dioxide 10–20 mesh granules. Quartz wool. Silica gel suitably treated *(8)*. Cobalto-cobaltic oxide granules prepared according to Gawargious and Macdonald *(5)*.

Apparatus

Oxygen cylinder. Pressure head consisting of a bell-chamber immersed in concentrated sulfuric acid contained in a cylindrical jar. Scavenging tubes for oxygen purification. Combustion tubes of transparent silica of length 35 [1] and 55 [2] cm. but otherwise of conventional shape. Microplatinum boats [1] and silica capsules [2] (7 × 0.5 cm) as sample containers. Ordinery [1] and Méker [2] burners. Tube furnaces, electric, of cylindrical shape, 15 and 7 cm long. The short furnace is a half-split type and used for the silica absorption tube. Standard Belcher-Ingram apparatus, commercially available from Baird and Tatlook Ltd., London, England. Standard Pregl and Flashentäger absorption tubes. Silica absorption tube specially designed and made in the workshop of the National Research Centere. As shown in Fig. 1, this tube is 8.5 cm long with internal and external diameters of 9 and 12 mm, respectively. The tube is provided, as conventional, with a ground-in-stopper for filling purposes and with a standard "Quickfit" cone and socket (5/20); its tare weight is 10–12 g. Aluminum carrier for the absorption tubes; guard tube; aspirator bottle; desiccator; stop watch.

Procedure

(i) *The rapid straight empty tube method (9)*. Assemble the flow train as usual *(1, 4)* connecting the silica absorption tube, filled with a silver gauze roll and kept at 550°C by means of the half-split furnace, externally between the combustion and water absorption tubes, and adjust the furnace temperature at 900°C and the flow rate of oxygen in to 30 ml/min. Weigh 3–7 mg of the sample into the quartz capsule which

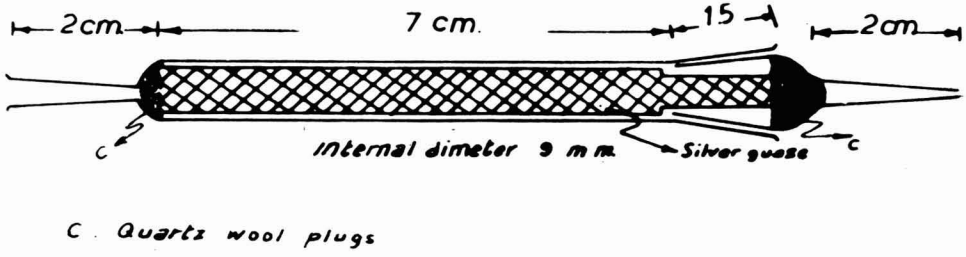

FIG. 1. The Silica absorption tube.

[1] For the cobalto–cobaltic oxide method.
[2] For the rapid straight empty tube method.

is inserted into the combustion tube with its open end facing the main heating zone. Start the pyrolysis and combustion by keeping a strong flame (butane gas and compressed air) under the open end of the sample tube for at least 2–3 minutes and then gradually moving it back towards the sample, i.e., in reverse direction to the oxygen stream. Then, heat along the whole length of the combustion tube for 2–3 minutes disconnect the absorption tubes and close them tightly with their "Quickfit" cone and socket. Cool the silica Ag-filled tube through rapid external exposure to a stream of air. Leave the tubes in the balance room for 4 minutes; meanwhile weighing the next sample. Wipe the tubes as conventional and record the weight of the water absorption tube on the third minute, the carbon dioxide absorption tube on the sixth minute and the halogen absorption tube on the tenth minute.

(ii) The rapid cobalto–cobaltic oxide method *(5)*. The procedure is exactly similar to that described previously *(4, 5)* except that the silica Ag-filled tube must be connected externally between the combustion and the water absorption tubes. The wiping and weighing of the absorption tubes is as described in the fore-mentioned method.

(iii) The rapid empty tube method of Belcher-Ingram *(2)*. Apart from covering the sample in the platinum boat with a 6–8-fold layer of powdered Co_3O_4 and connecting the silica absorption tube as mentioned above, the procedure followed is exactly similar to that described in Ref. *(2)*.

RESULTS AND DISCUSSION

(i) The Rapid Straight Empty Tube Method (9).

Awad *et al. (1)* found that on applying the ordinary procedure, originally developed by Korshun–Klimova *(9)*, for the analysis of highly halogenated organic compounds, unsatisfactory carbon and hydrogen results were obtained. They *(1)* recommended the use of a layer of silver wool, about 10 cm long and kept at 450°C for complete retention of halogen. But, although accurate carbon and hydrogen figures were obtained, yet this modification does not allow the determination of the halogen content. However, this modification *(1)* attracted our attention towards the use of silver wool in an insertion tube of silica (10 × 0.6 cm) to enable weighing before and after combustion. High carbon accompanied with low halogen values were obtained. This is most probably due to incomplete retention of halogen on the limited surface of the silver now contained in an insertion tube. Electrolytic silver wool, claimed *(10)* to be of higher activity, was then tried but unfortunately did not improve the results except for the first run.

At this stage the efforts were directed towards absorbing the halogen

on coming out of the combustion tube. For this purpose, an absorption tube of silica was joined externally between the combustion and water absorption tubes. The silica tube is filled with silver and maintained at 550°C [3] by means of a special short half-split furnace (7 cm long). From the increase in weight of the tube, the halogen percentage is calculated. Owing to the variable halogen recoveries obtained, a vast study had to be performed. Obviously, two main factors were operating, namely the weight of the silver packed tube and the form of silver.

Silica tubes of various weights and designs were tried, since the weight ratio of the tube: halogen was found to be critical. The smaller the weight of the tube the more accurate are the results. Tubes (85 × 9 mm) weighing not more than 13–15 g, after packing with silver, were found to be quite suitable.

However, although satisfactory results were obtained with the electrolytic silver wool packing *(10),* yet the silica wall of the halogen tube became severely attacked at the operating temperature (550°C) after only but a few analyses. Presumably, this attack is due to the sublimation of the silver halide, produced from the fine particles of the hot electrolytic silver, which is then progressively deposited or fused with the hot silica wall. Alternately, silver wool [4] or gauze [4] were then tried and the two gave equally correct results. However, the silver gauze roll, held in place by two quartz wool plugs, was preferred since it had the least attack on the silica absorption tube. One tube filled with a small roll of silver gauze had a useful life of about 6–8 analyses depending on the halogen content. After use, the exhausted tube was easily cleaned so that its useful life could be extended to be as long as that of the combustion tube.

Analysis of a representative series of highly chlorinated or brominated organic compounds gave satisfactory results for the three elements (Table 1). The average error obtained for carbon, hydrogen and halogen is ±0.13, +0.13 and ±0.14, respectively. These results offered true evidence both for the complete decomposition of such compounds and also for the quantitative retention of halogen.

(ii) The Cobalto–Cobaltic Oxide Method (5).

A review of the literature showed that the method has not been tested for the analysis of highly chlorinated or brominated organic compounds. The only work *(6)* available that dealt with the simultaneous determina-

[3] Found from studying temperatures ranging between 450 and 650°C (stepwise increase of 50°C); 550°C proved to be the best silver working temperature for quantitative retention of chlorine or bromine combustion products.

[4] Activated by treatment with (1:1) nitric acid for 2 minutes before use.

TABLE 1

SIMULTANEOUS DETERMINATION OF CARBON, HYDROGEN AND HALOGEN IN HIGHLY HALOGENATED ORGANIC COMPOUNDS BY THE RAPID STRAIGHT EMPTY TUBE METHOD USING AN EXTERNAL SILICA AG-FILLED TUBE

Compound	Wt (mg)	C (%)		Error (%)	H (%)		Error (%)	Halogen (%)		Error (%)
		Calc	Found		Calc	Found		Calc	Found	
p-Chlorobenzoic acid M.A.R.	6.120	53.70	53.80	+0.10	3.22	3.37	+0.15	22.65	22.41	−0.24
	5.950		53.91	+0.21		3.35	+0.13		22.52	−0.13
Chloral hydrate	6.220	14.52	14.61	+0.09	1.83	1.89	+0.06	64.31	64.21	−0.10
	6.535		14.63	+0.11		1.91	+0.08		64.19	−0.12
Chloranil	4.625	29.31	29.42	+0.11	0.00	0.16	+0.16	57.68	57.50	−0.18
	6.965		29.38	+0.07		0.18	+0.18		57.79	+0.11
Perchloroethane	5.520	10.15	10.23	+0.08	0.00	0.11	+0.11	89.85	89.67	−0.18
	6.110		10.40	+0.25		0.17	+0.17		89.97	+0.12
α,α-Heptachlorotoluene	6.155	25.23	25.32	+0.09	0.30	0.46	+0.16	74.47	74.23	−0.24
	6.500		25.33	+0.10		0.43	+0.13		74.22	−0.25
Perchloronaphthalene	4.825	29.75	30.01	+0.26	0.00	0.15	+0.15	70.25	70.26	+0.01
	5.120		29.80	+0.05		0.16	+0.16		70.12	−0.13
Hexachloronaphthalene	6.265	35.87	35.69	−0.18	0.60	0.69	+0.09	63.53	63.65	+0.12
	5.640		35.65	−0.22		0.73	+0.13		63.50	−0.03
p-Bromobenzoic acid M.A.R.	5.415	41.82	41.95	+0.13	2.51	2.62	+0.11	39.75	39.68	−0.07
	6.125		41.93	+0.11		2.65	+0.14		39.60	−0.15
Tetrabromo-*o*-xylene	6.380	22.78	22.66	−0.12	1.43	1.55	+0.12	75.79	75.55	−0.24
	6.775		22.65	−0.13		1.45	+0.02		75.94	+0.15
Pentabromotoluene	6.920	17.27	17.35	+0.08	0.62	0.80	+0.18	82.10	81.94	−0.16
	6.620		17.37	+0.10		0.81	+0.19		82.25	+0.15

tion of carbon, hydrogen, and halogen by this method is mostly on partially halogenated compounds. In this work *(6)*, the Co_3O_4 catalyst was used at 800°C together with an external tube containing silver wool at 580°C for absorption of halogen. However, of the compounds analyzed only 3–4 can be considered as highly halogenated and even those contained iodine but not chlorine or bromine.

However, before applying the external silica Ag-filled tube with the Co_3O_4 method, the oxidation efficiency of the catalyst had to be tested first for complete decomposition of the highly halogenated compounds under normal conditions *(5)* but along with a 10-cm layer of silver wool for halogen retention *(1)*. Although correct hydrogen values were obtained by this method, yet the carbon figures were always low by about 0.4%. This may be due to traces of the compound failing complete decomposition since on raising the catalyst temperature to 750°C reasonably accurate carbon values (only 0.2% low) were obtained.

Now, replacing the internal silver wool layer by the external silica tube containing silver gauze,[5] though gave correct hydrogen figures, yet the carbon and halogen values were constantly low by 0.2 and 0.5%, respectively. The observation that the percentage error in halogen is greater than that of carbon is explained by the fact that whereas the carbon is amplified, through combustion to CO_2, the halogen being retained as halide does not show any amplification.

The slightly low halogen results cannot be due to incomplete retention of the halogen, as then high carbon values would have been obtained, i.e., cannot be due to the tube modification which has already proved successful with the rapid straight empty tube method where a higher rate of oxygen is used. Therefore, the slightly low carbon and halogen figures are most reasonably attributed to incomplete decomposition of traces of the organic substance under the conditions of the Co_3O_4 method. However, this view was substantiated since on covering the sample in the platinum boat with a layer of powdered Co_3O_4 satisfactory results (Table 2) were found showing an average error of ± 0.14, $+0.16$ and ± 0.17% for carbon, hydrogen, and halogen, respectively.

(iii) The Rapid Empty Tube Method of Belcher–Ingram (2).

Expectedly, there should be no difficulty and indeed highly satisfactory carbon and hydrogen results were obtained by the straightforward method except for covering the sample with Co_3O_4 powder. Worth mentioning in this connection is that Ingram *(7)* raised the temperature of

[5] For determining the halogen simultaneously with carbon and hydrogen as recommended previously with the Korshun–Klimova method.

TABLE 2

SIMULTANEOUS DETERMINATION OF CARBON, HYDROGEN AND HALOGEN IN HIGHLY HALOGENATED ORGANIC COMPOUNDS BY THE COBALTO-COBALTIC OXIDE METHOD USING AN EXTERNAL SILICA AG-FILLED TUBE

Compound	Wt (mg)	C (%)		Error (%)	H (%)		Error (%)	Halogen (%)		Error (%)
		Calc	Found		Calc	Found		Calc	Found	
p-Chlorobenzoic acid M.A.R.	4.220	53.70	53.74	+0.04	3.22	3.34	+0.12	22.65	22.35	−0.30
	4.505		53.79	+0.09		3.31	+0.09		22.41	−0.24
Chloranil	3.475	29.31	29.15	−0.16	0.00	0.12	+0.12	57.68	57.39	−0.29
	3.945		29.45	+0.14		0.12	+0.12		57.54	−0.14
Perchloroethane	3.300	10.15	10.19	+0.04	0.00	0.10	+0.10	89.85	89.98	+0.13
	3.890		9.96	−0.19		0.19	+0.19		90.00	+0.15
α,α-Heptachlorotoluene	2.880	25.23	25.52	+0.29	0.30	0.42	+0.12	74.47	74.17	−0.30
	3.100		25.43	+0.20		0.46	+0.16		74.21	−0.26
Perchloronaphthalene	4.005	29.75	29.91	+0.16	0.00	0.11	+0.11	70.25	70.01	−0.24
	4.315		29.88	+0.13		0.14	+0.14		70.09	−0.16
p-Bromobenzoic acid M.A.R.	4.295	41.82	42.00	+0.18	2.51	2.65	+0.14	39.75	39.70	−0.05
	3.595		41.74	−0.08		2.70	+0.19		39.90	+0.15
Tetrabromo-*o*-xylene	4.335	22.78	23.04	+0.26	1.43	1.64	+0.21	75.79	75.89	+0.10
	4.310		22.83	+0.05		1.69	+0.26		75.70	−0.09
Pentabromotoluene	4.405	17.27	17.35	+0.08	0.62	0.90	+0.28	82.10	82.20	+0.10
	4.640		17.47	+0.20		0.82	+0.20		82.11	+0.01

TABLE 3

SIMULTANEOUS DETERMINATION OF CARBON, HYDROGEN AND HALOGEN IN HIGHLY HALOGENATED ORGANIC COMPOUNDS BY THE BELCHER-INGRAM METHOD USING AN EXTERNAL SILICA AG-FILLED TUBE

Compound	Wt (mg)	C (%)		Error (%)	H (%)		Error (%)	Halogen (%)		Error (%)
		Calc	Found		Calc	Found		Calc	Found	
p-Chlorobenzoic acid M.A.R.	4.885	53.70	53.51	−0.19	3.22	3.40	+0.18	22.65	22.90	+0.25
	3.910		53.74	+0.04		3.35	+0.13		22.59	−0.06
Chloranil	3.930	29.31	29.52	+0.21	0.00	0.14	+0.14	57.68	57.76	+0.08
	4.440		29.44	+0.13		0.11	+0.11		57.52	−0.16
Perchloroethane	3.820	10.15	10.27	+0.12	0.00	0.12	+0.12	89.85	89.53	−0.32
	3.600		10.19	+0.04		0.09	+0.09		89.48	−0.37
α,α-Heptachlorotoluene	4.095	25.23	25.40	+0.17	0.30	0.42	+0.12	74.47	74.62	+0.15
	4.230		25.31	+0.08		0.47	+0.17		74.33	−0.14
Perchloronaphthalene	4.575	29.75	30.00	+0.25	0.00	0.13	+0.13	70.25	70.42	+0.17
	3.405		29.92	+0.17		0.15	+0.15		70.10	−0.15
Hexachloronaphthalene	3.410	35.87	35.74	−0.12	0.60	0.71	+0.11	63.53	63.70	+0.17
	4.105		35.72	−0.14		0.66	+0.06		63.68	+0.15
p-Bromobenzoic acid M.A.R.	4.700	41.82	41.89	+0.07	2.51	2.63	+0.12	39.75	39.59	−0.16
	4.135		41.94	+0.12		2.66	+0.15		39.62	−0.13
Tetrabromo-*o*-xylene	4.225	22.78	22.70	−0.08	1.43	1.52	+0.09	75.79	75.60	−0.19
	3.615		22.84	+0.06		1.60	+0.17		75.72	−0.07
Pentabromotoluene	3.420	17.27	17.30	+0.03	0.62	0.77	+0.15	82.10	82.00	−0.10
	4.190		17.41	+0.14		0.81	+0.19		81.97	−0.13

the combustion chamber to 1000°C and used a layer of platinised asbestos in order to achieve complete decomposition of perchlorodiphenyl.

Similarly, when the silica tube containing silver gauze was incorporated externally in the absorption train, for determining the halogen simultaneously with carbon and hydrogen, correct and reproducible results (Table 3) were obtained. The mean accuracy calculated to $\pm 0.12\%$ for carbon, $+0.13\%$ for hydrogen and $\pm 0.16\%$ for halogen. The quantitative recoveries obtained for halogen proved that the relatively high flow rate of oxygen (50 ml/min) did not disturb the absorption efficiency of the silver gauze.

In conclusion, the external silica tube packed with a silver gauze roll and kept at 550°C by means of a separate half-split furnace is recommended for determining the halogen, whether chlorine or bromine, simultaneously with carbon and hydrogen. A further advantage of this modification is that it proved quite suitable for use with any of the three methods for the analysis of aliphatic or aromatic compounds whether partially or highly halogenated.

SUMMARY

The simultaneous determination of chlorine or bromine together with carbon and hydrogen in highly halogenated organic compounds was achieved by dry-oxidation of the sample with oxygen either in an empty or packed combustion tube. The carbon dioxide and water produced were absorbed in the usual way in soda-asbestos and anhydrone. The halogen was absorbed on a silver gauze roll kept at 550°C, placed in a specially-designed silica absorption tube, connected externally between the combustion and water absorption tubes, and determined gravimetrically. The method is simple, rapid, and accurate.

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MICROCHEMICAL JOURNAL 14, 373–375 (1969)

Direct Microdetermination of L-Lysine Separately and in a Mixture of Amino Acids

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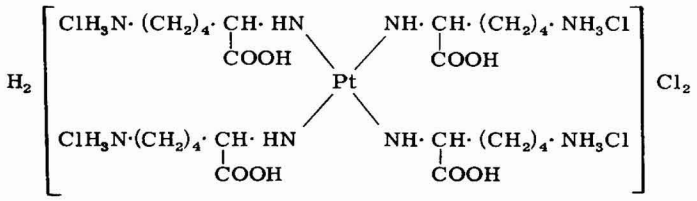
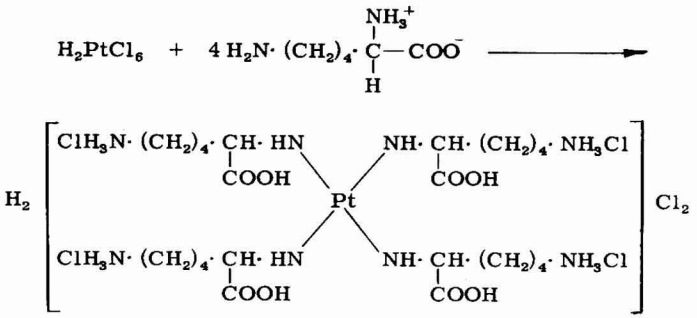
Available literature does not give any information towards the direct determination of L-lysine. However, the best known available methods for the determination of L-lysine are by Cu(II) ion exchange resin *(1)*; electrophoretic method *(2)*; the use of ninhydrin reagent *(3)*; photometric method *(4)*; chromatography *(5, 6)*; colorimetric method *(7, 9)*; iodometric titration of their copper salts *(10)*.

L-Lysine was determined directly in microamounts with platinic chloride, using bromcresol green as indicator. Potentiometric titration and results of analysis show that the reaction between platinic chloride and L-lysine results in the formation of a complex,



Micro–716 (fe) page 705

in the ratio of 1:4. Probably the following reaction takes place:



Micro–716 (fe) page 2

EXPERIMENTAL METHOD

Reagents Used

L-Lysine and bromcresol green (B.D.H. grade); platinic chloride, H_2PtCl_6 (Johonson and Methew grade); micropipette and burette used had LC = 0.01.

Saturated solution of L-lysine was prepared by dissolving the exactly weighed amount in distilled water. Platinic chloride solution was prepared by means of back titration with ferrocyanide *(11)*.

Solution of bromcresol green was prepared by dissolving the indicator in distilled water.

PROCEDURE

A known volume of a known standard solution of L-lysine taken in a beaker from a micropipette and diluted with distilled water. A few drops (2–3) of a solution of bromcresol green was added to the above mentioned solution in the beaker, when the whole solution assumed a sky blue color. A standard of platinic chloride was then run in the beaker from a microburette till the sky blue colored solution in the beaker turned light yellow at the end point.

L-lysine also was titrated in a mixture of serine, histidine, arginine, L-leucine, and DL-valine (0.5 ml of 0.005 *M* amino acids were added).

RESULTS AND DISCUSSIONS

Results in Table I show that the reaction between platinic chloride and L-lysine takes place in the ratio of 1:4. Hence, the resulting complex shows that after the formation of the mentioned complex in the ratio of 1:4 any further addition of platinic chloride changes the color of the indicator from sky blue to light yellow. Maximum error in the present series of experiments was 1%. It was observed that very small amounts of serine, histidine, arginine, leucine, and DL-valine do not in-

TABLE 1

MICRODETERMINATION OF L-LYSINE

L-Lysine 0.02 *M*	H_2PtCl_6 0.0055 *M*	L-Lysine ($\times 10^4$ mg–liter)		Error (%)
		Taken	Found	
0.5	0.45	1.8266	1.8083	
1.0	0.90	3.6532	3.6166	
1.5	1.35	5.4798	5.4249	1
2.0	1.80	7.3064	7.2332	
2.5	2.25	9.1330	9.0415	

terfere. If serine, histidine, and arginine are present in sufficient quantities, it is necessary to separate them before titrating L-lysine with platinic chloride. Since similar experimental values were obtained when very small amounts were present, no separate Table is given. The present method is simple, accurate, and less time consuming than the other methods mentioned.

SUMMARY

L-Lysine was determined by directly titrating against platinic chloride separately as well as in the presence of certain amino acids. Titration results, potentiometric titrations and results of analysis confirm the formation of a complex between platinic chloride and L-lysine in the ratio of 1:4. Maximum error observed is 1%. Interference by excess of amino acids is observed.

ACKNOWLEDGMENT

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

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MICROCHEMICAL JOURNAL 14, 376–379 (1969)

On the Mechanism of Detecting Selected Organic Substances by Means of Fuchsine Dyes and the Method of Thin-Layer Chromatography

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The previous publications described the detection on thin layers (by means of fuchsine dyes) of the following organic compound groups: higher saturated and unsaturated fatty acids *(1)*, higher fatty alcohols *(2)*, some pesticides *(3)*, esters of *o*-phthalic acid *(4)*, barbiturates *(5)*, naphthalene derivatives *(6)* as well as cholesterol and its esters *(7)*.

In the present publication the researches on the mechanism of detecting organic substances of the above-mentioned groups have been presented.

EXPERIMENTAL METHOD

Selected substances coming into the groups of organic compounds mentioned above have been analyzed on a thin layer according to conditions as described previously *(1–7)*.

After developing particular substances, the chromatographic spots received were scratched away in turn from the adsorbent surface and treated with 2 ml of chloroform.

The resulting mixture was spread on the adsorbent Kieselel G. Its layer was 0.5 mm thick and had been activated for 45 minutes in a temperature of 110°C.

As the mobile phase benzene + acetone were applied in a volume relation of 9:1.

The separation of fuchsine from the substance previously developed was confirmed (Table 1).

The fact of separating the colored chromatographic spot into new fuchsine and the substance previously developed testifies, that the colored chromatographic spot is not the product of a chemical reaction. It comes out from the above tests, that new as well as basic fuchsine being the developing substances do not set apart the chemical structure of substances being chromatographed. The above fact gives ample possibilities of further analytic research of the developed substances.

TABLE 1

THE CHROMATOGRAPHIC RESOLUTION OF THE COLORED COMPOUND

Colored compound	R_f	
	Substance	Developer
DDT + new fuchsine	1.00	0
DMDT + new fuchsine	0.80	0
Narcosan + new fuchsine	0.41	0
Cholesterol + new fuchsine	0.35	0
Stearic alcohol + new fuchsine	0.32	0
Stearic acid + new fuchsine	0.21	0

During further tests the a/m substances were separated in a preparative way and developed by means of new or basic fuchsine.

In order to separate the developer from the developed substance the following test was carried out: after developing the substance, respective strips were scratched out and put on the top of the adsorbent in a chromatographic column. The column (0.8-cm diam) was filled up to the height of 7 cm with silica gel of 100-200 mesh granulation. Next a phase consisting of benzene and acetone in 9:1 volume relation was let through the column. The dye remained on the top of the column whereas the substance tested along with the effluent went into the collection cell (Table 2).

After driving away the solvent a spectrophotometric test in infrared radiation was made and the received spectrum was compared with that of the reference substance.

TABLE 2

THE COMPARISON OF THE AMOUNT (mg) OF THE SUBSTANCE DROPPED ON THE THIN LAYER WITH THE AMOUNT (mg) OF THE SUBSTANCE RECOVERED AFTER PASSAGE DOWN THE COLUMN

Substance	Amount of substance			Mobile phase (ml)
	Dropped on thin layer (mg)	Recovered		
		(mg)	(%)	
Stearic acid	20	18.1	90.5	125
Stearic alcohol	20	19.8	99	75
Narcosan	20	20	100	75
Cholesterol	20	19.8	99	75
DMDT	20	20	100	60

Full conformity of both spectra was noticd giving an additional proof of the fact, that chromatographed substances do not react with fuchsine dyes. For example, the spectrum of stearyl alcohol (A) used as reference spectrum and that of stearyl alcohol separated from the day (B) are shown in Fig. 1.

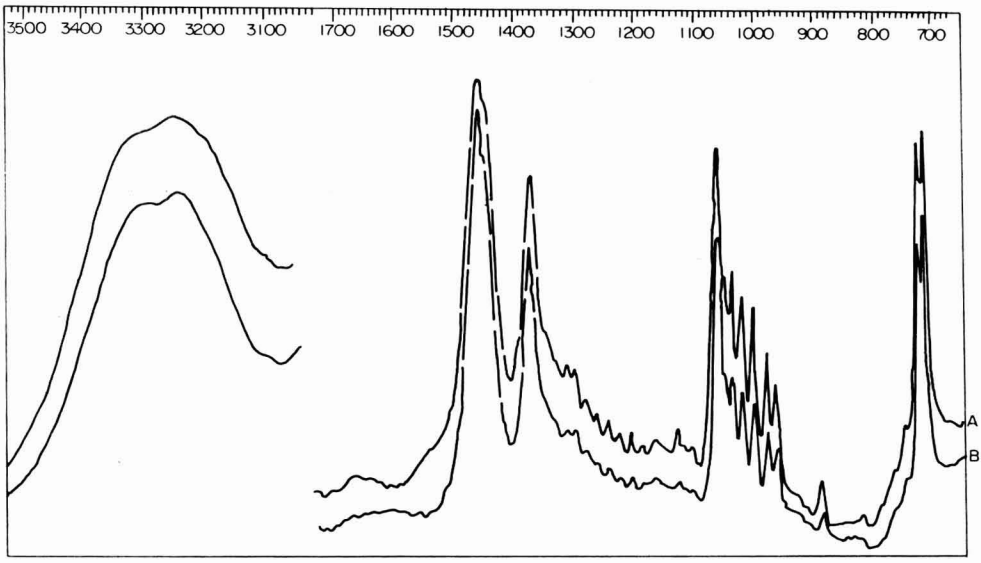

FIG. 1. The IR absorption spectrum of stearyl alcohol (A) and stearyl alcohol (B), separated from new fuchsine. The measurement was done in Nujol on the spectrophotometer Unicam SP–100.

It may be presumed from analysis of the above tests, that the mechanism of developing selected organic compounds by means of new or basic fuchsine, is based on a differential way of settling of the dye between the substance set on the adsorbent and th pure adsorbent. Colorimetric tests of settling of new fuchsine on the adsorbent as well as an adsorbent impregnated by an organic substance can be the confirmation of the hypothesis presented. The adsorbent was impregnated by adding benzine solutions of organic substances to Kieselgel G. The resulting data are shown as an example in Fig. 2.

As shown in Fig. 2, the adsorbent impregnated by an organic substance offers conditions for more extensive settling of the dye than pure adsorbent does. Thus the hypothesis of the mechanism of developing organic substances by means of fuchsine dyes appears to be correct and justified.

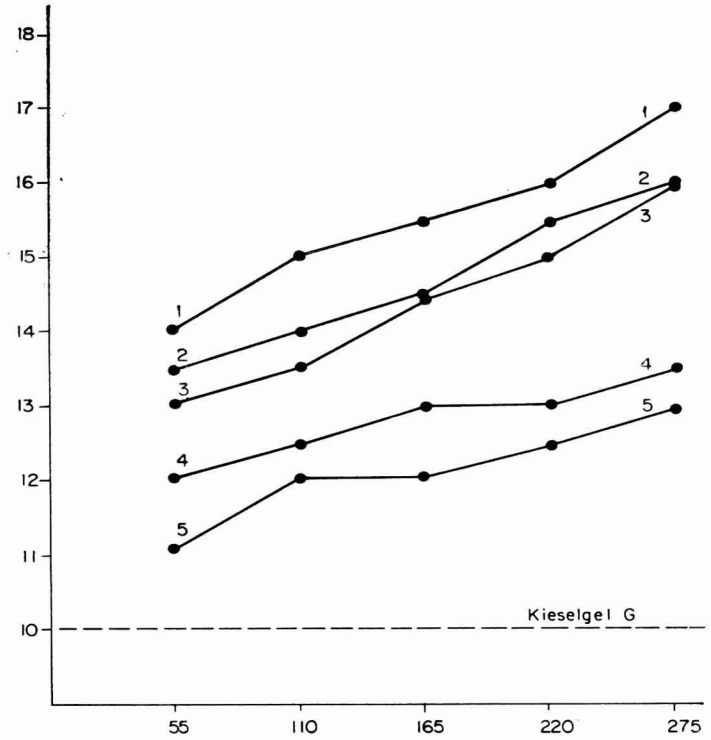

FIG. 2. The amount (μg) of fixed new fuchsine from the amount (μg) of organic substance, used to impregnate the adsorbent; 1, stearic acid; 2, stearic alcohol; 3, cholesterol; 4, DMDT; 5, DDT.

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MICROCHEMICAL JOURNAL 14, 380–384 (1969)

Analytical Applications of Isonicotinoyl Hydrazones II. Spectrophotometric Determination of Aluminum with 1-Isonicotinoyl-2-Salicylidene Hydrazine as Chromogenic Reagent

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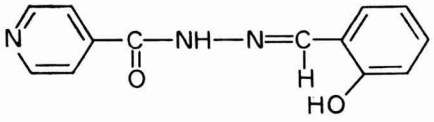
INTRODUCTION

The use of organic reagents in analysis is increasing because of their selectivity and sensitivity in the determination of elements. To find some new chromogenic reagents for the microdetermination of metals, research work was undertaken in our laboratories. Isonicotinoyl hydrazones form colored complex compounds with several cations *(1, 2)* and the present paper presents the results on the behavior of the 1-isonicotinoyl-2-salicylidene hydrazine (INSH) (I) with aluminum ion and the microdetermination of aluminum in the presence of beryllium.



(I)

EXPERIMENTAL METHODS

Apparatus

A Unicam SP 700A recording spectrophotometer was used for absorption spectra and a Zeiss M 4 Q III spectrophotometer was used

for absorbance measurements. In both cases 10-mm silica cells was used. For pH measurements a Metrohm E 396 pH meter equipped with glass-calomel combination electrode was used.

Reagents

Preparation of INSH. INSH was prepared according to Sah and Peoples (*3*) and it was recrystallized twice from methanol. The mp was in agreement with this given in the literature (*3*). The INSH solution was prepared in concentration of 2×10^{-3} *M* by dissolving the reagent in absolute ethanol (B.D.H. reagent).

Standard aluminum solution. A 0.1 *M* solution was prepared by dissolving the appropriate weight of aluminum perchlorate (K and K reagent) in distilled water and it was standardized with EDTA in the presence of Chromazurol S (*4*).

Metal ion solutions. Nitrates and chlorides of metals were used in the preparation of solutions of cations and their concentration were determined before use by the conventional methods. All reagents used were Merck's G.R. grade.

RESULTS AND DISCUSSION

Absorption Spectra

The absorption spectra of INSH complex of aluminum, aluminum and reagent, measured against a solvent blank are presented in Fig. 1. An absorbance maximum at 375–385 mμ was found for INSH solutions containing various excesses of aluminum ion in the pH range of 3.5–5.5 (μ = 0.02, $NaClO_4$) while INSH does not exhibit appreciable absorbance above 370 mμ. Therefore, further measurements were made at 375 and 390 mμ.

Effect of pH and Time on the Color Development

The effect of pH on the color development of the Al-INSH complex was examined by measuring the absorbance of the complex at two wavelengths 375 and 390 mμ, where the concentration of aluminum ion and INSH was 4×10^{-5} and 2×10^{-5} *M* respectively. The results showed that the maximum color development, yellow, was over the pH range of 3.5–5.5. In this pH range the full color development takes place in 40 minutes at 25°C and it is very stable. The color of the complex was stable for 12 hours in direct light and after 24 hours the absorbance decreased by approximately 5%. In the dark, the complex was stable for at least 48 hours. Under these conditions the reagent itself is colorless.

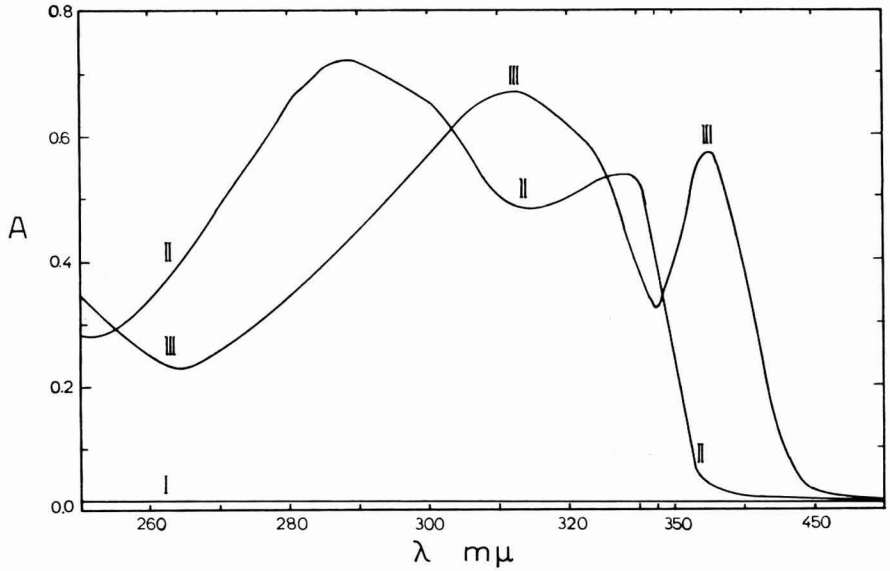


FIG. 1. Absorption Spectra of (I), $Al^{3+} = 2 \times 10^{-4}$ *M*; (II), INSH = 2×10^{-4} *M*, and (III), Al–INSH complex ($Al^{3+} = 4 \times 10^{-4}$ *M* and INSH = 2×10^{-4} *M*).

Composition of the Complex and Its Formation Constant

The composition of the complex was determined by applying the Job's continuous variations method (*5*). A maximum for a 1:1 ratio of Al:INSH results on the Job curves for solutions of C_{total} 2.4×10^{-5} *M*, 4.0×10^{-5} *M* and 5.6×10^{-5} *M* as shown in Fig. 2. The apparent formation constant at pH 5.0 was then calculated according to Turner and Anderson (*6*) and was found to be of the order of 3×10^{6}.

Beer's Law and Sensitivity of the Reagent

Calibration results at pH 5.0 showed that the Beer–Lambert law was obeyed from 0.5 μg/ml up to 3.5 μg/ml of aluminum. The sensitivity of the reaction between INSH and aluminum, calculated by using Sandell's definition (*7*), is 0.0021 μg/cm². This corresponds to a molar extinction coefficient of 12,700. These figures may be compared with the sensitivities of alizarin S (0.003 μg/cm²), aurintricarboxylic acid (0.002 μg/cm) and Eriochrome cyanine R (0.0015 μg/cm²) (*8*).

Recommended Procedure

To a sample solution containing aluminum in a 25-ml volumetric flask add 2 ml of a 2×10^{-3} *M* reagent solution (in absolute ethanol)

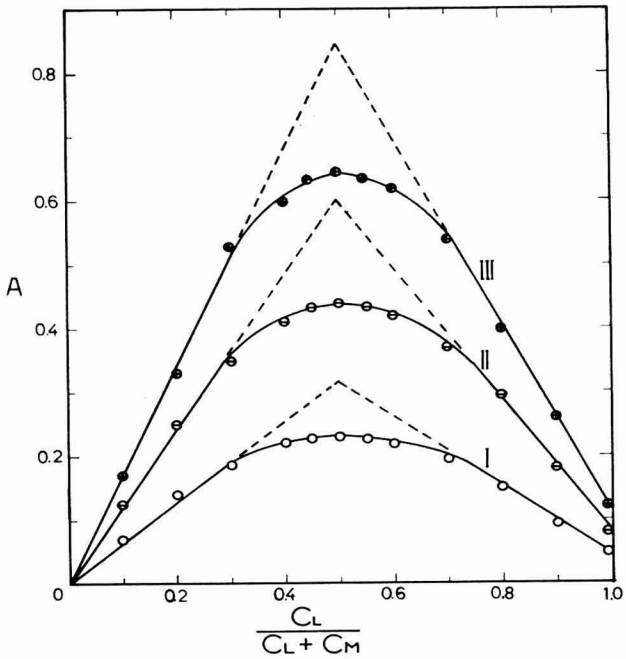


FIG. 2. Continuous variation method: total aluminum and INSH concentration (I), 2.4 × 10^{-5} *M*; (II), 4.0 × 10^{-5} *M*; (III), 5.6 × 10^{-5} *M*, pH = 5.0, μ = 0.02($NaClO_4$).

and 0.5 ml of 1 *M* sodium perchlorate solution. Dilute with distilled water, adjust pH to 5.0 with 0.01 *M* perchloric acid or 0.01 *M* sodium carbonate and set it aside for 40 minutes. Measure the absorbance of the solution against a similarily prepared reagent blank at 375 mμ.

The Effect of Diverse Ions

A number of foreign ions was studied to make the procedure as diversified as possible. The interference of ions was studied by adding various cations or anions to a quantity of aluminum and following the outlined procedure. A maximum error of 4% was considered tolerable.

In the determination of 2.1 ppm of aluminum, beryllium do not interfere when present up to 200 ppm. Similar results were obtained for lithium, sodium, potassium, and magnesium.

Calcium barium and strontium do not interfere when present up to 100 ppm. Cobalt(II), chromium(III), zinc, cadmium, and mercury(II), do not interfere when present up to 20 ppm. Copper(II), nickel, iron(III), lead, chromium(VII), molybdenum, vanadium(V), tungsten(VI), thorium and phosphates interfere. Chlorides, nitrates, perchlorates and sulfates do not interfere, when present up to 200 ppm.

SUMMARY

A spectrophotometric method is described for the microdetermination of aluminum in acidic medium (pH 5.0) with the title compound. Spectrophotometric studies show the formation of 1:1 (ligand:Al) water-soluble complex and the reaction is suitable for photometric determination of 0.5–3.5 ppm of aluminum. Beryllium does not react with this reagent and aluminum can be determined in the presence of 100-fold quantity of beryllium. The molar absorptivity of the complex at 375 mμ is $\varepsilon \backsim$ 12,700 and the apparent formation constant at pH 5.0 is of the order of 10^6. The interference due to a number of ions has been studied.

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MICROCHEMICAL JOURNAL **14**, 385–390 (1969)

Direct Titrimetric Microdetermination of L-Asparagine and D L-Valine

I. Determination of -Asparagine and DL-Valine in Presence of Each Other without Separating

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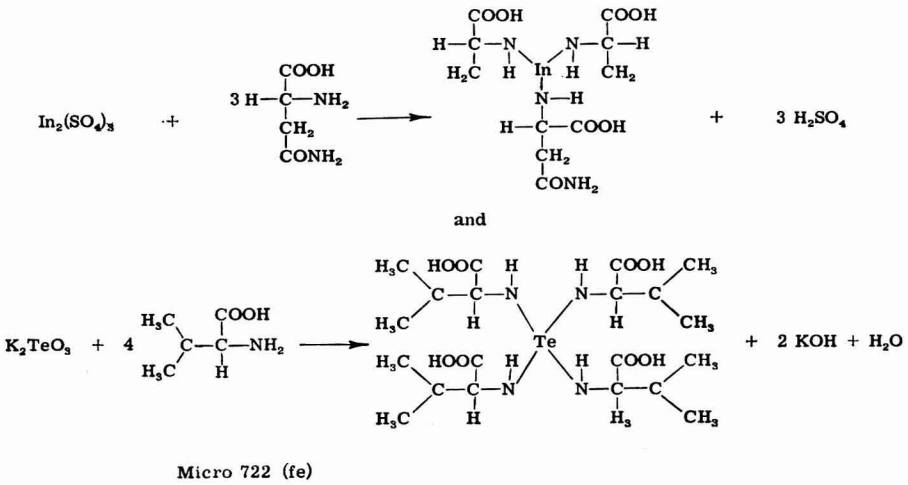
Literature concerning the determination of L-asparagine and DL-valine is not very plentiful; and no direct and simple method for their quantitative determination either separately or in presence of each other was found. However, L-asparagine is generally determined chromatographically (*1, 2*) titrimetrically (*3, 5*); manometrically (*7*); microbiologically (*8*); and enzymically (*9*). DL-Valine is, generally, determined titrimetrically by oxidizion with potassium permanganate (*12*); by a modified potentiometric ninhydrin method (*13*); by oxidizion with ninhydrin to aldehydes (*14*); by circular chromatography (*10*); by direct photometry (*11*); by partition chromatography on silica gel (*6*); and by gas chromatography (*4*).

Most of the methods are difficult, lengthy, and time consuming with the exception of only one direct photometric method (*11*) described for DL-valine. But there is no successful method which determined them either after complete separation from a mixture of these two or in one solution without separation.

The present method deals with the direct determination of L-asparagine and DL-valine in micro quantities separately, as well as in presence of each other in the same solution without separation. Separately, L-asparagine and DL-valine were determined by direct titration with indium sulfate and potassium tellurite using congo red and xylenol orange as indicators, respectively. From potentiometric titrations and results of analysis it was confirmed that complexes are formed between indium and L-asparagine, and between tellurium and DL-valine in the ratios 1:3 and 1:4, respectively. Probably the following reactions take place:



Micro 722 (fe)

EXPERIMENTAL METHODS

Reagents used

L-Asparagine, DL-valine, and potassium tellurite (E. Merck grade); indium sulfate, congo red, and xylenol orange (B.D.H. grade).

Apparatus

Micro pipette (graduated of 1-ml capacity with LC = 0.01.
Micro burette (graduated) of 2-ml capacity with LC = 0.01.

PROCEDURE

Direct Determination of L-*Asparagine and* DL-*Valine Separately* L-*Asparagine.* A known volume of a standard solution of L-asparagine was taken in a beaker and diluted to about 30 ml. A few drops (1–2) of congo red solution were added in the beaker and the whole solution assumed a red color. This solution was titrated against a standard solution of indium sulfate from a microburette. The endpoint was marked by a sharp change in color from red to violet.

DL-*Valine.* A known volume of a standard solution of DL-valine was taken in a beaker and diluted to about 30 ml. A few drops of a solution of xylenol orange weer added and the whole solution was either rose colored or pink red depending on the concentration of DL-valine and xylenol orange. The pink red solution was titrated against a standard solution of potassium tellurite run from a microburette, where at the endpoint a sharp change in color took place from pink red to purple.

Determination of L-*Asparagine and* DL-*Valine in Presence of Each Other without Separation*

Known volumes of standard solutions of L-asparagine and DL-valine were taken in a beaker in the ascending and descending order from the top, respectively. A few drops of xylenol solution were added after dilution with distilled water to about 30 ml, the whole solution was pink. Then a standard solution of indium sulfate was run from a microburette, when the first change was marked by a light rose color. To this same solution and with those few drops of the indicator xylenol orange a standard solution of potassium tellurite was allowed to run in to titrate DL-valine. At the endpoint a purple red color appeared sharply. Hence, in the same solution first L-asparagine was titrated an dthen DL-valine using the same indicator.

RESULTS AND DISCUSSIONS

The results are shown in Tables 1–3. Ranges for L-asparagine and DL-valine varied from 6.0537×10^{-4} to 18.1608×10^{-4} mg/liter; and from 5.8478×10^{-4} to 29.2390×10^{-4} mg/liter, respectively.

TABLE 1

MICRODETERMINATION OF L-ASPARAGINE

L-Asparagine 0.02 *M* (ml)	$In_2(SO_4)_3$ 0.0084 *M* (ml)	L-Asparagine ($\times 10^{-4}$ mg/liter)		Error (%)
		Taken	Found	
0.2	0.16	6.0056	6.0537	
0.3	0.24	9.0084	9.0804	
0.4	0.32	12.0112	12.1074	0.8
0.5	0.40	15.0140	15.1340	
0.6	0.48	18.0168	18.1608	

TABLE 2

MICRODETERMINATION OF DL-VALINE

DL-Valine 0.01 *M* (ml)	K_2TeO_3 0.0104 *M* (ml)	DL-Valine ($\times 10^{-4}$ mg/liter)		Error (%)
		Taken	Found	
0.5	0.12	5.8575	5.8478	
1.0	0.24	11.7150	11.6956	
1.5	0.36	17.5725	17.5434	0.16
2.0	0.48	23.4300	23.3912	
2.5	0.60	29.2875	29.2390	

TABLE 3

MICRODETERMINATION OF L-ASPARAGINE AND DL-VALINE IN PRESENCE OF EACH OTHER WITHOUT SEPARATION

L-Asparagine 0.02 *M* (ml)	$In_2(SO_4)_3$ 0.0084 *M* (ml)	L-Asparagine ($\times 10^{-4}$ mg/liter)		DL-Valine 0.01 *M* (ml)	K_2TeO_3 0.0104 *M* (ml)	DL-Valine ($\times 10^{-4}$ mg/liter)	
		Taken	Found			Found	Taken
0.2	0.16	6.0056	6.0537	2.5	0.6	29.2875	29.2390
0.3	0.24	9.0084	9.0804	2.0	0.48	23.4300	23.3912
0.4	0.32	12.0112	12.1074	1.5	0.36	17.5725	17.5434
0.6	0.48	18.0168	18.1608	0.5	0.12	5.8575	5.8478

Since the complex between indium sulfate and L-asparagine is formed in the ratio of 1:3, the calculations were done accordingly by multiplying the values obtained by 3. But in the case of tellurium and DL-valine the complex is formed in the ratio of 1:4 hence the values obtained are multiplied by 4. In separate determinations of L-asparagine and D.-valine two different indicators congo red and xylenol orange were used, which give a sharp change from red to violet and from pink to purple, respectively.

Peculiarity of this method lies in determining L-asparagine and DL-valine together without separating and titrating in one solution using only xylenol orange as indicator. Further, it is peculiar that pink color developed by adding xylenol orange to a mixture of L-asparagine and DL-valine, under these concentration ratios, L-asparagine dominates. Hence, the reason why, L-asparagine is titrated first against a standard solution of indium sulfate, a light rose color marks the endpoint. Then, in the same solution without adding any further indicator DL-valine is titrated against standard solution of potassium tellurite, when purple color is developed at the endpoint. An important precaution is taken while titrating L-asparagine: the total volume in each titration should be the same, and, also, every time the same number of drops of the indicator should be added.

Results show the maximum error in the case of L-asparagine and DL-valine by 0.8 and 0.16% respectively. Present method has advantages over other methods that these amino acids have been determined separately and in presence of these two without separating quantitatively in micro amounts, are simple in itself, accurate, less time consuming.

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MICROCHEMICAL JOURNAL 14, 391–395 (1969)

Purification of Labeled ^{203}Hg-Neohydrin

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The preparation of labeled Neohydrin has been carried either by the exchange (*1*) or the synthetic method (*2*). The preparation of the labeled compound by synthesis using radio mercuric acetate gives a favorable yield over 95% when certain precautions are taken (*3*).

Purification by crystallization has the disadvantage that Neohydrin crystallizes out very slowly and tends to remain in solution if the quantity of mercury acetate used is below 400 mg. This places a restriction on the specific activity attainable and on the preparation of the short lived ^{197}Hg labeled Neohydrin. A method for the removal of unreacted radiomercury from the reaction mixture containing the labeled Neohydrin has been developed by using chromatographic adsorption on alumina column (4). One of the inconveniences of this method is that the total sample of labeled Neohydrin collected by elution is contained in a large volume of the eluate and contaminated with $Al(OH)_3$.

The use of small chromatographic columns which enables purification to be carried in one step in a final volume of (5–10 ml) and at the same time the adsorbing material has a high capacity for the removal of ionic mercury and none for the organic compound is the aim of this study. Two cation exchangers besides Al_2O_3 were investigated in batch and in continuous operations for this purpose.

EXPERIMENTAL METHODS

Batch Studies

A weak acidic cation exchange resin Amberlite IRC–50 (active group-COOH, total capacity 10 meq/g of dry resin); and a strong acidic cation exchanger Dowex 50 (active group SO_3H, total capacity 5 meq/g of dry resin); with the usual aluminium oxide (B.D.H.) for chromatographic adsorption analysis were used in these experiments.

Portions (0.25 g) of the dry resins or of Al_2O_3 are conditioned in the Na^+ form or OH^- form by washing with 2 *N* HCl, d. H_2O, then 2*N* NaOH, d. H_2O till pH 8. To each exchanger is added 20 ml of clear solution of radiomercuric hydroxide $\simeq$0.67 mg of Hg^{2+} at

pH 8 in a pear-shaped flask and gently shaken. Samples are taken from the solution at different times and their activities are counted.

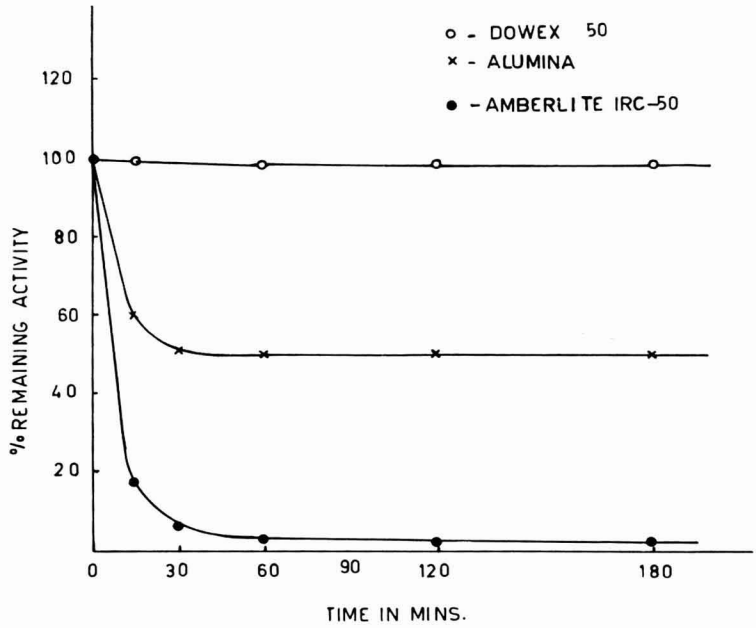

FIG. 1. Removal of $^{203}Hg^{2+}$ ions from solution.

Column Studies

Since Dowex 50 resin gave negative results in batch operation, only Amberlite IRC–50 and Al_2O_3 are used in column experiments.

(a) Behavior og Hg^{2+} ions on Amberlite resin. A 0.250-g aliquot of Amberlite IRC–50 conditioned in the Na^+ form as above is placed in the chromatographic column shown in Fig. 2. Aqueous solutions of radiomercury hydroxide at pH 8 are passed through the column. A flow rate of 1 ml in 40 minutes gives a final solution containing no ^{203}Hg activity. Washing the loaded resin with 4 ml of H_2O of pH 8 does not bring any activity in the eluate.

(b) Behavior of reaction mixture of ^{203}Hg-Neohydrin on Amberlite IRC–50. A 3-mil aliquot of reaction mixture of 95% labeled Neohydrin at pH 8 are passed on a fresh conditioned column of amberlite resin at a flow rate of 1 ml/45 minutes. A total activity of 98.5% of the ^{203}Hg Neohydrin in a 7-ml sample containing no free Hg^{2+} ions is obtained as shown in Fig. 3 (B).

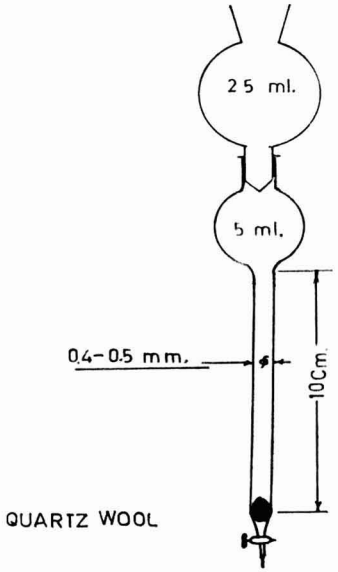

FIG. 2. Chromatographic column.

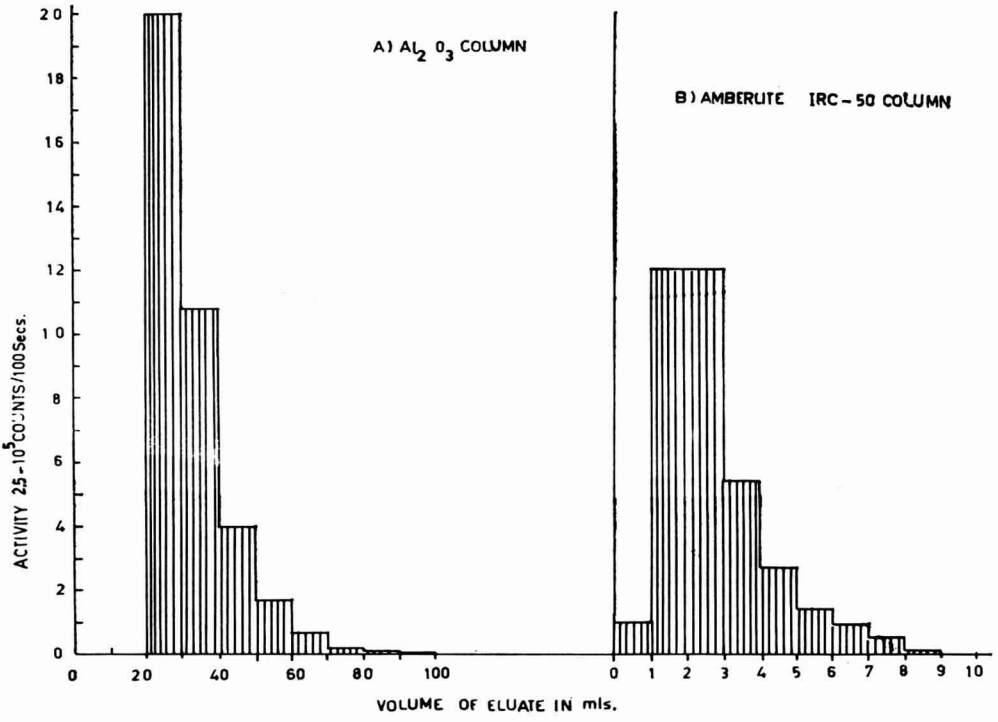

FIG. 3. Chromatographic purification of ^{203}Hg-Neohydrin on Al_2O_3 and on Amberlite IRC—50.

(c) Behavior of reaction mixture of ^{203}Hg-Neohydrin on Al_2O_3 Column. When a similar reaction mixture [as in (b)] is passed on 10 g of alumina on a column of 1 cm^2 × 10 cm conditioned in the OH^- form, 97.6% of the ^{203}Hg-Neohydrin activity in a volume of 50 ml as in Fig. 3 (A) are collected.

DISCUSSION

It is quite clear from Figs. 1 and 3 that Amberlite IRC–50 has a very high selectivity for the removal of Hg^{2+} from the reaction mixture in a neutral or slightly alkaline solution and none for the organic mercury. It allows, in one step, the collection of the pure labeled Neohydrin in a small final volume that does not need further treatment. This exceptional affinity that Amberlite IRC–50 presents can be explained by the following arguments:

The sequestering of divalent ions by soluble carboxylic acids offers an explanation for the high affinity of the carboxylic cation exchange resins for divalent cations (*5*).

We might possibly relate this unusual affinity to the acidity of the polymer and to the ability of the –COOH group to form covalent bonds with H^+ and extend this ability to form covalent bonds with Hg^{2+} ions.

Counter ions as Hg^{2+} ions which tend to associate with the fixed ionic group by say forming ion pairs or complexes are preferred by the resin.

The ionization of mercuric hydroxide $Hg(OH)_2$ may involve the following consecutive reactions (*6*):

$$Hg(OH)_2 \rightleftharpoons HgOH^+ + OH^- \rightleftharpoons Hg^{2+} + 2OH^-$$

and tendency or Hg^{2+} to form complex ion is quite known.

This might explain why with Dowex–50, a resin so tightly cross-linked that allows only small ions to diffuse, exchanges very poorly or not at all with Hg^{2+} ions.

Alumina has a low exchange capacity thus a larger amount of the adsorbing material is needed and consequently a larger volume of eluate. During elution $Al(OH)_3$ may dissolve and so the final solution has to be neutralized, centrifuged, then evaporated to a small volume.

We may conclude that purification with Amberlite IRC–50 is a very attractive method and has eliminated many hazardous steps.

SUMMARY

An easy method for the purification of labeled Neohydrin was studied by the use of the weak cation exchange resin, Amberlite IRC–50, in a small chromatographic column. The resin shows a high selectivity for the removal of mercury

ions from a neutral or alkaline solution. This method allows, in one step, the collection of the pure labeled Neohydrin in a small volume, avoiding evaporation, neutralization, and centrifugation when using the alumina method.

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Titrimetric Microdetermination of Hippuric Acid

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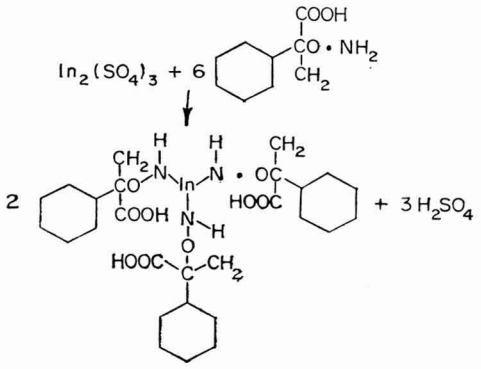
Few methods for the determination of hippuric acid are available in the literature. However, the methods, which are commonly used, are colorimetric methods (*1–3*); potentiometric titration (*4*); gel filtration spectrophotometric technique (*5*).

The present method deals with the determination of hippuric acid in micro amounts by direct titration with indium sulfate. Potentiometric data and results of analysis show that a complex between indium and hippuric acid is formed in the ratio 1:3. Probably the following reaction takes place:



EXPERIMENTAL METHODS

Reagents used: Hippuric acid (E Merck grade); indium sulfate (B.D.H. grade); and catechol violet (B.D.H. grade).

Standard solution of hippuric acid was prepared by dissolving the exactly weighed amount in distilled water. This solution was further standardized colorimetrically (*2*). Indium sulfate was prepared by dissolving the exactly weighed amount in distilled water.

PROCEDURE

A known volume of a standard solution of hippuric acid is taken in a beaker through a micropipette and diluted to 25 ml. A few drops (2–3) of catechol violet solution is added and the solution assumes a

light yellow color. Then a standard solution of indium sulfate is added from a microburette until the light yellow color changes sharply to a light ash green color.

RESULTS AND CONCLUSION

Results are shown in Table 1. The range in which hippuric acid was estimated varied from 2.6877×10^{-4} to 26.8770×10^{-4} mg/liter. Since the complex between indium and hippuric acid is formed in the ratio of 1:3, hence calculations have been done by multiplying the observed calculated values by three. Maximum error in these experiments is 1.4%. This method supersedes other methods in the sense that it is direct, simple, and less time consuming.

TABLE 1

MICRODETERMINATION OF HIPPURIC ACID

Hippuric acid 0.0076 *M* (ml)	$In_2(SO_4)_3$ 0.01 *M* (ml)	Amount of hippuric acid Taken × 10^4mg/liter	Found	Maximum error (%)
0.2	0.05	2.8235	2.6877	
0.4	0.10	5.4470	5.3754	
0.8	0.20	10.8941	10.7508	1.4
1.0	0.25	13.6176	13.4385	
2.0	0.50	27.2353	26.8770	

SUMMARY

Hippuric acid was determined quantitatively in micro amounts with indium sulfate, using catechol violet as indicator. Potentiometric results and present results confirm that a complex between indium and hippuric acid is formed in the ratio of 1:3. Maximum error in these experiments is 1.4%. Determinations up to 26.8770×10^{-4} mg/liter were made.

ACKNOWLEDGMENT

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Application of Back Titration of EDTA with Mercury(II) to the Analysis of Inorganic Pigments

II. Analysis of Zinc Pigments

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INTRODUCTION

In previous studies the potentiometric back titration of EDTA with mercury(II), was successfully applied to the determination of metal ions for which evidence of chelation with EDTA exists, the analysis of cation mixtures, various types of alloys (*20–22*) and lead pigments (*18*). The present work is an extension of the same application to the analysis of zinc pigments and mixtures of zinc oxide with copper phthalocyanine.

Based on selective control of pH, Zn has been determined volumertically in presence of large amounts of Mg (*8*). The same mixture was successfully analyzed by back titrating excess CDTA with mercury(II) once at pH 8 and once at pH 11 (*17*). EDTA removes Zn from the redox system Zn–ferro–ferricyanide, thereby causing a reduction in potential at the end point, which is shown by a suitable indicator as 3, 3′-dimethyl naphthidine. The Mg is then titrated, in the same solution, using Eriochrome black T (EBT) (*9, 14*). The Zn can be separated from other cations by means of anion exchange and solvent extraction prior to EDTA titration (*15*). It can be determined potentiometrically by back titrating excess EDTA with iron(III) chloride in ammonium acetate buffer, pH 5–6 (*30*). The potentiometric method of determining Zn and Pb with disodium–barium–EDTA (*35*) is complicated, time and reagent consuming. Crisan (*13*) exploited the fact that Zn–EDTA complex is stable within a critical range of pH, to analyze its mixture with Fe(III) and Cr(III) in a catalyst used in petroleum industry. After adsorption on and elution from an anion exchange resin, Zn can be titrated with EDTA using xylenol orange at pH 6 after addition of NH_4F, KCN, and $Na_2S_2O_3$ (*2*).

Tomoyuki (*3*) reported that titration of Zn with EDTA and EBT at pH 10 in the solution of Zn NH_4PO_4 in tartrate, suffers from coprecipitated Cu, Co, and Ni cations.

Khalifa (*23*) successfully analyzed mixtures of Zn, Cu, Pb, Mg, Ca, Sr, or Ba by a procedure involving potentiometric back titration of excess EDTA with mercury(II) at pH 11 for total and at pH 8–10 for the heavy metals; and an additional titration with EDTA and EBT after masking Zn and Cu with cyanide followed by addition of acetone and titration of the liberated Zn to the EBT end point. Among the indicators used in direct titration of Zn with EDTA are to be mentioned EBT (*7, 40*), PCV (*27, 38*) and PAN (*26*).

Pribil (*31*) determined Cu by potentiometric back titration of EDTA with iron(III) chloride. Belcher (*5*) recommends the removal of Fe(III), Al, Cd, Zn, Mn, Ni, Co and Pb prior to the potentiometric titration of Cu with EDTA in acetate buffer pH 5–6. Bermejo (*6*) determined 0.064 to 96 mg of Cu with CDTA with an accuracy of $\pm 0.5\%$ in absence of Ni, Co, Zn, Cd, and Mn. Khalifa (*16, 17*) determined copper and analyzed its mixtures with other cations by potentiometric back titration of excess EDTA or CDTA with mercury(II). Ali (*4*) determined micro amounts of Cu with EDTA after extracting into phenyl bromide its complex with pyridine and thiocyanate to increase the sharpness of the end point.

Sierra (*36*) used a platinium electrode in the potentiometric determination of mixtures of Cu and Ag with Ba–EDTA. Although there are, available, some methods for the determination of Cr(III), yet most of them involve some drawbacks. For instance the reaction of dichromate—resulting from oxidation of Cr(III)—with iodide does not take place instantaneously, but the speed increases appreciably with increasing hydrogen ion concentration. However, the high acidity may introduce an error through the air oxidation of HI (*25*).

The titrimetric procedure making use of excess EDTA and titrating such an excess with a standard Mn^{2+} solution (*24*) involves the addition of many reagents and further the results which are hardly reproduced are always lower by at least 2% than the expected ones. The method of Szeckers (*39*) involves oxidation of Cr(III) with permanganate, dissolution of MnO_2 in ascorbic acid, and titration of the resulting Mn^{2+} with EDTA. Crisan (*12*) converted Cr(III) into $PbCrO_4$ and titrated excess of lead ions with EDTA and PCV. Khalifa (*19*) recently investigated a reliable potentiometric procedure by aid of which he analyzed multicomponent chromium mixtures. The present procedures for analysis of zinc pigments are not only reliable, but also applicable to such an analysis with the additional use of masking agents as dimercaptosuccinic acid (*28*) or dithiocarbaminoacetic acid (*10*) for copper and methyl ketone in presence

of sodium pyrophosphate (*33*) or triethanolamine and ascorbic acid (*32*) for chromium.

EXPERIMENTAL METHODS

The water used was always de-ionized; the chemicals were of the highest purity available. These were nitrates of mercury(II) and silver; EDTA; urotropine; diethyldithiocarbamate; carbonate, hydroxide, and citrate of sodium; citrate and persulfate of ammonium; diammonium hydrogen phosphate; ammonia; potassium permanganate; ferrous ammonium sulfate; hydrochloric nitric, sulfuric, and phosphoric acids; zinc metal; electrolytic copper metal; (Eriochrome black T) indicator; and absolute alcohol.

The samples analyzed involved zinc oxide alone or adulterated with calcium, magnesium, or barium carbonates; zinc sulfide alone or adulterated with calcium carbonate or silica; lithopone alone or adulterated with calcium carbonate; high strength lithopone; titanated lithopone; copper phthalocyanine alone or mixed with zinc oxide; copper phthalocyanine plus zinc oxide adulterated with calcium, magnesium, or barium carbonates; zinc chromate alone or mixed with zinc oxide or adulterated with calcium or barium carbonates.

Solutions

The 0.05 *M* mercury(II) solution (16.25 g/liter containing 3.5 ml of conc nitric acid) was standardized potentiometrically with standard 0.05 *M* EDTA solution prepared normally and standardized against 0.05 *M* zinc solution.

The 0.05 *M* zinc solution was prepared from cleaned arsenic-free zinc metal (3.269 g/liter containing 12 ml of conc nitric acid).

The 0.1 *N* permanganate solution was prepared according to the procedure cited in 1967 Book of ASTM standards (*1*).

The 0.1 *N* ferrous ammonium sulfate solution was about 40 g/liter of 5% sulfuric acid solution containing a 5 g of high purity aluminum sheet to minimize the tendency for possible oxidation of the solution (*37*).

The 0.1 *M* sodium citrate solution was 29.4 g/liter. The diammonium hydrogen phosphate reagent was prepared as recommended by Scott (*34*).

The sulfuric phosphoric acid mixture was prepared as mentioned before (*18*).

The copper solution (1 ml $\equiv$ 1.0 mg) was prepared by dissolving 0.500 g of electrolytic copper foil in 10 ml of 50% (v/v) nitric acid, boiling till all brown fumes are expelled, cooling, slightly diluting and

adding 0.2 g of urea to neutralize nitrous acid, and diluting to 500 ml in a volumetric flask.

The copper solution (1 ml ≡ 0.1 mg) was prepared by accurate dilution from the more concentrated one.

The 0.1% solution of sodium diethyl dithiocarbamate was always freshly prepared by dissolving 0.5 g in 500 ml of water.

The 0.5 *M* sodium hydroxide solution was prepared by dissolving 10 g in 500 ml of previously boiled and cooled water.

The urotropine solutions were 2 and 5%.

The cell and equipment including pH and potentiometer were essentially the same as those before mentioned (*18*).

Procedures

I. *Zinc oxide.* Weigh 0.5 g (dried at 105–110°C for 2 hours) into a 250-ml beaker, moisten with water followed by few drops of alcohol (to increase the wetting property and to enhance the dissolution of the pigment), add 10 ml of 20% (v/v) nitric acid, warm to dissolve; in case any insoluble matter is present, add 25 ml of water and boil, filter, and wash with hot water, test the residue (if in quantity) for silica, barium sulfate, and silicates; make the original solution or filtrate with water up to 250 ml in a volumetric flask.

A. Place a suitable aliquot (5–10 ml) into a 150-ml beaker, add 5 ml of 0.05 *M* EDTA solution, 40 ml of 2.0% (w/v) urotropine solution, 1.0 ml of 0.05 M mercuric nitrate solution (the same titrant as used in the back titration), a few drops of 0.5 *M* sodium hydroxide solution required to keep the pH value at 8.0–8.3 (initial emf = +0.04 V) and finally continue the back titration with mercury(II) solution using the silver amalgam as indicator electrode. This titration gives the content of zinc.

B. Place a suitable aliquot (5–10 ml) into a 150-ml beaker, add 5.0 ml of 0.05 *M* EDTA, 40 ml of 2.0% urtropine, few drops of 0.5 *M* sodium hydroxide required to keep the pH value at 11.0 (initial emf = +0.13 V) and finally back titrate with mercury(II) solution. This titration gives the content of zinc plus the one or more of the alkaline earth metals (AEM).

II. A. *Copper phthalocyanine.* Weigh 0.5–1.0 g in a silica crucible (capacity 30 ml), ignite at a low flame, then at elevated temperature to disintegrate and to convert copper into copper oxide, cool, add 5 ml of 33.0% (v/v) nitric acid, warm to dissolve; in case any insoluble matter is present, transfer into a 150-ml beaker; add 25 ml of water and boil, filter and wash with hot water, test the residue (if in quantity) for silica, barium sulfate, and silicates; make the

original solution or filtrate with water to a known volume (100–250 ml).

(i) To determine copper, place a suitable aliquot (5–10 ml) into a 150-ml beaker, add 3–5 ml of 0.05 M EDTA, 40 ml of 2.0% urotropine, a few drops of 0.5 *M* sodium hydroxide required to keep the pH value at 8.0–9.0 (initial emf = +0.04–0.055 V) and finally back titrate with mercury(II) using the silver amalgam as indicator electrode.

B. *Copper phthalocyanine plus Zinc oxide* with or without AEM carbonates; follow Procedure IIA. To determine copper in presence of zinc with or without AEM, two methods are applicable depending on the copper content of the sample.

(i) Electrolytic method; take a suitable aliquot, (usually half the sample solution) add 3–5 ml of conc nitric acid and 5–10 ml of conc sulfuric acid for each 100 ml of the aliquot, electrolyze at room temperature with current densities of 2–5 A at 2–6 V using the usual platinium alloy gauze cylindrical cathode and spiral rotating anode. The usual precautions regarding washing and removing electrodes before switching off the current and drying in alcohol should be observed.

(ii) Colorimetric method; take a suitable aliquot of the sample solution (containing 0.1–0.01 mg of copper) into a Nessler tube; add 5 ml of 20% (w/v) ammonium citrate solution followed by 5 ml of conc ammonia (sp gr 0.880), dilute to 70 ml, add 10 ml of freshly prepared 0.1% (w/v) sodium diethyldithiocarbamate solution, complete to 100 ml and compare the brown color with that of standards containing known amounts of copper.

To determine copper plus zinc, follow procedure IA.

To determine copper plus zinc plus AEM follow procedure IB.

III. Zinc sulfide and lithopones.

A. Zinc sulfide alone or adulterated with silica; lithopone; high strength lithopone and titanated lithopone. Weigh 0.5–1.0 g into a 250-ml beaker, moisten with water followed by a few drops of alcohol, add 5–10 ml of 50% (v/v) nitric or sulfuric acid, heat to dissolve, add 25 ml of water and boil to expel all gases, filter and wash with hot water; test the residue for silica, barium sulfate, silicates and titanium dioxide; make the filtrate with water up to 250 ml.

To determine zinc, follow procedure IA.

B. Zinc sulfide, lithopone, high strength lithopone and titannted lithopone adulterated with AEM carbonates (usually calcium carbonate). Weigh 0.5–1.0 g into a 250-ml beaker, moisten with water followed by a few drops of alcohol, add 10 ml of 50% (v/v) hydro-

chloric acid, heat to dissolve and to expel completely the hydrogen sulfide; evaporate on a water bath to dryness, add 2 ml of conc nitric acid and evaporate to dryness, repeat once again the evaporation with conc nitric acid; cool, add a few drops of dilute nitric acid, 25 ml of hot water; boil, filter, and wash with hot water; test the residue for silica, barium sulfate, silicates, and titanium dioxide; make the filtrate with water up to 250 ml.

To determine zinc, follow procedure IA.

To determine zinc plus calcium follow procedure IB.

Barium which is present in the above samples as $BaSO_4$, can be determined gravimetrically.

In an attempt to determine Ba by the potentiometric back titration procedure, we analyzed two samples by the procedure described under IV:

IV. Lithopones. Weigh 0.5 g in a silica crucible [do not use a platinium crucible because platinium is attacked by SO_3 and the platinium surface becomes coated with platinous sulfide(11)], ignite at a low flame then, at elevated temperature, cool, dissolve in 10 ml of 2% (v/v) nitric acid, wash with hot 1.0% nitric acid, reserve the filtrate (f_1).

Transfer the residue to a platinium crucible; ignite at a low temperature until free from carbonaceous matter and finally at elevated temperature, cool, mix with 5 g of anhydrous sodium carbonate and fuse at 1000°C for 10 minutes; cool, extract by boiling with 100 ml of water, and digest at incipient boiling for 1 hour to coagulate the precipitated iron and titanium oxides (if present) plus barium carbonate; filter through a loose filter paper and wash with hot 1.0% sodium carbonate solution; the filtrate contains soluble silicate and sulfate; dissolve the residue in the least amount of hot dil nitric acid, filter, wash with hot water, test the residue for oxides of titanium and iron; add this filtrate (f_2) to the main one (f_1) and make with water up to 250 ml.

To determine zinc, follow procedure IA.

To determine zinc plus barium follow procedure IB.

V. Zinc chromate ($4\ ZnO \cdot 4CrO_3 \cdot K_2O \cdot 3H_2O$). Weigh 0.5 g (dried at 105–10°C for 2 hours) into a 250-ml beaker, moisten with water add 10 ml of 10% (v/v) nitric acid, cover with a watch glass and agitate on a water bath, add 10 ml of alcohol in aliquots, leave covered on the water bath to dissolve and to reduce hexavalent to trivalent chromium; remove the cover and evaporate the solution nearly to dryness to get rid of alcohol, cool, add 25 ml of hot water; boil; in case any insoluble matter is present, filter, and wash with hot water:

test the residue (if in quantity) for silica, barium sulfate, and silicates; make the original solution or filtrate with water up to 250 ml.

A. To determine zinc (in absence of AEM), place a suitable aliquot (5 ml) into a 150-ml beaker, add a few milliliters of 0.5 *M* sodium hydroxide, enough to precipitate chromium as chromium hydroxide and to keep zinc in solution as sodium zincate (about 2 ml); add 40 ml of 2.0% urotropine, stir well, add 5 ml of 0.05 *M* EDTA, 1 ml of 0.05 *M* mercury(II) (the same titrant as used for back titration); a few drops of dilute nitric acid required to keep the pH value at 8.0–8.3 initial emf = +0.04V), and finally complete the back titration with mercury(II) using silver amalgam as the indicator electrode.

Note: In presence of calcium and/or barium, determine chromium volumetrically; zinc and chromium potentiometrically following Procedure VB; zinc is calculated by the difference.

B. To determine zinc plus chromium, place a suitable aliquot (5ml) into a 150–ml beaker, add 6 ml of 0.05 *M* EDTA, a few milliliters of water, cover with a watch glass; boil for 15 minutes on a hot plate, cool to room temperature, add 20 ml of 0.05 *M* mercury(II), stir while adding a few drops of 0.5 *M* sodium hydroxide required to keep the pH value at 8.0–8.3 (initial emf = +0.04 V), and finally complete the back titration as above.

C. To determine Zn + Cr + Ca and/or Ba follow procedure VB without addition of citrate, using 40 ml of 2.0% (w/v) urotropine and a few milliliters of 0.5 *M* sodium hydroxide required to keep the pH value at 1.0 (initial emf = 0.13 V), and back titrate with mercury(II) solution.

RESULTS AND DISCUSSION

The results obtained by applying Procedure IV (Table 4) to the analysis of lithopones exhibit a deviation in the barium content ranging from −0.7 to 3.0%. This is attributed to the fact that when the filtrate (f_2) is added to the main one (f_1), a turbidity or a precipitate forms depending upon the concentration of the sulfate radical present in the main filtrate (f_1) as $ZnSO_4$. The latter may be formed as a result of (i) oxidation of ZnS when heated to redness (*29*), (ii) manufacture of lithopones by interaction between BaS and $ZnSO_4$, and (iii) slow air oxidation of the pigment during long periods of storage. Thus the presence of $ZnSO_4$ will lead to conversion of barium nitrate into the insoluble sulfate, leading thereby to the above error.

In the analysis of zinc chromate by the classical and the potentiometric back titration procedures we obtained concordant results

TABLE 1

ANALYSIS OF ZINC OXIDE

No.	Zn (mg)		ZnO (%)		pH 8–8.3 (mV/0.1 ml of titrant)	AEM (mg)		AEM carbonate (%)		pH 11 (mV/0.1 ml of titrant)
	a [c]	b	a	b		a	b	a	b	
1 [a]	7.954	7.949	99.00	98.94	198	—	—	—	—	—
2 [b]	6.365	6.354	78.42	78.28	207	0.826 Ca	0.826	20.40	20.40	76
3	4.704	4.707	59.30	59.35	167	1.570 Ca	1.582	39.70	40.03	54
4	6.786	6.767	83.54	83.27	202	0.429 Mg	0.432	14.70	14.84	69
5	5.123	5.120	63.60	63.57	236	0.982 Mg	0.981	34.00	33.95	67
6	6.736	6.741	83.94	84.00	190	0.839 Ba	0.823	12.10	11.84	57
7	5.573	5.568	68.80	68.73	218	1.593 Ba	1.608	22.70	22.92	58

[a] Not adulterated, result of 6 detn with SD of $\pm 0.34\%$.

[b] Nos. 2–7, zinc oxide samples artificially adulterated by proper mixing with commercial samples of alkaline earth metal (AEM) carbonates, mean result of 3 parallel detn.

[c] The a's were determined by classical methods before adulteration; and the b's were determined potentiometrically, after adulteration, following Procedure I; time required, 2 hours.

with chromium (columns a and b, respectively, Table 5) but not with zinc, the potentiometric results of which (column b) were compared to calculated ones (column a_1) on basis of the formula $4\ ZNO \cdot 4CrO_3 \cdot K_2O \cdot 3H_2O$ and the percentage of chromium found.

The results in Tables 1, 2, 3, and 5 show that the procedures recommended by us for the analysis of zinc pigments and extenders are quite simple and extremely reliable. Nearly all the probabilities of adulteration are taken into consideration. The apparatus used is simple and includes no elaborate or expensive pieces of equipment. All titrations had very sharp end points lying in the immediate vicinity of the expected ones. However, it was noted, in the determination of zinc in adulterated zinc pigments, and adulterated zinc pigments mixed with copper phthalocyanine, that the more the alkaline earth metals, specially calcium, were present, the less was the magnitude of the potential end point breaks. This was overcome by using about sevenfold the amount of EDTA required to chelate with the heavy metals, when steady, rapid, and sharp end points were obtained. Mercuric solutions used as back titrants should be standardized within each set of experiments with the requisite buffer and pH. Our technique to obtain a strong Zn–EDTA complex was so simple that we used the same back titrant to enhance the chelation of zinc.

Trials to determine Zn alone potentiometrically, in zinc chromate pigments containing an AEM were not successful. On treatment of the three component sample with enough alkali hydroxide and determining zinc in the filtrate, the results were lower by 2.0%. Although Zn can be accurately determined in presence of Cr(III) by potentiometric back titration of their solution after treating it with enough alkali, excess EDTA and urotropine, yet its determination under the same conditions in presence of an AEM gave low results. Addition of urotropine to the three component sample precipitates zinc hydroxide which did not dissolve in excess of EDTA. If in addition, citrate was added the same phenomenon was observed. Other sequences of addition led to the same results.

It is possible that the procedures are capable of further development and that other materials may subsequently be included or alternatively substituted for some of those already investigated.

SUMMARY

The potentiometric back titration of excess EDTA with mercury(II) was used very successfully for the analysis of zinc pigments and mixtures of zinc oxide with copper phthalocyanine, especially when adulterated. This new method eliminates the use of hydrogen sulfide separation and is thus characterized by simplicity and

TABLE 2
ANALYSIS OF COPPER PHTHALOCYANINE OR ITS MIXTURE WITH ZnO PURE OR ADULTERATED

No.	Cu (mg)		Zn (mg)		Titrant (mV/0.1 ml)	AEM (mg)		pH 11 (mV/0.1 ml of titrant)
	a [d]	b	a	b		a	b	
1 [a]	4.797	4.803	—	—	163	—	—	—
2 [b]	0.966	0.970 [f]	8.694	8.704	140	—	—	—
3	0.240	0.240 [e]	6.002	5.991	151	—	—	—
4	0.095	0.095 [e]	7.285	7.283	144	—	—	—
5 [c]	0.795	0.792 [f]	7.247	7.269	93	1.600 Ca	1.590	54
6	0.440	0.440 [e]	5.678	5.702	130	1.099 Mg	1.085	66
7	0.115	0.115 [e]	6.245	6.251	152	1.667 Ba	1.664	48

[a] Copper phthalocyanine, result of 7 detn with SD of ±0.62%.

[b] Copper phthalocyanine samples artificially mixed by proper weighing with commercial samples of zinc oxide, mean result of 3 detn, involving but negligible error.

[c] Copper phthalocyanine plus zinc oxide samples, artificially adulterated as mentioned above, mean result of 3 detn, involving but negligible error.

[d] The a's were determined by classical methods before adulteration and/or mixing and the b's potentiometrically, after adulteration. e colorimetrically following procedure IIBii; and f by electrolysis following procedure IIBi.

TABLE 3

ANALYSIS OF ZINC SULFIDE AND LITHOPONES

No.	Sample	Zn (mg)		Total zinc (%)		pH 8.0–8.3 (mV/0.1 ml of titrant)	Ca (mg)		Calcium (%)		pH 11 (mV/0.1 ml of titrant)
		a [d]	b	a	b		a	b	a	b	
1 [b]	Zinc sulfide	6.642	6.645	65.50	65.53	116	—	—	—	—	—
2 [a]		5.566	5.575	55.43	55.51	177	—	—	—	—	—
3 [b]	+ Silica	4.646	4.615	46.76	46.45	168	—	—	—	—	—
4 [c]	+ $CaCO_3$ + Silica	2.222	2.216	22.20	22.13	84	2.026	2.041	20.24	20.39	46
5 [b]	Lithopone	3.821	3.819	19.12	19.11	210	—	—	—	—	—
6 [b]	high strength	7.797	7.806	32.20	32.24	188	—	—	—	—	—
7 [b]	titanated	9.080	9.087	39.00	39.03	152	—	—	—	—	—
8 [c]	+ $CaCO_3$	2.601	2.601	12.91	12.91	102	2.478	2.527	12.30	12.54	54

[a] Dissolved in sulfuric acid, result of 6 detn with SD of ±0.18%.

[b] Dissolved in nitric acid; not adulterated by AEM carbonates; mean result of 3 parallel detn.

[c] Artificially adulterated as mentioned in Table 1, footnote *b*, a mean result of 3 parallel detn with an average error of −0.10% zinc, and +0.20% calcium.

[d] The a's were determined by classical methods before adulteration; and the b's potentiometrically after adulteration.

TABLE 4

ANALYSIS OF LITHOPONES

No.	Sample	Zn (mg) a	Zn (mg) b	Total zinc (%) a	Total zinc (%) b	pH 8–8.3 (mV/0.1 ml of titrant)	Ba (mg) a	Ba (mg) b	Barium (%) a	Barium (%) b	pH 11.00 (mV/0.1 ml of titrant)
1 [a]	Lithopone	3.846	3.799	19.12	18.89	156	8.374	8.247	41.63	41.00	40
2 [b]	High-strength lithopone	6.595	6.591	32.67	32.65	130	5.915	5.334	29.30	26.42	52

[a] Mean result of 3 parallel detn, with an average error of −0.2% zinc, and −0.7% barium.

[b] Mean result of 3 parallel detn, involving but negligible error for zinc, and −3.0% barium.

TABLE 5

ANALYSIS OF ZINC CHROMATE

No.	Zn (mg)		(mV/0.1 ml of titrant)	Cr (mg)		(mV/0.1 ml of titrant)	AEM (mg)		pH 11.00 (mV/0.1 ml of titrant)
	a_1	b		a	b		a	b	
1 [a]	3.166	3.122	162	2.514	2.465	96	—	—	—
2 [b]	7.086	7.083	162	0.519	0.543	110	—	—	—
3 [c]	2.534	2.484	—	2.011	2.011 [a]	90	0.817 Ca	0.825	56
4	2.522	2.465	—	2.004	2.004 [a]	130	1.037 Ba	1.004	44

[a] Not adulterated, mean result of 3 detn, with an average error of −0.5% zinc, and −0.5% chromium.

[b] Artificially mixed as mentioned in Table 2 footnote *b*, mean result of 3 detn, involving but negligible error with zinc, and +0.27% with chromium.

[c] Artificially adulterated as mentioned in Table 1, footnote *b*, mean result of 2 detn, with an average error of −0.5% zinc, +0.08% calcium, and −0.5% barium.

[d] The a's were determined by classical methods before adulteration; a_1 calculated and b potentiometrically before adulteration.

rapidity; 28 different industrial samples were analyzed, with many advantages over the classical procedures used for analysis of such materials.

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Chemical Microscopy of 1,5-Naphthyridine: Reactions with Platinum Metals and Gold[1]

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In continuing the study of various heterocyclic nitrogen bases as micro reagents for noble metals (*1*) it was decided to investigate the behavior of 1,5-naphthyridine because its structure, having two nitrogens in a pair of fused rings (Fig. 1), appeared to offer interesting possibilities.

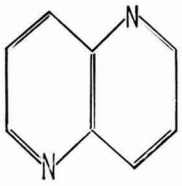

FIG. 1. 1,5-Naphthyridine.

EXPERIMENTAL METHODS

The 1,5-naphthyridine was purchased as a white crystalline solid. After the container had been opened, however, there was a tendency for the remainder of the contents to undergo discoloration during storage. In subsequent batches of the compound this discoloration was avoided by storage in a refrigerator. For use as a precipitating agent the naphthyridine was made up as a 0.75 *M* solution in 1 *N* hydrochloric acid; this solution remained stable indefinitely. Test solutions of the various metals were prepared by appropriate dilution of their respective stock solutions with 1 *N* hydrochloric acid. Most of the stock solutions were based on known concentrations of the chlorides of Au(III), Pt(IV), Pd(II), Ir-(III), Rh(III), and Ru(III), in 1 *N* HCl. In the case of Os(VI), the stock solution was based on potassium osmate. The tests were carried out by allowing a 20-μl drop of reagent from a capillary pipet to flow into a similar drop of sample solution on a microslide. A given test was considered positive if a characteristic crystalline product separated withn 2 or 3 minutes.

[1] The present paper was adapted from one presented at the Southwest Regional Meeting of the A.C.S., Shreveport, Louisiana, December, 1964.

RESULTS AND DISCUSSION

For the microscopic identification of members of the platinum group, and gold, the 1,5-naphthyridine reagent proved most efficient with platinum, which yielded comparatively long, highly refractive, yellow prisms. The latter appeared singly, and also in burr-like clusters. As viewed through a 16-mm objective and 10× ocular, some of the crystals exhibited an elongated rectangular outline. Some appeared to be parallelepipeds; however, many of the crystals which were differently oriented presented outlines whose opposite ends were tapered like a chisel (Fig. 2). It should be mentioned that the yellow color of this derivative was not in agreement with the "orange red" color of the chloroplatinate reported by Bobranski and Sucharda (*2*); however, these authors also in-

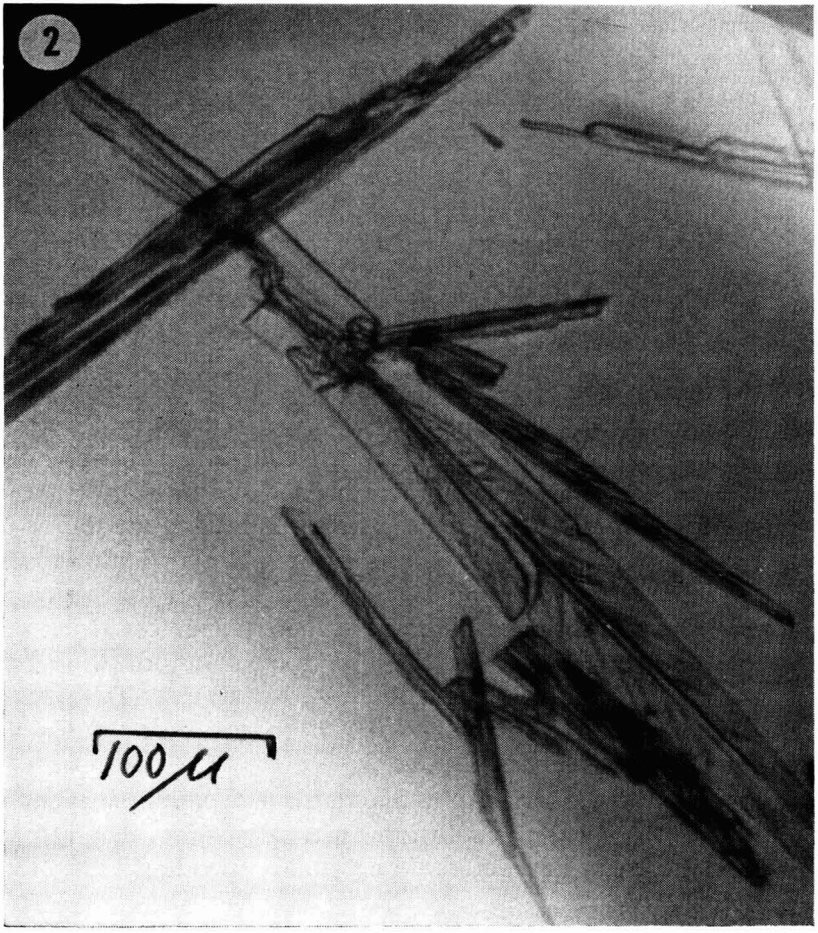

FIG. 2. One type of crystalline derivative formed with platinum; metal concentration, 1:5000.

dicated that their presumed pure 1,5-naphthyridine was in the form of "yellowish" needles, rather than the white crystalline product used in the present work.

Extinction of the platinum derivative was very nearly parallel; between crossed polars, the crystals which were not in extinction position exhibited bright polarization colors. With the laboratory temperature at 25° the platinum in a test drop could be identified when the metal ion concentration was equal to as little as one part in 15,000. For a 20-μl sample this was equivalent to identifying approximately 1.3 μg. However, it should be mentioned that the reaction appeared to be temperature sensitive to a greater degree than are some other micro crystalline reactions, so that the sensitivity may decrease appreciably above the 20–25° range.

Under similar conditions acidified solutions of gold(III) chloride reacted to form yellow prisms, along with crosses and daggers having irregular edges. In addition, there were occasionally some very fine granular particles which appeared to be octahedra (Fig. 3). The prisms, which generally appeared in groups, exhibited parallel extinction; any cruciform aggregates which were not in extinction position generally appeared yellow. Good positive tests for gold were obtained in solutions containing as little as one part metal in 10,000.

With acid solutions of palladium(II) chloride the reagent caused a fairly prompt separation of an apparently amorphous precipitate, which, however, gradually yielded rather small, pale yellow crystals (Fig. 4A and B); many of these were narrow prisms, or needles, which exhibited oblique extinction. Crystals not in extinction position assumed a yellow color. Use of an 8-mm objective is recommended for their observation. Palladium was readily identified at a dilution of one part in 5000, or the equivalent of 4 μg in a 20-μl sample.

Under the observed conditions, the chlorides of iridium(III), rhodium(III), and ruthenium(III) did not effect the separation of any crystalline product with the naphthyridine reagent. This was likewise true for an acidified solution of potassium osmate containing one part osmium per thousand. Since copper is a component of certain precious metal alloys, it was of interest to find that an acidified solution of cupric chloride, even at a concentration of eight parts Cu(II) per thousand, failed to yield a precipitate with the reagent.

In connection with the metals which failed to yield a precipitate with the reagent it was considered worth while to determine whether their presence would interfere with the identification of platinum, palladium, and gold in mixtures. While platinum, in the absence of any other metal, could readily be identified at a concentration of one part in 15,000, it

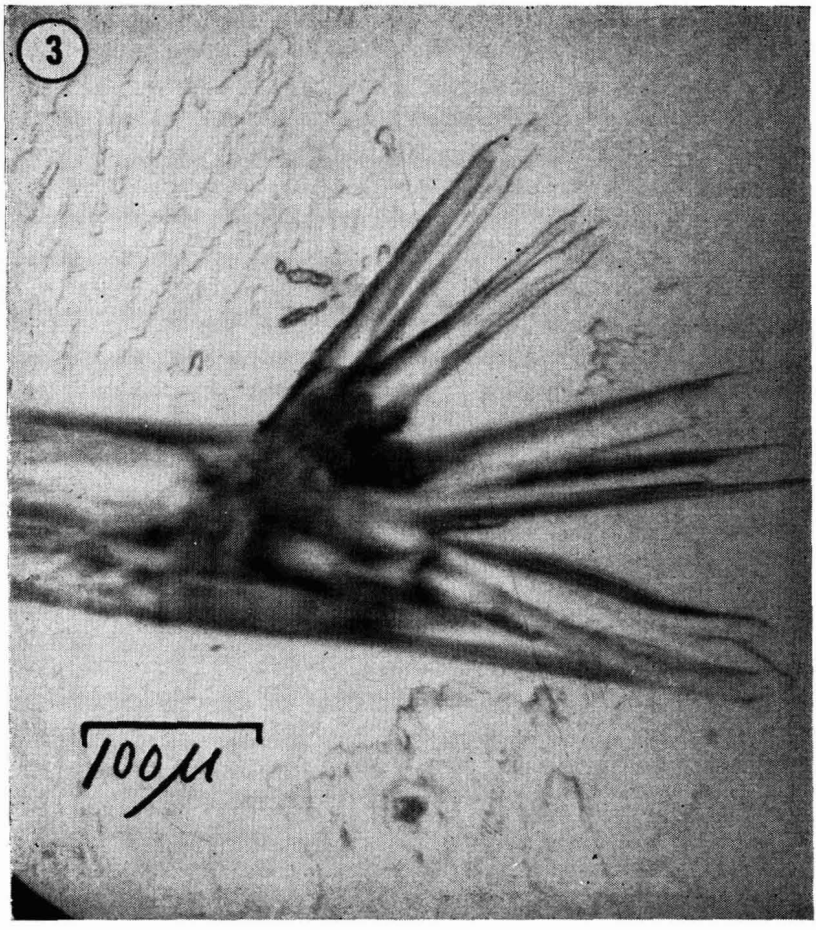

FIG. 3. Types of crystals representing the gold complex; metal concentration, 1:1000.

was found that the presence of cupric ion interfered with the reaction. In a solution containing 0.5 part platinum per thousand, along with 8 parts of copper, no crystalline precipitate was formed. Iridium also interefered with the detection of platinum. A test solution having a platinum content of 0.5 part per thousand, in the presence of an equal quantity of iridium caused no separation of the crystals characteristic of the platinum derivative previously mentioned. Although a few short prisms eventually separated along the periphery of the reaction mixture, their color was brown instead of yellow, and the crystalline form was not that of the platinum complex.

Appreciable amounts of rhodium lowered the efficiency of the test considerably. Addition of naphthyridine reagent to a mixture containing

0.5 part per thousand of platinum, and an equal amount of rhodium, yielded the characteristic prisms of platinum derivative very slowly. Furthermore, the product was less abundant than that produced in a solution containing the same platinum concentration in the absence of rhodium. Apparently small concentrations of potassium osmate offered no interference in the test for platinum, since a solution containing both these metals at a concentration of 0.5 part per thousand gave a good yield of the typical yellow crystals of platinum derivative within 3 minutes. Some of these crystals seemed a bit less slender than those formed in the absence of osmate. Ruthenium, on the other hand, interfered not only with the test for platinum, but also in tests for gold and palladium.

In solutions of palladium(II) ion at a concentration of 0.5 part per thousand, the presence of a corresponding concentration of either rhodium or iridium offered no interference with the separation of the palladium complex. As usual, there was a fairly prompt separation of an amorphous precipitate, followed by the formation of the fine crystals denoting palladium. When the palladium solutions contained appreciable amounts of osmate or cupric ion, the addition of naphthyridine reagent resulted in the formation of an amorphous product, but this was not followed by the separation of the typical crystalline palladium derivative.

In general the formation of the gold complex seemed more susceptible to interference than the other two. In a solution with a gold(III) concentration of 0.5 part per thousand, in the presence of a similar amount of iridium, rhodium, ruthenium, osmate, or cupric ion, the naphthyridine either failed to yield a crystalline product, or it caused the separation of crystals which were not really characteristic of the gold derivative.

SUMMARY

As summarized in Table 1, by the formation of characteristic crystalline derivatives under the microscope a hydrochloric acid solution of 1,5-naphthyridine may serve as a reagent to identify low concentrations of platinum(IV), gold(III), and palladium(II) in acid solutions of their chlorides, whereas rhodium(III), ruthe-

TABLE 1

NOBLE METALS via 1,5-NAPHTHYRIDINE

	Pt	Au	Pd
Minimum conc.	1:15,000	1:10,000	1:5000
μg/drop	1.3	2	4
Crystal color	Pale yellow	Yellow	Pale yellow
Extinction	Nearly parallel	Parallel	Oblique
Color between crossed polars [a]	Varicolored	Yellow	Pale yellow

[a] Refers to crystals which are not in extinction position.

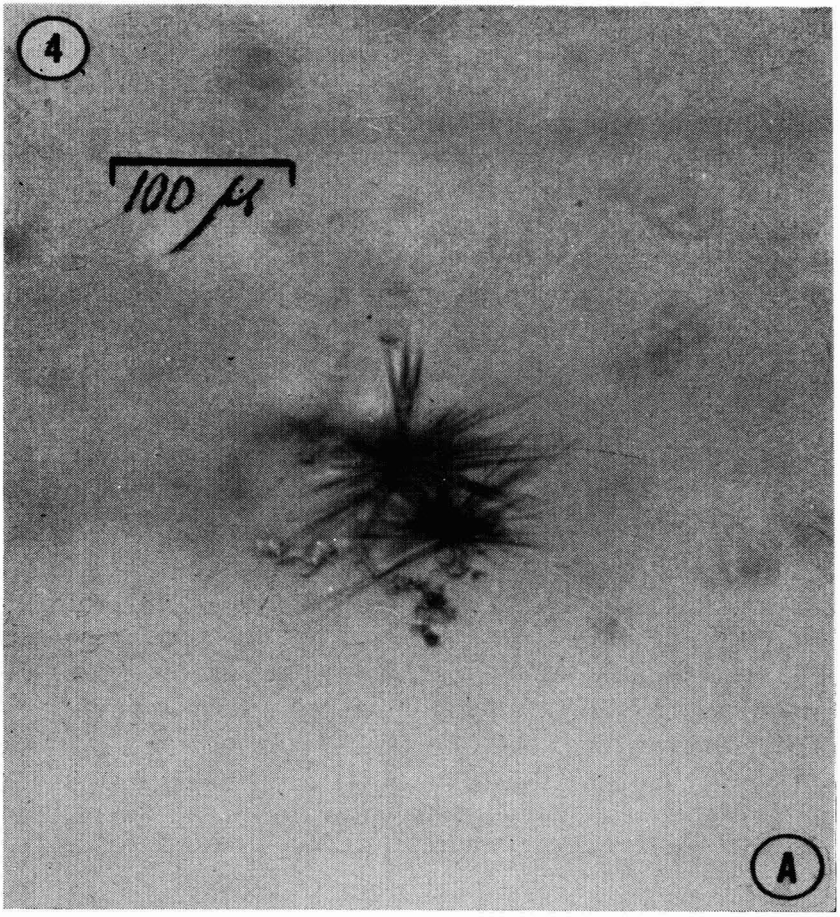

FIG. 4A. Crystalline derivative of palladium; metal concentration, 1:1000. (B). Palladium derivative, from same preparation shown in (A), but from different field.

nium(III), and osmium(VI) do not form corresponding precipitates. As a reagent for the noble metals, therefore, naphthyridine appears more versatile than the related compound, pyridine, when applied under similar conditions. As shown previously (*3*), gold was the only member of the group to yield a crystalline precipitate with the pyridine reagent. Unfortunately it is not always feasible to identify platinum, gold, or palladium in a mixture of precious metal ions. In most instances the presence of one of the metals which do not form a precipitate with the reagent will interfere with the identification of platinum, palladium, and gold (Table 2).

ACKNOWLEDGMENT

The work reported in this paper was made possible, in part, by a grant from The Research Corporation.

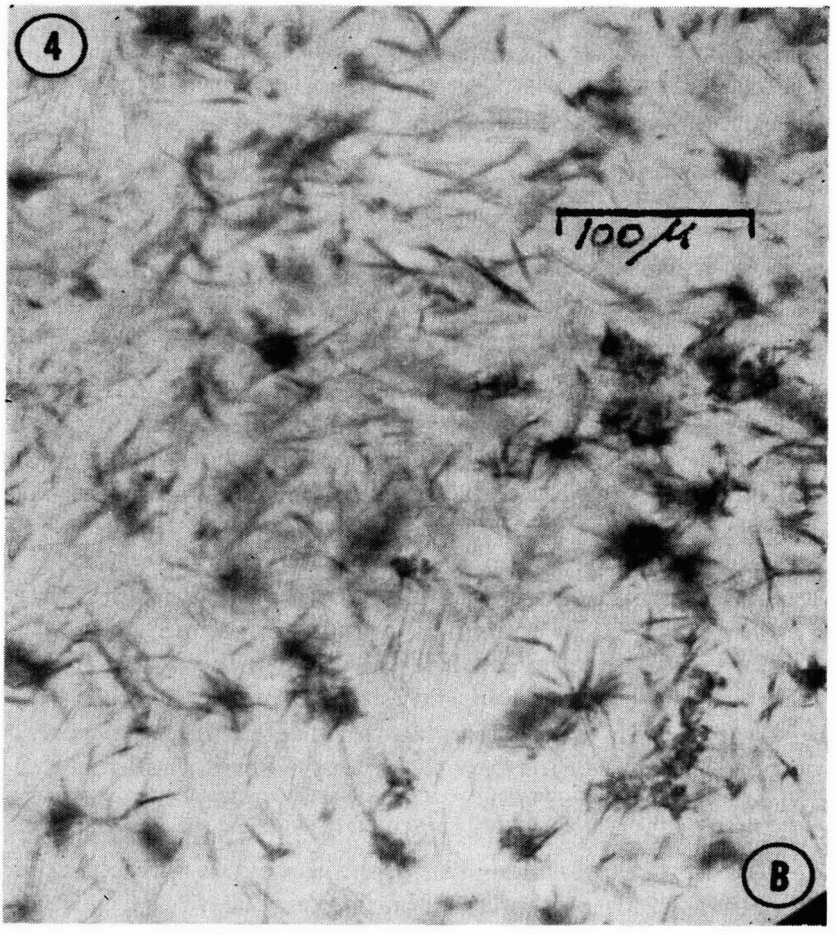

TABLE 2
SUMMARY OF INTERFERENCES

Metal to be identified	Other metal present [a]				
	Ru	Rh	Ir	Os	Cu
Pt	+	+	+	−	+
Pd	+	−	−	+	+
Au	+	+	+	+	+

[a] + = interference; − = identification in mixture feasible.

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MICROCHEMICAL JOURNAL **14,** 422–429 (1969)

On the Preparation of Alkali Bicarbonates Labeled with Carbon-14

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Received March 10, 1969

INTRODUCTION

Alkali hydrocarbonates labeled with carbon-14 are important tools in tracer studies applied in science, biology, and medicine. Different techniques have been described for their preparation based on the absorption of radioactive carbon dioxide, liberated by the acid decomposition of labeled barium carbonate, in a stoichiometric amount of an alkali hydroxide solution.

Methods of bubbling carbon dioxide in the alkaline solution, similar to the technique proposed by Calvin *et al.* (*4*), were tested. Thus, bubbling carbon dioxide in an equivalent amount of sodium hydroxide solution placed in an absorption scrubber resulted in the absorption of 59% of the total amount of carbon dioxide passing; of which 14% only were found present in the bicarbonate form while the rest were present in the carbonate form. Using two scrubbers in series, additional 31% of carbon dioxide were trapped in the second scrubber, mainly in the carbonate form. These results showed that the bicarbonate formation does not occur by a simple one-stage absorption process as was expected.

On the other hand, methods depending on the forced diffusion and trapping of radioactive carbon dioxide in contact with an alkali hydroxide solution described by many authors (*1, 4–7, 9, 10*) seemed to be more reliable. However, data presented in such procedures are generally of qualitative nature. The mechanism of the process, the precise formulation of products and also the efficiency of the techniques used are generally not specified. In addition, the working procedures and equipment used are too complicated.

In the present work, the absorption process of radioactive carbon dioxide in different solutions containing alkali hydroxides or carbonates is comprehensively studied with special emphasis on the kinetics and mechanisms of the processes leading to maximum yields of labeled alkali bicarbonates. A simple apparatus for the transformation of barium carbonate into sodium bicarbonate is described. A simple technique is also described to separate solid sodium bicarbonate from solution in order to overcome the activity loss during evaporation of bicarbonate solutions.

EXPERIMENTAL METHODS

AnalaR grade B.D.H. laboratory chemicals were used. The carbonate-free sodium hydroxide solution was prepared according to the method described by Vogel (*11*). Sodium carbonate was always heated up to 250°C before use.

The apparatus used for the bicarbonate preparation is schematically shown in Fig. 1. It consists of a carbonate destruction flask 500-ml capacity and a relatively smaller carbon dioxide absorption flask both connected with a tube provided with a facility for acid addition and a side arm attachment to a vacuum water pump.

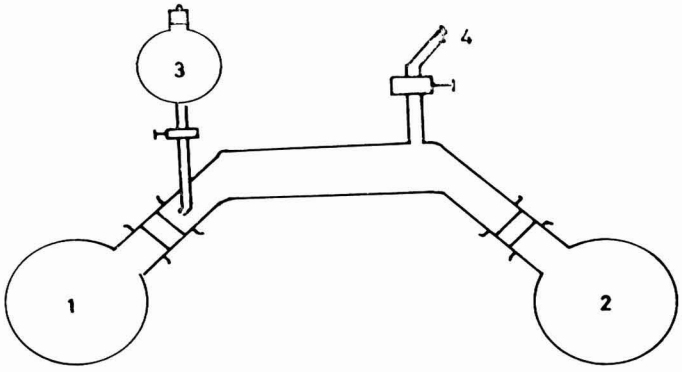

FIG. 1. Diffusion absorption apparatus for the preparation of alkali bicarbonates labeled with carbon-14: (1) carbonate destruction flask; (2) carbon dioxide absorption flask; (3) Acid delivery funnel; and (4) vacuum stopcock.

Working Procedure

An amount of barium carbonate (100–500mg) to be transformed was placed in the carbonate destruction flask and a stoichiometric amount of sodium hydroxide or sodium carbonate in solution was placed in the absorption flask. The apparatus was then evacuated, flushed with argon, and evacuated agin. Concentrated sulfuric acid was then added from the acid delivery funnel and after the reaction flask was heated gently, the apparatus was left, unattended, for different periods of time ranging from 2 to 48 hours. At the end of the exposure period, the absorption solution was removed and analyzed.

Potentiometric acid base titrations were always carried out to follow the formation of different labeled products with time. A radiometer type P03 pH meter was adjusted by a standard buffer solution with pH 9.3 before use.

Isolation of Solid Sodium Bicarbonate

It was reported that evaporation of sodium bicarbonate solutions is usually accompanied with appreciable loss in radioactivity attributed to the occurrence of an exchange reaction with atmospheric carbon dioxide (*4, 8*). Analysis of the solid product obtained in our laboratory by gentle evaporation proved the presence of varying amounts of carbonates in the bicarbonate residue obtained. This showed that the radioactivity loss is more probably due to the occurrence of the following reaction:

$$2NaHCO_3 \rightarrow Na_2CO_3 + CO_2 + H_2O. \quad (1)$$

To overcome this it was found better to separate the solid bicarbonate from solution on cold by the addition of an excess amount of acetone to a given amount of the bicarbonate solution in the ratio 12:1. In this way almost quantitative (96–100%) separation of the bicarbonate occurred. The solid was then filtered by a vacuum filteration assembly and dried by gentle air suction. Drying the solid by gentle air blowing, using a fan at room temperature, resulted in a 10% transformation into the carbonate; whereas, when drying was carried out, using a heating lamp, at 65–70°C it was found that the transformation extent increased to 76%.

Radioactivity Measurements

Thin solid samples of sodium bicarbonate were counted using a low voltage halogen quenched Phillips GM tube type no. 18506 having a window thickness of 2.5–3.5 mg/cm^2 and a background of 20 cpm. Samples were prepared by filtering a sodium bicarbonate suspension in acetone through the glass filter assembly usually used for the preparation of uniform thin solid samples on filter paper discs (*2*). All counted samples were placed exactly in the same position with respect to the counter window. The radioactivity of samples was corrected for self-absorption and also for any radioactivity loss due to the partial transformation into the carbonate.

Radioactivity of solid sodium bicarbonate samples was also determined using the flow monitoring counter erected in our laboratory for measuring the radioactivity of carbon dioxide-^{14}C liberated by the acid destruction of labeled carbonate samples using the technique that was previously reported (*3*).

RESULTS AND DISCUSSIONS

For the preparation of labeled sodium bicarbonate, radioactive carbon dioxide liberated by the acid destruction of barium carbonate was absorbed in either sodium hydroxide or sodium carbonate solutions.

Using 1 mole of sodium hydroxide/mole of carbon dioxide liberated and following the composition of the absorption solution with time by potentiometric titrations it was possible to find out the mechanism of the absorption process. The results obtained are shown in Fig. 2. It could be observed that in about 5 hours, 48% of the total amount of carbon dioxide present in the system reacted practically with all the hydroxide according to the following classical reactions leading mainly to the formation of the carbonate:

$$CO_2 + NaOH \rightarrow NaHCO_3, \tag{2}$$

$$NaHCO_3 + NaOH \rightarrow Na_2CO_3. \tag{3}$$

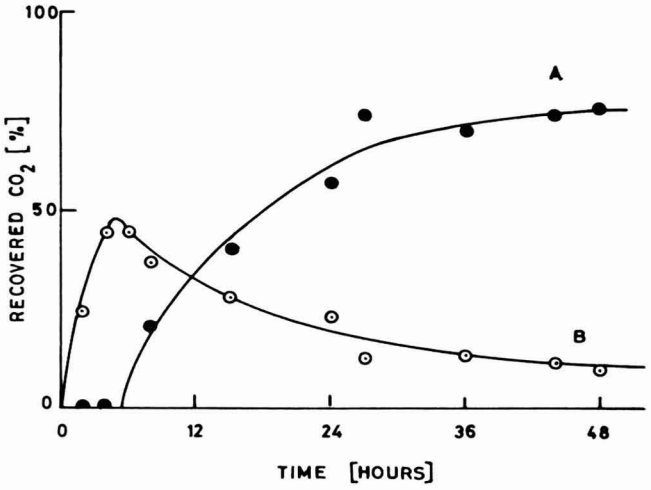

FIG. 2. Time dependence of the carbon dioxide recovery values in the hydroxide absorption solution: (A) in the bicarbonate form; and (B) in the carbonate form.

The absence of the bicarbonate at this stage—manifested by the fact that the titration curves of the absorption solution consisted of three inflections, the first of which was the result of the neutralization of excess sodium hydroxide present whereas the other two were the characteristic inflections of the carbonate neutralization—clearly shows that reaction 3 is an instantaneous reaction. This was further confirmed by titration of prepared mixtures containing increasing amounts of the hydroxide in a given amount of the bicarbonate. Two step titration curves were obtained similar to those obtained for the titration of carbonate–bicarbonate mixtures until the amount of the hydroxide was greater than the stoichiometric amount whereby a three-step titration curve was obtained characteristic for the hydroxide–carbonate mixtures.

TABLE 1

RESULTS OF EXPERIMENTS ON THE PREPARATION OF SODIUM BICARBONATE LABELED WITH CARBON-14

Exp. no.	Barium carbonate-^{14}C used		Sodium bicarbonate-^{14}C obtained		Recovered CO_2 (%)	Radioactivity recovered (%)	Exp. duration (hours)	Carbon dioxide absorber
	Amount (mg)	Sp. act. (mμCi/mg)	Amount (mg)	Sp. act. (mμCi/mg)				
				Series A				
1	187	14.59	67.2	36.0	84.5	88.7	48	Sodium
2	186	5.54	61.9	15.5	78.2	93.1	48	hydroxide
3	187	8.88	66.6	23.1	83.8	92.6	48	
4	150	8.33	48.5	25.1	75.9	97.4	24	
5	150	8.33	47.0	24.5	73.6	92.2	24	
				Series B				
6	140	14.64	85.9	22.6	72.1	94.7	24	Sodium
7	140	5.50	85.1	8.1	71.4	89.5	24	carbonate
8	90	11.55	57.2	15.2	74.6	83.6	24	
9	150	8.33	98.1	11.6	76.7	91.1	24	
10	150	8.33	96.2	11.5	75.3	88.5	24	

According to data shown in Fig. 2, the formation of stable bicarbonate occurs only after the completion of reaction 3 according to the following scheme:

$$Na_2CO_3 + CO_2 + H_2O \rightarrow 2\ NaHCO_3. \quad (4)$$

This reaction occurs with a relatively slow rate. Thus, about 43 hours were necessary in order that an additional 38% of carbon dioxide could interact with the fomred carbonate to yield hydrocarbonate containing 76% of radioactive carbon dioxide while 10% of radioactive carbon dioxide remained in the carbonate form. This minor carbonate amount could be neutralized to increase the overall bicarbonate yield to 86%. On the basis of these results it is possible to explain the failure of the flow system of absorbers in preparing the required bicarbonate. The short time of contact of carbon dioxide with the hydroxide solution in the scrubbers used leads mainly to the carbonate formation by reactions 2 and 3 while reaction 4 leading to the bicarbonate formation occurs only to a very limited extent.

Since the bicarbonate preparation went through the intermediate carbonate formation, pure sodium carbonate solutions were used for the absorption of carbon dioxide. The results shown graphically in Fig. 3 represent the amount of carbon dioxide absorbed in a given amount of sodium carbonate, taken in a mole per mole ratio, during increasing time intervals up to 15 days. It is obvious that after 24 hours about 75% of the carbon dioxide present were absorbed in the carbonate solution. In-

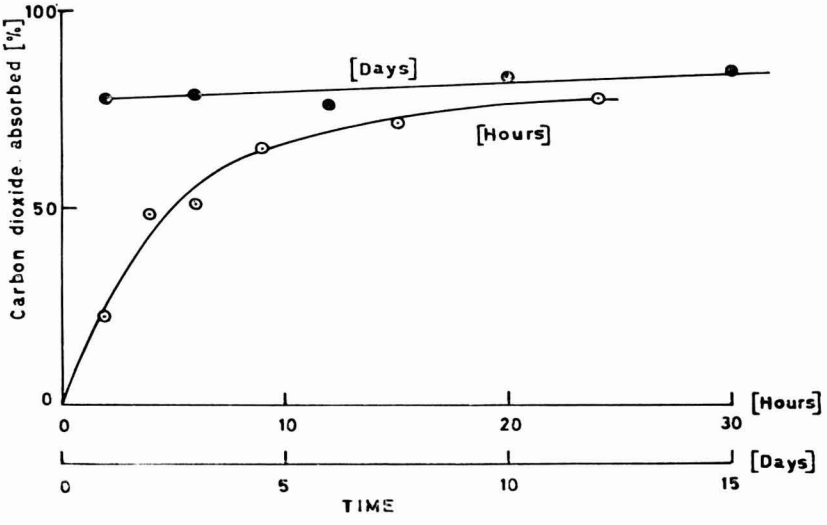

FIG. 3. Time dependence of the carbon dioxide recovery values in the carbonate absorption solution.

creasing the absorption time was not advantageous since the bicarbonate formation, after 24 hours, continues with a very slow rate of 0.5%/day. Again, increasing the carbonate concentration was not effective in markedly increasing the bicarbonate yield. Thus, using 1.3 mole of the carbonate/mole of carbon dioxide resulted in only a 4% increase in the bicarbonate yield.

Table 1 shows tne results of experiments on the transformation of barium carbonate into sodium bicarbonate labeled with carbon-14. In experiments of series A, sodium hydroxide solutions were used, whereas, experiments of series B were carried out using sodium carbonate solution as absorber for radioactive carbon dioxide. It could be observed that in all experiments the values of recovered radioactivity were always greater than the values of carbon dioxide recovery. This could be taken as an indication of the occurrence of isotopic exchange reactions during the long time of contact of carbon dioxide with the absorption solutions.

From the results presented it is obvious that both sodium hydroxide or sodium carbonate could be used equally well as absorbing solutions in the preparation of bicarbonates labeled with carbon-14. In this respect, sodium carbonate could be more recommended due to the ease with which it could be obtained in a pure state.

SUMMARY

A simple technique for the preparation of alkali bicarbonates is described. Carbon dioxide, liberated by the acid decomposition of labeled barium carbonate, was absorbed in alkali hydroxide or carbonate solutions. Kinetics of the processes and also mechanisms leading to the formation of labeled bicarbonates are discussed in detail. A simple procedure is also described for isolation of solid bicarbonates from aqueous solutions on cold to overcome the radioactivity loss of bicarbonate solutions occurring on evaporation.

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MICROCHEMICAL JOURNAL 14, 430–431 (1969)

Use of Microcosmic Salt as a New Titrant for the Microdetermination of Sulfanilic Acid

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Received March 18, 1969

Sulfanilic acid has been determined potentiometrically (*1*), polarographically (*2*) or by iodometric titration (*3*). Recently Bellen (*4*) suggested a method for its determination by titration with sodium hydroxide in a mixture of urotropine. The present paper describes its determination by titration with a new titrant microcosmic salt.

EXPERIMENTAL METHODS

Reagents used. Sulfanilic acid (A. R. Italy), Microcosmic salt (E. Merck) and bromcresol purple (B.D.H.)

Procedure

To 5 ml of a solution of Sulfanilic acid, add some distilled water to raise its volume to about 20 ml, followed by 1 or 2 drops of a 0.1% solution of bromcresol purple indicator. The solution is yellow at this point. Then titrate it with a standard (*5*) microcosmic salt solution till the yellow color is completely discharged and the solution becomes a faint purple color.

RESULTS

The results are given in Table 1; sulfanilic acid was estimated over a concentration range of 0.00057–0.00347 mmoles. The results are concordant and precise.

TABLE 1

MICRODETERMINATION OF SULFANILIC ACID

Sample n.	Vol of sulfanilic acid taken 0.0024 *M* (ml)	Vol of 0.001 *M* microcosmic salt soln used (ml)	Sulfanilic acid (mg)		
			Found	Theoretical value	Error (mg)
1.	3.0	3.44	0.6138	0.6101	0.0037
2.	2.0	2.28	0.4067	0.4067	0.0000
3.	1.0	1.12	0.1998	0.2033	0.0035
4.	0.5	0.56	0.0999	0.1016	0.0017

SUMMARY

Sulfanilic acid was determined in micro quantities with a new titrant, i.e., microcosmic salt solution using bromcresol purple as indicator. Estimations were carried out in the range of 0.1016–0.61 mg with a maximum error of ±0.0037 mg.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 14, 432–436 (1969)

An Improved Absorption System for Carbon and Hydrogen Determination

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Received March 21, 1969

In order to utilize fully the advantages of the newer high speed combustion systems for the determination of carbon and hydrogen in organic materials it is necessary that the products of combustion, carbon dioxide and water, be separated and measured by a method faster than the conventional Pregl procedure. Other than the methods involving thermal conductivity, change in conductance, or other instrumental methods, very little work has been done on improving this phase of the determination. Because of the advantages of simplicity and specificity we would prefer to use a gravimetric finish up, at least for routine work.

Presuming that the balance room environment and weighing equipment is equal to the task, the problem resolves to a choice of absorption system. For many years we have found the use of the American Chemical Society standard absorption tube intolerable. The design incorporating very small capillaries and a baffle section does not lend itself to increased flow rates. Also, the quality of purchased tubes has been so poor that only a small fraction of the tubes have been usable. The absorption tubes as recommended by Schöniger [1] and supplied by P. Haack of Vienna [2] and at least one supplier [3] in the United States are a decided improvement; however, these tubes still require connection by means of silicone rubber connectors, and no means is provided to seal the tubes during the equilibration and weighing operation. In a previous paper the author (*2*) employed the Flaschenträger type absorption tube (*1*) in an Ingram type (*4*) combustion system. These tubes perform very well, they have no capillary constriction and can be sealed to prevent displacement of gases during the weighing process. Serious objections to the use of these tubes are: they are very expensive, the location of the side arms is critical, and they have a rather small absorbent capacity due to the weight of the glass parts of the tube. Of all the absorption tubes

[1] Schöniger, W., private communication, 1957.

[2] P. Haack, Garnisongasse 3, A-1090 Wien, Austria.

[3] The Arthur H. Thomas Company, Vine Street at Third, P.O. Box 779, Philadelphia, Pennsylvania 19105.

examined those supplied by the Coleman Instruments Corporation [4] for use with their carbon and hydrogen analyzer appeared to be the most desirable. They are simple in design, they have no critical glass parts, and are relatively low in weight. Because of the rubber stopper closure they lend themselves to mechanical connection to the combustion train.

Evaluation of these tubes proved disappointing, weighings could not be made with sufficient accuracy and reproducibility to meet the standards of current practice. It was suspected that the variability was due to the rather large opening in the rubber stopper, allowing gas displacement during the weighing process. Plugging these openings during equilibration and weighing effected some improvement, but still not enough to be acceptable. Also, it was felt that the use of stoppers would add additional sources of error in routine use. Additional experiments indicated that the difficulty appeared only when the tubes were used on the combustion train to absorb carbon dioxide and water, and not when they were merely weighed serially, allowing them to stand by the balance between weighings. In a paper by Gustin (*3*), it was pointed out that most of the variability in the weighing of absorption tubes was due to the change in temperature of the tube arising from the heat of absorption of the gaseous products on the absorbent; the absorption of water on magnesium perchlorate and the absorption of carbon dioxide on sodium hydroxide or lithium hydroxide are strongly exothermic processes. Cooling the tubes with an electric fan during the absorption as advised by Gustin (*3*) effected remarkable improvement.

Because of the possibility of creating drafts it was felt that the use of an electric fan directed on the apparatus was not good practice in the laboratory. A rugged and very simple device that allowed the use of the Coleman absorption tubes and at the same time provided very efficient and localized cooling of the tubes while attached to the combustion train was fabricated. The cooling air, in our case, was provided by connection to the laboratory compressed air supply pipe; however, if clean dry air is not available a small instrument type electric blower could just as easily be attached to the air plenum of the device.

The absorption tube holding device is shown in Figs. 1 and 2. None of the dimensions are critical, except that the spring loaded connectors should be placed far enough apart to insure easy removal of the absorption tube, and yet close enough together so that the tube, when inserted, is held under full pressure of the compression spring. The nonmovable connecting tubes are ⅛-inch o.d. standard wall stainless steel tubing.

[4] Coleman Instruments Corporation, 42 Madison Street, Maywood, Illinois 60154.

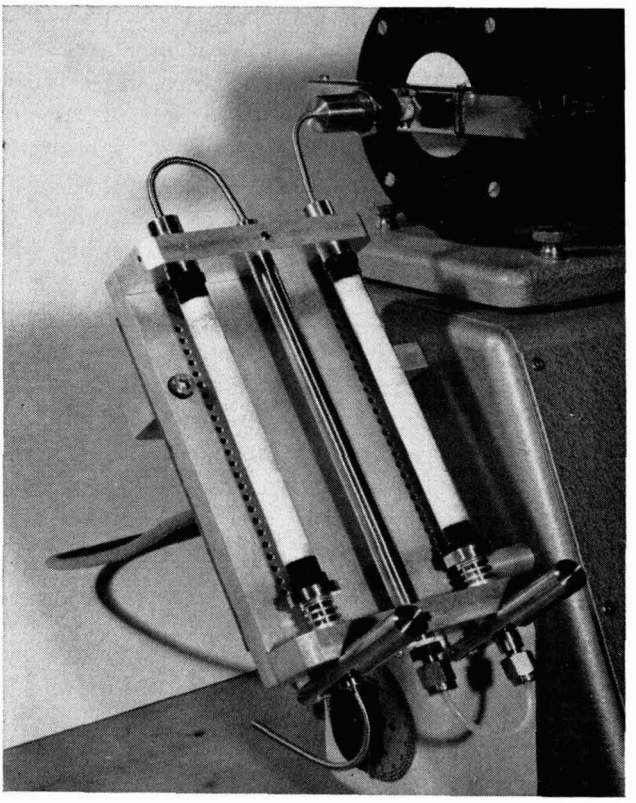

FIG. 1.

The flexible connecting tubing is ⅛-inch o.d. Tetran [5] tubing held in place by modified Swagelok connectors. The holder is attached to the combustion train by means of a firm bracket as shown in Fig. 2. This can be made to suit individual applications.

As sufficient heat is conducted from the combustion tube by the all metal connector [6] and tubing, no provision is necessary for extra heating to prevent condensation of water at the exit end of the combustion tube. An added advantage of using the metal combustion tube connector is that standard Vycor tubing may be used for combustion tubes instead of the special combustion tube with capillary end.

Incorporated in the absorption tube holder is a stainless steel tube for containing the manganese dioxide required for the removal of certain nitrogen oxides.

[5] Tetran is the registered trademark of the Pennwalt Corporation for polytetrafluoroethylene.

[6] A & N Corporation, 45 New Street, Worchester, Massachusetts 01006.

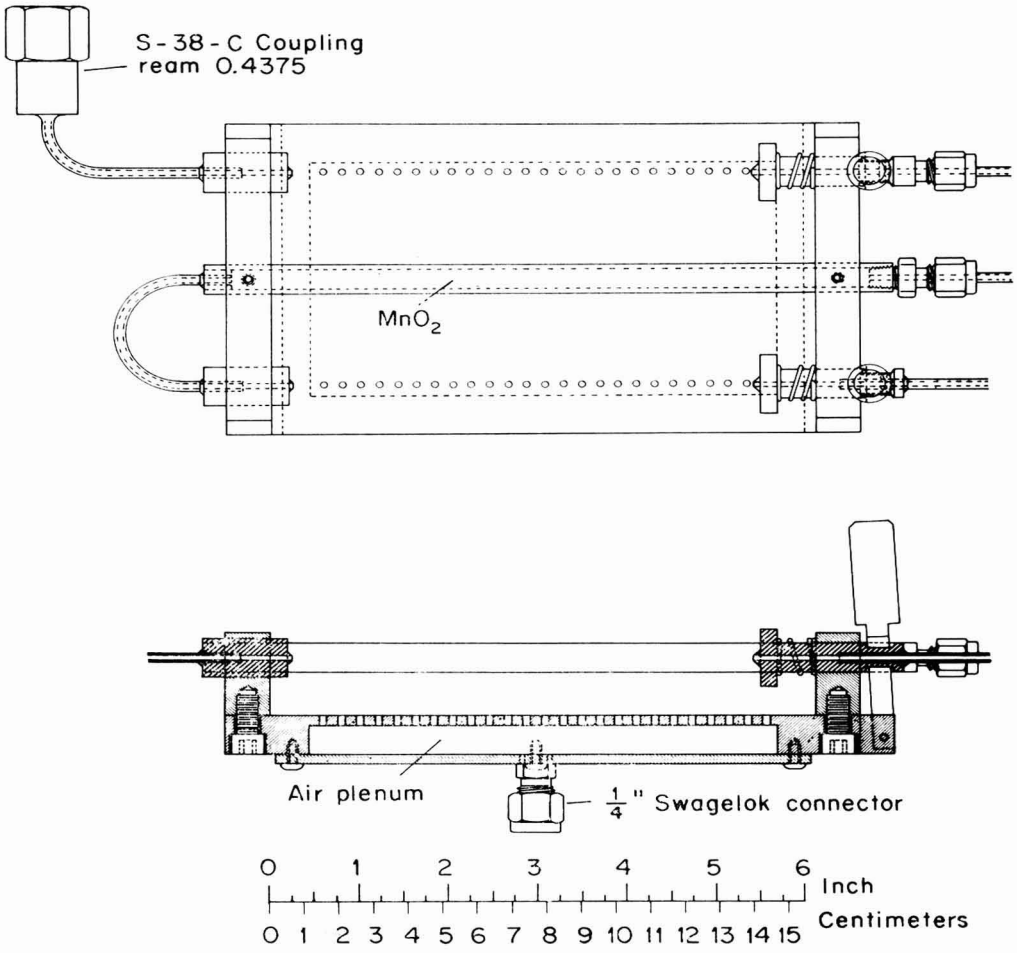

FIG. 2.

For use, the water absorbing tube is prepared by placing a rubber closure, Coleman no. 7229-V, in one end of a clean dry absorption tube, Coleman no. 33-330. A small section of quartz wool is placed next to the stopper followed by a 2-cm section of Anhydrone (magnesium perchlorate), a 0.5-cm section of quartz wool, and the remainder of the tube is filled with additional Anhydrone allowing sufficient room for another 0.5-cm section of quartz wool and the rubber closure (Fig. 3). We have found it expedient to sieve the Anhydrone in a dry box before use, taking that portion passing through No. 10 and being retained on No. 20 U.S. Standard Mesh screens.

The carbon dioxide tube is prepared in a similar manner. A rubber

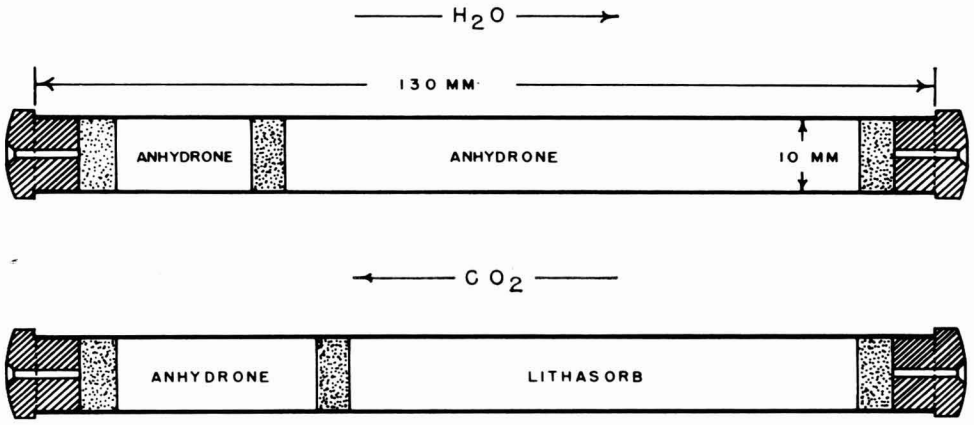

FIG. 3.

closure is placed in the tube end followed by a 0.5-cm section of quartz wool, followed by a 7-cm section of Lithasorb, a 0.5-cm section of quartz wool, a 3-cm section of Anhydrone, a 0.5-cm section of quartz wool, and finally the rubber closure. The above quantities of absorbent are sufficient for 2–3 days of normal use.

In use the carbon dioxide absorbing tube is weighed 2 minutes after removal from the train, and the water absorption tube in 4 minutes. Stability of the tubes during weighing has been much better than was experienced when using the normal Pregl technique.

SUMMARY

The determination of carbon and hydrogen was improved by cooling the absorption tubes to eliminate weighing errors caused by temperature fluctuation. Absorption tubes were simplified to allow efficient rapid combustion.

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TLC-Fluorimetric Analysis for Atmospheric Scopoletin

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Although polynuclear compounds, such as arenes (*6, 8*), aza arenes (*13, 14, 16*), imino arenes (*1*), and ring-carbonyl (*12, 17, 18*) compounds have been found and determined in particulate samples from the atmosphere and from air pollution sources, very few individual members of the more polar hydroxy compounds shown to be present in the atmosphere (with the help of nonspecific general colorimetric and fluorimetric methods) (*7, 9, 15*) have been unequivocally identified. The eight identified compounds of this class are polynuclear aromatic and heterocyclic phenols (*10*). Because of the interest in airborne particles of biological origin and their physiological effects on human beings, methods of characterization and assay for these types of compounds need to be developed.

There are two main types of pollution in which the hydroxylated organic compounds could be present in large amounts. The first, called "blue haze" is found in natural wilderness areas usually in the summer and fall seasons. It is derived from plants and their falling leaves. The second is derived from cleared wilderness and from farm or urban areas where a new type of ecology has taken hold. This latter type is one of the main causes of hayfever, the culprits being pollen from trees, grasses, and ragweed; spores; protozoan cysts; bacteria; virus; etc.

Since these particles could be assayed indirectly through determination of a component, the presence of these hydrophilic constituents in these particles and in air needs to be ascertained. One such hydrophilic constituent of biological origin is scopoletin. This hydroxycoumarin derivative has been found in tobacco (*3*) and tobacco smoke (*19*). In the present paper the separation, characterization, and assay of scopoletin found in air, house dust, and coffee roast effluents are discussed.

EXPERIMENTAL PROCEDURE [1]

Equipment. The fluorescent colors obtained on a chromatogram or a pherogram were examined in a Chromato-Vue Cabinet (Kensington Scientific Corp., Berkeley 10, California) under a 3660 Å light source.

Absorption spectral characterizations were performed with a Cary recording spectrometer, model 11, equipped with 3-ml cells of 1-cm path length. An Aminco-Bowman spectrophotofluorimeter was used with the following settings: Sensitivity 50, slit arrangement No. 2, and phototube RCA type 1P21. An Aminco thin-film scanner equipped with a prototype reflectance attachment was used in scanning and in obtaining the fluorescence excitation and emission spectra of the TLC or PE spots.

Chemicals. Solvents, such as acetic acid, acetone, ethyl acetate, formic acid, benzene, and methaonl were obtained in the purest form from commercial sources. Usually methanol had to be distilled before fluorimetric use. Reagents, such as 40% aqueous tetraethylammonium hydroxide (K and K Laboratories) and sulfuric acid, were obtained in the purest grade. Scopoletin was obtained from Mann Research Laboratories and used without further purification.

Separation media. Silica gel F 254 plates (Brinkman) were used in thin-layer chromatography and Whatman No. 1 paper (Gelman Instrument Company) was used in the paper electrophoretic studies.

Developers. For thin-layer chromatography: toluene–ethyl acetate–formic acid, 5:4:1 (v/v/v). For paper electrophoresis: 0.1 *N* aqueous sodium hydroxide solution.

Extraction. Particulates obtained from the urban atmosphere or from air pollution source effluents were extracted in a micro-Soxhlet extractor for 6 hours with benzene or methanol. For the urban atmospheric samples, approximately 20–50 mg of airborne particulates representing $\sim$200–500 m^3 of air was the minimum amount necessary for an analysis. The extracts were evaporated to dryness at room temperature under vacuum.

Thin-Layer Chromatographic Procedure

The organic residue from airborne or other particulates was dissolved in chloroform (20–50 mg/ml), and 100 μl was spotted on the silica gel plate with the help of a drier. Special care was taken to prevent spreading of the samples. For analysis of organic material from the minimum amount of particulates or air, this material was obtained as a residue in a centrifuge tube. It was then dissolved in five to six 50-μl portions of

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

chloroform and transferred quantitatively each time to the silica gel plate in one small spot. Separate spots containing 50 and 175 ng of pure scopoletin were also placed on the plate.

The plate was developed in toluene–ethyl acetate–formic acid, 5:4:1 (v/v/v). Time of development was approximately 60 minutes. The wet plate was sprayed with a solution of toluene–40 % aqueous tetraethylammonium hydroxide, 49:1 (v/v). The scopoletin and adjacent unknown spots were outlined quickly under ultraviolet light. They were then scraped from the plate into 50-ml beakers and immediately covered with 5–10 ml of hot methanol–40% aqueous tetraethylammonium hydroxide, 49:1 (v/v). Following trituration and gentle warming on a hot plate the mixture was filtered through a sintered glass filter (M) by suction. A Fisher Filtrator is convenient here. The remainder of the silica gel in the beaker was rinsed into the filter with three 4-ml portions of the hot alkaline methanol. The filtrates were collected in a 125-ml suction flask so that the final volume of eluent was not less than 25 ml. Smaller amounts tend to give lower recoveries. The samples were evaporated to about 5 ml with suction in a water bath at 50–55°C and transferred quantitatively to a 10-ml glass-stoppered volumetric flask with methanol. The mixture was made to the mark with methanol and then read on the fluorimeter at F = 390/458. A small spot of silica gel approximately equivalent in area to each standard and unknown spot and with the same R_f value was removed and treated in the same fashion. For greater sensitivity the standards, unknowns, and blanks can be diluted to 1 ml.

A graph should be prepared relating the total nanograms of standards versus the meter multiplier times transmittance ($MM \cdot T$) values obtained for a range of concentrations, Fig. 1.

RESULTS

Stability of scopoletin. In neutral or alkaline methanol scopoletin is stable at room temperature in the dark for at least 3 weeks when its concentrations are greater that 10^{-6} *M*. At lower concentrations some standard solutions were stable, and others showed some decrease in fluorescence.

Scopoletin is sensitive to light. Four methanolic solutions of the compound were exposed to room light for 4 hours. The fluorescence intensity was decreased by 7%.

Scopoletin decomposes on the silica gel plate. Standard samples left on the plate under room light for 1.5 hours gave 52% recovery. Scopoletin (0.19 μg) is decomposed fairly rapidly when separated on a silica gel plate and then exposed to the ultraviolet light of the fluorimeter

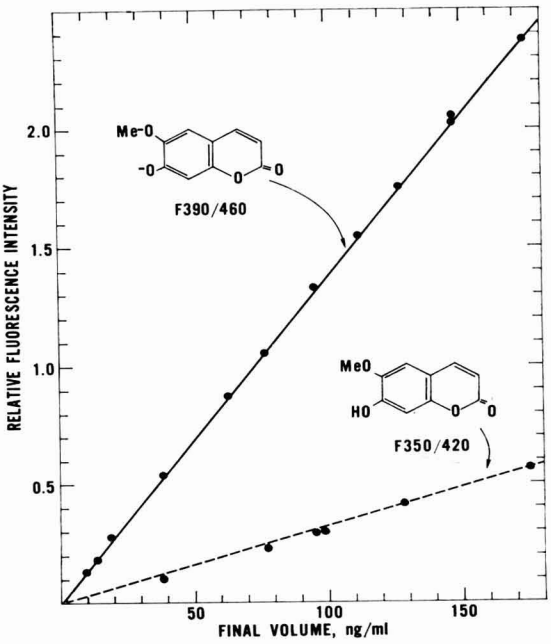

FIG. 1. Fluorescence intensity versus concentration of scopoletin in methanol (— — —); and in methanol containing 1% of 40% aqueous tetraethylammonium hydroxide (———).

(monochromator set at 330 mμ), Fig. 2. Under these conditions the fluorescence excitation and emission spectra also change gradually.

Characterization of scopoletin. The identity of scopoletin obtained from air samples and from air pollution source effluents is based on the similarity of the R_f values obtained by silica gel thin-layer chromatography in the toluene–ethyl acetate–formic acid solutions and the mobility values obtained by paper electrophoresis in 0.1 *N* NaOH of the unknowns and pure scopoletin. Even when thin-layer chromatography was followed by paper electrophoresis for separation of the pollution samples, the unknown spot that ran with scopoletin gave similar fluorescence spectra. The types of separation obtained by TLC and PE are shown in Fig. 3.

The fluorescence spectra of the spot separated by thin-layer chromatography and the standard scopoletin spot with the same R_f value are closely similar in methanol or alkaline methanol, Fig. 4. In addition, after two thin-layer chromatographic separations pure scopoletin and the air pollutant spot adjacent to it gave an excitation band at 365 mμ and an emission band at 446 mμ in concentrated sulfuric acid.

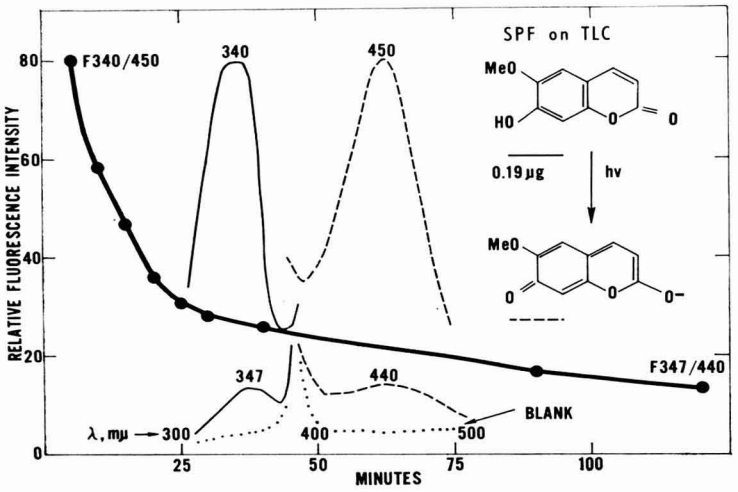

FIG. 2. Photodecomposition of scopoletin (0.19 μg) on silica gel F254 (after separation) at λ330 mμ: direct fluorimetric spectra of fresh scopoletin spot (top curves) and after 120 minutes (bottom curves); blank for all spectra (····).

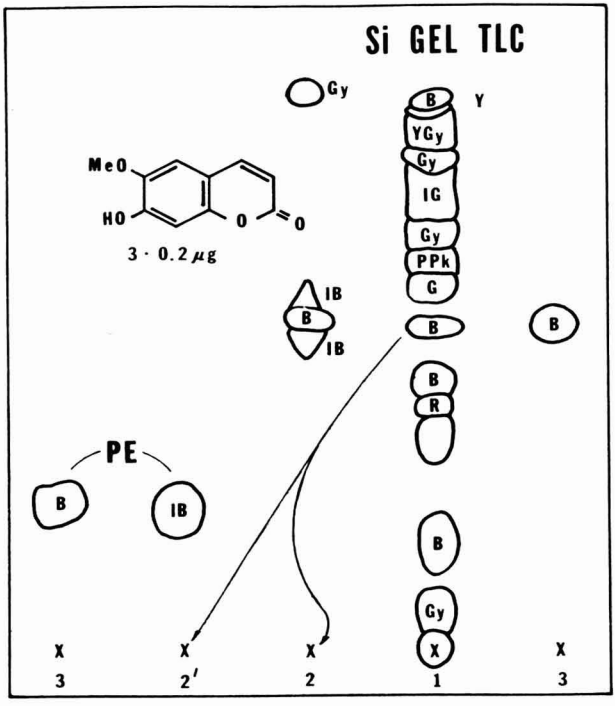

FIG. 3. Thin-layer chromatographic separation, 1, followed by a second thin-layer chromatographic separation of the spot opposite scopoletin, 2. Thin-layer chromatographic separation of pure scopoletin, 3 (on right). Paper electrophoretic separation of pure scopoletin, 3 (on left) and TLC spot from 1, 2.

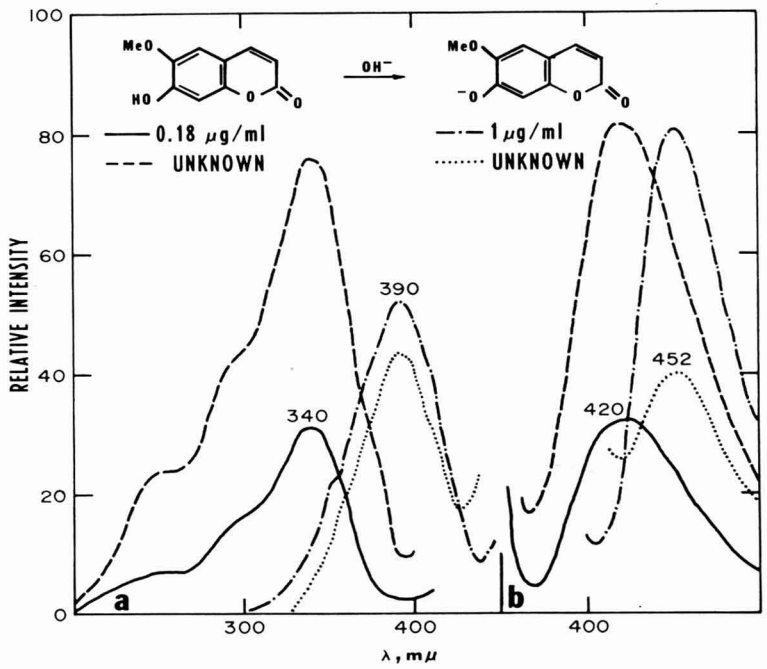

FIG. 4. Fluorescence spectra obtained after thin-layer chromatographic separation: scopoletin in methanol at F = 340/420 and meter multiplier reading, MM = 0.003 (———) and in alkaline methanol at F = 390/452 and MM = 0.03 (—·—). TLC spot (opposite scopoletin) from urban organic air particulate sample, in methanol at F = 340/420 and MM = 0.003 (— — —) and in alkaline methanol at F = 390/452 and MM = 0.01 (·····).

It is necessary to corroborate all fluorescence excitation spectra with the analogous absorption spectrum of the compound under investigation. There are three main reasons for this necessary confirmation. The fluorescence excitation spectrum obtained may be that of a highly fluorescent impurity. If too high a concentration of the fluorescent compound is present, spurious peaks may be obtained because of self-absorption. Since the supposedly neutral solvent can leach out alkali from the glass of the container, the fluorescence spectrum can be that of the anion.

The absorption maxima of scopoletin in methanol and alkaline methanol occurred at 345 and 390 mμ, respectively, while the fluorescence excitation spectra showed bands at 340 and 390 mμ, respectively. Values of 346 and 395 mμ have been reported as the absorption maxima of scopoletin in methanol (*5*) and alkaline methanol (*2*), respectively. Scopoletin absorbs at 418 mμ in alkaline dimethylformamide. This red shift with increased solvent basicity is what one would expect (*11*).

To obtain the fluorescence spectrum of neutral scopoletin in methanol

or dimethylformamide, these solutions must be made weakly acidic. This is done by adding a drop of concentrated hydrochloric acid to a few ml of the solution.

For some mixtures the fluorescence spectra of separated scopoletin could be obtained directly from the plate. For example, following alumina thin-layer chromatography the fluorescence spectra was that of the anion F = 385/445. On paper or on glass-fiber paper impregnated with silica gel the fluorescence spectra was that of the neutral compound, $F = 348/422$. The fluorescence spectra of the cation on silica gel or alumina could be obtained by spraying with concentrated sulfuric acid. The fluorescence spectra of the anion could be obtained by spraying any of these media with acetone contaiing 1% of 40% aqueous tetraethylammonium hydroxide; the fluorescence spectra of the neutral compound could be obtained by spraying with trifluoroacetic acid fumes. Excited state ionization can also take place on the plate as shown in Fig. 2, where the excitation spectrum is that of the neutral compound and the emission spectrum is that of the anion.

It is necessary to emphasize that the quantum yield in fluorescence of neutral scopoletin depends strongly on the intermolecular hydrogen bonding properties of the solvent with scopoletin. Scopoletin can form two types of intermolecular hydrogen bonds. One involves the attachment through its hydroxyl H atom to a pair of free electrons on the hetero atom of a solvent; the other involves the attachment of the hydroxyl H of the solvent to the free electrons of the oxygen atoms.

The effect of the latter type of bond is shown by the fact that the fluorescence intensity of scopoletin in cyclohexane–*o*-dichlorobenzene–propionaldehyde, 49:1:50 (v/v/v) is more than 30 times greater than in cyclohexane–*o*-dichlorobenzene, 49:1 (v/v), where a hydrogen bond cannot take place (*4*).

The effect of the other type of intermolecular hydrogen bond is similar in that dioxane–water, 1:1, solutions of scopoletin have ~20 times the fluorescence intensity of dioxane solutions. The intermolecular hydrogen bond of scopoletin with water has a greater fluorescence-intensifying effect than does the hydrogen bond of scopoletin with propionaldehyde since the dioxane–proprionaldehyde, 1:1, solution of scopoletin has only twice the fluorescence intensity of the dioxane solution. Scopoletin is nonfluorescent in neutral and alkaline carbon disulfide and nitromethane solutions.

Variables in Assay Procedure

Although the investigator works as quickly as possible to remove the scopoletin, the samples removed last are frequently slightly lower in rela-

tive fluorescence intensity than the first eluted spots. Therefore, a standard is spotted at each end of the plate. The amount of scopoletin in the air pollution samples is then calculated on the basis of the recovery of the standards.

Following the elution step the extract is filtered through a sintered glass filter. After several such filtrations the silica gel tends to stop up the sintered glass. Soaking in about 25% sodium hydroxide solution overnight will remove the silica gel particles.

Scopoletin separated on the plates was recovered quantitatively. Using the elution procedure scopoletin added to airborne particulates was recovered in 97% amounts; the range was from 94 to 100%.

As Fig. 1 indicates, a linear relation is obtained between the concentration of scopoletin and the fluorescence intensity. The analysis for the scopoletin anion is much more sensitive than for the neutral compound. Attempts to analyze for the scopoletin as cation showed that in our hands this method was less sensitive and much more erratic.

Scopoletin was also analyzed by fluorimetric scanning of the silica gel thin-layer chromatogram after separation. Fig. 5 shows a fluorimetric scan of a series of scopoletin standards and a spot of the same R_f value obtained following separation of a methanolic extract of an urban airborne particulate sample. The advantage of this type of assay is its speed and simplicity; however, sharp, definite spots were not always obtained in a separation. The lack of sharp spots made no difference in the elu-

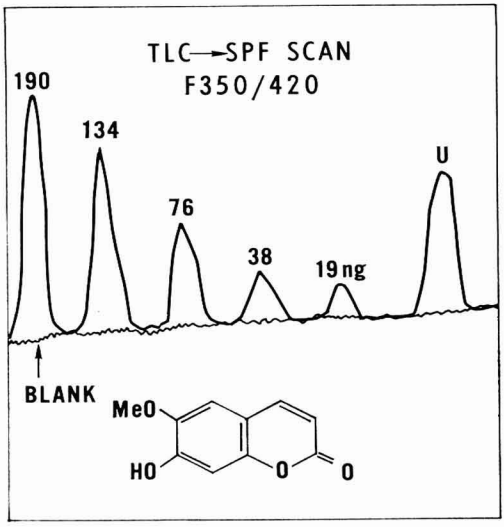

FIG. 5. Fluorimetric scanning at F = 350/420 after TLC separation of scopoletin standard spots and the unknown spot (opposite scopoletin) obtained from an urban organic air particulate sample.

tion method: reasonable assays were obtained whether the spots were sharp or not.

Application

A variety of air pollution particulates were analyzed by the elution method, Table I. Where enough sample was available, three to four determinations were made. The highest values were obtained in a sample of coffee roast effluent obtained from a commercial plant in New Orleans; the lowest value was from a sample of house dust obtained from Jackson, Mississippi. These data indicate that the method can be used for various types of samples.

Probably the most striking factor in this study is the presence of a large number of "oxygenated" fluorescent unknowns in the various samples. The study of these various compounds from the allergy viewpoint will be continued.

TABLE I

FLUORIMETRIC ASSAY FOR SCOPOLETIN

Sample	Extraction Solvent [a]	Concentration (μg/g of dust)
Manhattan airborne particulates	Methanol	6.4 ± 0.2 [b]
	Benzene	7.2 ± 0.3 [c]
Jackson, Mississippi house dust	Methanol	0.7
Coffee roast effluent	Methanol	27.2

Urban air samples

City		(ng/m^3 of air)
Altoona	Benzene	1.3
E. Chicago	Benzene	0.2
Birmingham	Benzene	0.8
New York	Benzene	0.8

[a] Six hours Soxhlet extraction.
[b] Average of four determinations.
[c] Average of three determinations.

SUMMARY

The evidence for the presence of scopoletin in airborne particulates, house dust, and coffee roast effluents consists of R_f values obtained with silica gel thin-layer chromatography and mobility values obtained with paper electrophoresis, as well as fluorescence spectra obtained from the chromatogram and from methanolic, alkaline methanolic, and sulfuric acid solutions. By a procedure involving TLC SPF, scopoletin has been assayed in these various samples. Recovery of scopoletin

from enriched airborne particulates was 97%. These various separations and fluorimetric examinations have shown that a large number of unknowns was also present in these samples.

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A Rapid Colorimetric Method of Estimation of Micro-Quantities of Diphenylamine

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INTRODUCTION

Diphenylamine has had a variety of applications both in industry and laboratory. A few important and large scale applications are: in pesticide formulations containing Lindane, as fungicide and scald inhibitor in fruits, in the manufacture of phenothiazine, as an intestinal disinfectant for cattle and poultry, and in dye manufacture.

Many methods are reported in the literature to detect diphenylamine and other aromatic amines as spot tests by polarography, phosphoroscopy, and chromatography. Bruce and co-workers (*1*) and Sawicki and co-workers *(2)* developed a spectrophotometric method of estimating diphenylamine and other aromatic amines using 2,4-dinitroaniline and 5-nitroisatin chloride as color developing agent, respectively.

Hahn and his associates (*3*) suggested a method of determining diphenylamine by Lautch reaction. Yavorski (*4*) reported color reaction between xanthydrol and diphenylamine but could not determine the chemical colorimetrically due to instability of the color.

Grodzinski (*5*) suggested a method to determine the diphenylamine using its blue color formation with potassium dichromate and sulfuric acid. According to their observations, the results are 5% lower than the standard methods.

Keirs and co-workers (*6*) have standardized phosphorometric method of analysis. As shown by their data, the error is about 10% at concentrations of 10^{-2} to 10^{-6} *M*.

Usmani (*7*) has claimed that diphenylamine can be estimated up to 0.6 mmoles by polorographic method. Many of the methods reported in the literature are tedious, time consuming, and require lot of manipulations. The aim of the present investigation was to develop a simple, rapid but sensitive method of estimating diphenylamine using a colorimeter.

REAGENTS

All the chemicals used in this study are of A. R. grade. Diphenylamine (A.R.) obtained from *May & Baker Ltd.*, England, was used. *p*-nitrobenzene-diazonium fluoborate, obtained from Eastman Organic

Chemicals, Rochester, New York was used. Distilled ethyl alcohol was used to prepare the standard solutions and to dilute the colored solution to a known volume.

EQUIPMENT

Bausch and Lomb (Spectronic-20) was used to read the intensity of the colored solution.

PROCEDURE

Diphenylamine (O./g) was dissolved in 100 ml of ethanol and 1 ml of this solution was further diluted to 100 ml. Volumes, corresponding to 10–60 μg were transferred to separate test tubes. To each test tube 1 ml of 0.1g of *p*-nitrobenzene-diazonium salt dissolved in acetone (A.R.) was added and shaken well. The reaction mixture was left for 20 minutes until the orange color reached the maximum intensity. The volume in each test tube was made up to 25 ml with ethanol. The orange color was read at 490 mμ, the region of maximum absorption.

DISCUSSION

As shown in Fig. 1, the method obeys Beer's law up to 4 μg/ml of the solution. The optical density was recorded at various intervals of time and the results showed that the color complex was stable at room temperature (28–30°C) for about 24 hours and was not affected by light. Many of the common solvents (acetonitrile, dichloroethane, ether, benzene, butanol, etc.) had no effect on the color complex during dilution and therefore any solvent of choice can conveniently be used for dilution. Each estimation can be performed within 25 minutes. The orange

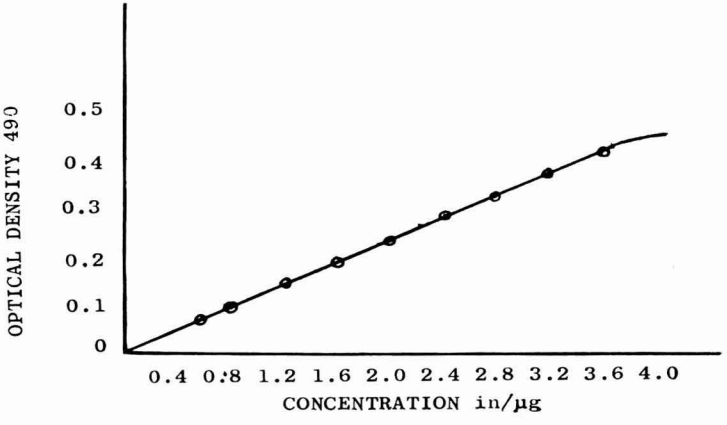

FIG. 1.

color of the solution was changed into violet-red (Table 2) on the addition of concentrated nitric acid, hydrochloric acid, and sulfuric acid, while acetic acid, formic acid, and propionic acid did not alter the orange color. This may be due to the break down of the colored complex by strong mineral acids while weak acids had no action. When the orange solution was treated with ammonium hydroxide, potassium hydroxide, sodium carbonate, the original color gradually turned brick red (Table 2). The appearance of red color is due to the alkali reacting with the unreacted reagent (*p*-nitrobenzene diazonium salt) in the reaction mixture and thereby the brick-red color is produced. Since the orange color is developed with the reagent by primary, secondary, and tertiary amines, their presence interferes in the estimation.

With the above method, diphenylamine was estimated in prepared samples and verified. As shown by the results (Table 1) the chemical can be estimated with very good accuracy, the error being ±0.3–2.3%.

SUMMARY

A rapid and convenient method for determining pure diphenylamine in microquantities has been developed. The orange colored solution formed between diphenylamine and *p*-nitrobenzene-diazonium salt is stable for sufficiently long time. At 490 mμ, the region of maximum absorption, Beer's law is obeyed up to 4 μg/ml of the solution. With the above method diphenylamine can be estimated in pure form with an accuracy of ±0.5-2%.

TABLE 1

ESTIMATION OF DIPHENYLAMINE IN ETHANOL SOLUTIONS

Sample:	I	II	III	IV	V
Diphenylamine	0.1844	0.1640	0.0264	0.0136	0.0094
Diphenylamine (g) (estimated by present method)	0.1836	0.1632	0.0268	0.0139	0.0096
Error	0.0008	0.0008	0.0004	0.0003	0.0002
Error %	−0.43	−0.5	+1.5	+2.2	−2.3

TABLE 2

EFFECT OF ACID–ALKALI ON THE ORANGE COLORED COMPLEX

Solvents	Color changes
10 *N* Potassium hydroxide	Orange color turned brick red
Liquor ammonia	Orange color turned into insoluble brick red precipitate
Hydrochloric acid	Violet-red
Nitric acid	Violet-red
Acetic acid	Orange color not affected

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Combined Micro Dry-Column Chromatography and Mass Spectrometry

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INTRODUCTION

Dry-column chromatography (DCC) is a preparative scale method for rapidly effecting separations by means of capillary solvent flow through a column of dry adsorbent (*9*). Both DCC and thin-layer chromatography (TLC) have the common feature of dry adsorbent and any TLC conditions can be directly transferred to DCC without loss of resolution. However, the advantages of extending DCC to the micro level appear to have received little attention.

Microcolumns can be broken into separated peak-segments following development then each peak can be studied by a variety of techniques. For example, a given peak-segment can be examined by mass spectrometry prior to or following derivativization. It can also be subjected to gas chromatography (*15*) or pyrolysis-TLC (*13*) or other combined techniques (*11, 5*). The very fast development time and ease of handling such microcolumns will minimize the oxidative alteration of labile compounds during the scraping-elution step needed for recovery of TLC bands.

In principal, there is no clear limit in the size to which microcolumns might be reduced other than the limits of the detection system used to characterize peaks. It may ultimately be possible to use them to resolve the constituents of single cells or organelles.

The present paper describes first a method for the rapid mass production of micro dry columns (MDC); second, a procedure for transferring TLC conditions to them; and third, examples of the mass spectrometric characterization of mixtures separated by means of MDC.

MATERIALS AND METHODS

Adsorbents

Most of the work in developing this technique was carried out on siliceous adsorbents but we have also packed microcolumns with alumina, magnesia, barium sulfate, calcium carbonate, and Teflon. The general

requirement is that the adsorbent must be very finely ground and sieved to a particular narrow particle size range to fit the column size chosen. The adsorbent chiefly used for this preliminary work was made up of a mixture of three adsorbents. These were: (a) Corning high purity porous glass, Code 7936 (with alumina binder and UV indicator) obtained from Corning Glass Works, Corning, New York; (b) Silica Gel Plain, Catalog 8076-1 obtained from Warner-Chilcott Laboratories, 200 S. Garrard Blvd., Richmond, California; and (c) magnesium silicate, AIC-CO SOL PG obtained from Alleghany Industrial Chemical Company, P.O. Box 786, Butler, New Jersey. These adsorbents were mixed in the weight percentages of 88.6:5.7:5.7 in the order a:b:c. The adsorbent (approx 150 g) was placed in a plain 1-liter glass-stoppered borosilicate glass reagent bottle together with 400 ml of chloroform and 2 sizes of grinding media: 80 porcelain cylindrical grinding pebbles 0.5 inch o.d. × 0.5 inch long; and 24 spheres 0.75 inch in diameter. The charge was then milled at a rate of 48 rpm for 16 hours, the solvent was filtered off and the adsorbent was dried for 8 hours in an oven at 110°C. The neutral porous glass adsorbent is a useful "carrier" for the other adsorbents as it does not pack down to an impermeable bed. It substitutes readily for Celite in admixture with soft adsorbents such as magnesia. We have also used Unisil activated silicic acid which is available in 325 minus mesh size as a special item from Clarkson Chemical Company, Williamsport, Pa. and which requires no pretreatment other than sieving. The adsorbents were sieved to the proper particle size cuts through Buckbee-Mears microsieves, obtainable from Buckbee-Mears Company, 245 E. 6th St., St. Paul, Minn. This was done by holding the sieve stack on the table of a magnetically driven vibrating table, the Model J-1A Paper Jogger, which is obtainable from the Syntron Co., Homer City, Pa. This apparatus is also essential to the packing of the columns.

Columns

Columns were made from commercially available disposable micropipettes (Microcaps) of uniform length and bore available from Drummond Scientific Co., Broomall, Pa. The 25 μl size which is convenient to handle is 64.5 mm long and 0.940 mm o.d. × 0.698 mm i.d., as determined by optical comparator measurement. We will describe the preparation in terms of the 25-μl columns because of their general utility. The 1-μl size which is 31.7 mm long and 0.588 mm o.d. by 0.191 mm i.d. can also be easily packed but it is inconvenient to handle without a micromanipulator. We have not attempted to pack columns smaller than the 1-μl size.

Packing the columns

Columns were conveniently packed in lots of 50, about 30 minutes being required to complete the entire operation. We will describe the 5 steps in this procedure in the consecutive order in which they are carried out: plugging one end, packing and fluidizing the bed, removal of excess adsorbent, "dancing," and plugging the other end.

The first step is to plug each column with a uniformly thick pad of ininert ceramic fiber so that it can be filled with adsorbent from the open end. The column is used as a cork borer to cut a plug from a 1 mm thick mat of Fiberfrax ceramic paper, Type 970-FH without binder, available from the Carborundum Company, Niagara Falls, New York. Fiberfrax paper is also available in 0.5 and 2 mm thick sheets as types 970-AH and 970-JH, respectively, an advantage in applying the sample by imbibition.

The second step is to drop the plugged columns, plug end down, into a graduated cylinder which is taped to the deck of the paper jogger, as shown in Fig. 1. The cylinder should be of such size that the columns stand in it in a nearly vertical position; a 10-ml graduate about 1 cm i.d. is convenient for 50 columns of the 25-μl size. A 60° conical glass funnel 7 cm in diameter with a stem length of about 2–3 cm is placed in the graduate and covered with a Buckbee-Mears sieve of 7.4 cm grid diameter. The sieve is charged with adsorbent which has been previously put through it and it is then covered with a sieve cover. The paper jogger is now energized at a power setting which causes the funnel–sieve–cover assembly to virbate freely thus making the adsorbent sift as free particles into the columns and graduate. This operation is carried out in a fume hood in order to minimize exposure of personnel to the fine adsorbent smoke formed in the process. We used $-45/+20$ μ sieved adsorbent for the 25-μl columns and -20 μ sieved adsorbent for the 1-μl columns at a power setting (on our instrument) of 4.0 for this step. The graduate is filled to a height of 1–2 cm above the tops of the columns and the sieve is then emptied. The setup is reassembled and jogged for about a minute at an energy level sufficient to fluidize the bed, usually at a power setting of 6–8. This procedure frees the microcolumns of any entrapped air. After the "fluidization," jogger power is reduced to a setting of about 4.5 to pack the bed to its maximum density and the bed is jogged until no further change in bed volume is seen.

The third step is that of removing the filled columns from the excess adsorbent in which they are embedded.

The graduate and its contents are laid horizontally on the jogger deck and jogged gently (at a power setting of 1–2) to loosen the columns and

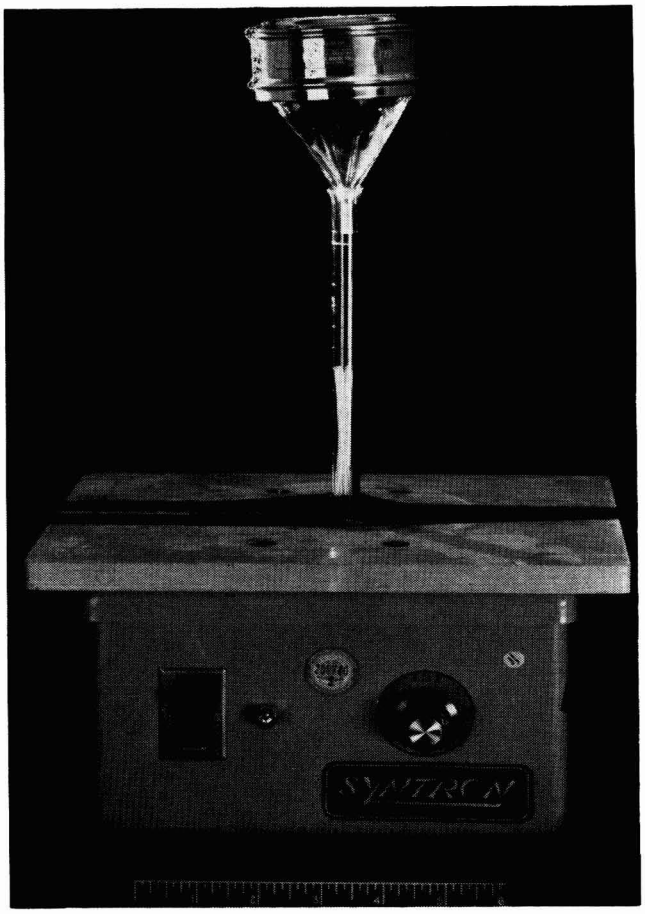

FIG. 1. The apparatus required for packing microcolumns: a group of fifty 25-μl columns in the 10-ml graduate receives presieved adsorbent from the loosely fitting funnel–sieve–cover assembly as the whole is vibrated; scale in inches.
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the adsorbent which is packed tightly around them. The loose adsorbent is poured off the top and the jogging–pouring operation repeated until the columns buried at the bottom of the graduate can be removed. This operation carried out correctly causes very little adsorbent to be lost from the columns. However, the adsorbent in the columns must be packed more tightly than it is at this point to ensure that no voids are present. This is done in the fourth step by "dancing" the columns. The columns are placed, plug end down, in a graduate which is large enough to enable them move freely when it is jogged. If 25-μl columns are placed in a 25-ml graduate of about 2 cm i.d., they lean against the wall

at an angle of 17° from the vertical. If the graduate, filled loosely with columns, is centered on the jogger deck and vibrated at the proper energy level, the columns will "dance" in a circular path in the plane of the deck at the same time that they jog vertically. This treatment carried out for about 5 minutes packs the adsorbent in the columns to a void-free maximum density. For example, Unisil of $-45/+20$ μ sieve size had a bulk density of 0.41 g/ml which was reduced 25–μl columns "danced" 5 minutes to 0.62 g/ml. These columns were full before "dancing" (63 mm of bed) but the bed was only 57 mm long after "dancing" leaving 6 mm of the column empty. An individual variation of 1–2 mm in the bed height of a given lot of columns is unimportant because the bulk density of the adsorbent which determines their separation efficiency will be the same for each.

The final (fifth) step in the packing consists of plugging the open end of the column with Fiberfrax as before, and this plug and air gap provides the means of applying a sample.

Applying the Sample

For qualitative studies, as for multiple tests of sample homogeneity, the plug is dipped into the sample solution, then held for a few seconds in a gas stream to evaporate the solvent. A 25-μl column Fiberfrax plug 2 mm long will imbibe 0.4—0.5 μl of solvent. The dipping-evaporation step can be repeated as often as is required to apply a proper load to the column. The availability of the different thicknesses of Fiberfrax mat enables one to choose a plug thickness which will imbibe more or less sample. During solvent evaporation about 20–30% of the solute is left outside the plug on the rim of the column. For exact studies this excess can be removed with Fiberfrax paper and the sample residue and column weighed on a microbalance, such as the Cahn gram balance, obtainable from Cahn Instrument Co., 7500 Jefferson St., Paramount, California. For example, a Unisil-packed 25-μl column weighs about 73.8 mg of which 13.4 mg is adsorbent and with a similar column used as a counterweight one can accurately weigh applied samples to the nearest microgram. Our loading factors for Unisil agree with those of Loev and Goodman (*9*) for macro DCC on silica. One can thus separate 50μg of their "very difficultly" separable mixture of *N*-(*p*-dimethylaminophenyl)–1,4–naphthoquinoneimine and *p*-dimethylaminoazobenzene on a 25-μl Unisil column with benzene as the eluent. Generally, the load which may be resolved by a column is proportional to its cross-sectional area and thus the 1-μl column should carry about $\frac{1}{13}$ the load that the 25-μl column can carry. However, a 1-μl column of bed height 28 mm (wt 20.6 mg) contains only 0.5 mg of adsorbent and its bed height is

only about half that of the 25-μl column. Thus the practical load for a 1-μl column must be only about $\frac{1}{50}$ (or less) that of the "large" column.

Activation

We generally activated the batches of columns at 110°C for 1 hour, then cooled them in a desiccator over saturated calcium chloride at a RH of 31%. "Deactivation" (9) was done simply by allowing a given column to equilibrate in a stoppered shell vial with a strip of filter paper soaked in the TLC solvent system to be used.

Development

After the sample is loaded on the plug it is pushed into firm contact with the bed. This is done with the aid of a 1-μl pipette which then becomes a wick. It may be necessary to ensure firm contact of the bed and wick by tapping the assembly gently on the bench top.

The wicks are conveniently held and handled in "wick holders" (which are not essential, however). The "wick holders" are made as follows: No. 24 gauge T-2X heat-shrinkable Teflon tubing, available from Penntube Plastics Co., Clifton Heights, Pa., is heated on a soldering iron, then drawn down to a loose force-fit for the 1-μl wick. A piece of the tubing 2.2 cm long including restriction is shrunken onto a pierced bifurcated soldering lug, such as Part No. X-3650-1 of Lyn-Tron, Inc., 1206 W. Chestnut St., Burbank, California. This assembly may easily be handled and the wick replaced with a clean one if necessary. The wick for the 1-μl columns is made from No. 34 gauge steel needle stock (0.007 inch o.d. × 0.003 inch i.d.) available from Hamilton Co., P.O. Box 307, Whittier, California. This full-hard temper tubing is cut by first rolling it under a razor edge across a block of tool steel then bending it across the score with tweezers until it breaks. The steel wick is best used without a "wick assembly."

Figure 2 shows a pair of 25-μl Unisil columns of different states of activation during development of the Desaga dye mixture in benzene. This dye mixture is butter yellow, Sudan red G, and indophenol blue (7). The column on the right was filled with activated adsorbent as received while that on the left was exposed to water vapor for 16 hours to "deactivate" it.

The "deactivated" column cleanly separated the dyes into 2 yellow bands (R_f 0.47 and 0.34), a red band at R_f 0.32, a pink band at R_f 0.22, 2 blue bands at R_f of 0.18 and 0.10 and a pink band at R_f 0.06 The active as-received adsorbent separated the mix only into a single yellow band at R_f 0.14, a red band at R_f 0.05 and a blue band at the origin. The "deactivated" columns' performance agrees well with that of

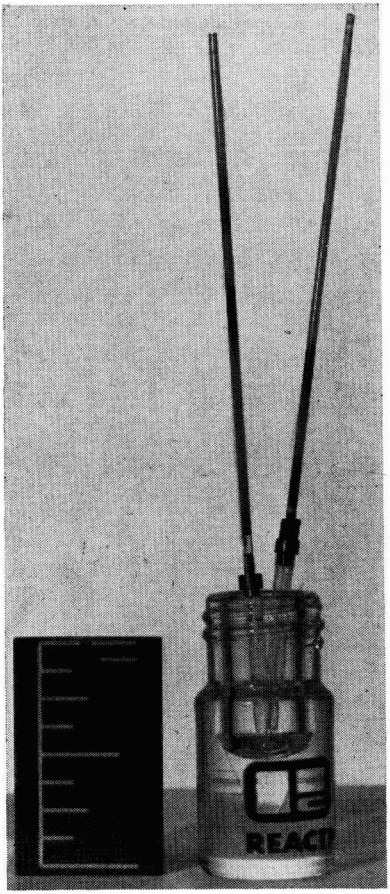

FIG. 2. A pair of 25-μl Unisil columns in the process of development of a sample of Desaga dyes in benzene: the 1-μl wicks can be seen through the walls of the Teflon wick holders; scale is 1 inch.
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Piel's microparticulate silica liquid chromatography column (*12*) for the same mixture.

Detection and Identification of Separated Species

The conventional techniques for visualization of colorless species are handicapped by their lack of sensitivity at the submicrogram level. This applies also to the adsorbents which contain fluorescent indicators, such as Corning Code 7936 glass. If readily sublimable reagents (e.g., iodine, tetracyanoethylene) are used, the column must first be freed of solvent by rapid evaporation in a vacuum. This causes band broadening which in itself is undesirable. Furthermore, if polar solvents have been used the

solvent removal process is incomplete under conditions that do not affect the component under investigation.

Therefore, the detection and identification by instrumental techniques is to be preferred. In the list of available techniques, mass spectrometry proves to be by far the most suitable due to its high sensitivity and specificity. Less than 0.01 μg can readily be detected and identified.

After development, the column is immediately broken into equal sized small sections which are subsequently introduced into the mass spectrometer via a direct insertion inlet system. Normally, the samples are analyzed at a relatively low resolution of $M / \Delta M \sim 2.000$ which allows only limited mass measurement. Nevertheless, this is sufficient for identification of the majority of the bands. If required, the sample can be rerun, since only a vary small amount of sample and time are consumed. This permits the analyzer to cut out a specific, still unidentified band and investigate it under conditions of high resolving power $M/\Delta M > 10{,}000$ and make an identification after precise mass measurement of all peaks of the spectrum (*3, 4, 6*).

The mass spectrometer employed in these experiments is a AEI Type MS-902 double focusing instrument fitted with a direct insertion probe for introduction of solid samples. The probe was modified to handle the microcolumn sections directly and to provide independent temperature control of the sample. Limits of detection and identification are 0.01 μg or less at a resolving power of less than 2000, and 0.1–1.0 μg at a resolving power of 10,000—20,000. The simple presence of a band can be detected below the 10 ng level.

Breaking the Column

The column is first scored with a diamond scribe, as in the sliding table apparatus shown in Fig. 3. It is then broken in the mass spectrograph solid-sample probe cup with the aid of a counterbored adapter which slips over the top of the cup, as in Fig. 4. The cups can be stored in the dark under an inert atmosphere at low temperature in large numbers until needed for analysis.

Applications Studied

1. A mixture of azulene, hydrocortisone, 17-β-estradiol, and estriol were separated on a 25-μl column of the mixed (glass carrier) adsorbent in the system chlorofrom–methanol–water 188:12:1 (v/v) with a development time of 8.00 minutes (*1*). About 1 μg of each compound was loaded on the column by the dip–evaporation method. The column bed which was 5.7 mm long was broken into 7 pieces 5 mm long and 2 pieces 10 mm long, the long pieces at the origin. The pieces were insert-

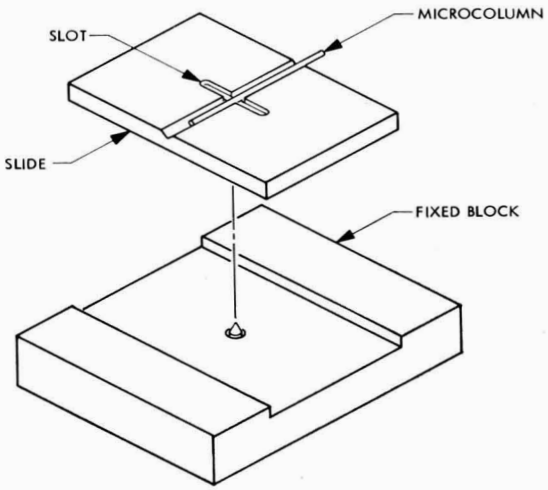

FIG. 3. Apparatus for scribing columns to be broken into segments: the Lucite plastic table slides across the fixed block within which a diamond point scribes the microcolumn through the slot provided.
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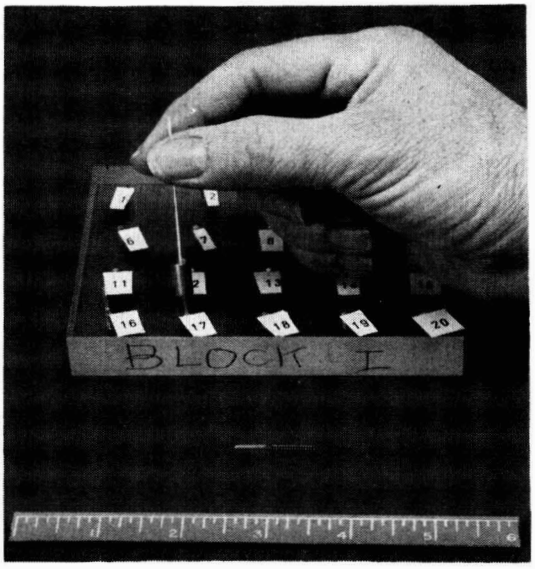

FIG. 4. Storage block for mass spectrograph solid-sample cups; with a loose cup in the foreground: a scribed microcolumn is being broken against the steel adaptor which is fitted over the cup beneath it by means of a counterbored recess.

ed into the mass spectrometer by means of the solid inlet probe and the analysis of the segments showed that the compounds had been cleanly separated on the column. It also showed that the cholesterol sample used contained cholestane contaminant.

2. A mixture of water-soluble bacterial stain dyes were well separated on both 25-μl and 1-μl columns of Corning Code 7936 glass alone. A total of 1 μg of each dye was loaded by dipping and separated in the system benzene–methanol–acetone–diethylether 4:2:1 (v/v). The porous glass columns were activated by heating at 120°C for 30 minutes, then equilibrated over saturated calcium chloride solution (RH 31%). Development time was 12.05 minutes for the large and 2.00 minutes for the small column. The dyes were: erythrosine B (C.I. 45430), crystal violet (C.I. 42555), rose bengal (C.I. 779), Nile blue A (C.I. 51180), Auramine O (C.I. 41000), brilliant green (C.I. 42040), acridine orange (C.I. 46005) and methyl violet 2B (C.I. 42535).

3. A whole acetone extract of *Chlorella pyrenoidosa,* strain 71105, was chromatographed on a 25-μl column of the mixed (glass carrier) adsorbent in the system petroleum ether (30°/60°)–acetone–chloroform 5:5:4 in 8.83 minutes (*8*). Carotene, lutein, violaxathin, pheophytins a and b, and chlorophyll a and b were cleanly separated and characterized in the total applied sample of 2.5 μg.

DISCUSSION

The use of this and related methods (*10*) has been restricted in the past chiefly to noninstrumented studies of inorganic mixtures (*14, 17*). However, its chief value is that of an adjunct to instrumental methods. Used this way MDC is superior to TLC in terms of economy in time of preparation and manipulation as well as in protection of labile mixtures from oxidation. It would be an advantageous substitute for TLC in microclinical applications, such as the separation of urinary corticosteroids (*16*) and such applications would be broadened by the use of reaction techniques (*2*) with the method. Corning porous glass is a useful carrier for soft adsorbents such as magnesia as it does not "pack down" in the columns unduly nor does it shrink under the stress of solvent capillary flow. The glass alone, however, shows molecular sieve properties in that, for example, it sorbs butter yellow from the Desaga dyes in benzene.

SUMMARY

The preparative scale method of dry-column chromatography has been adapted to the micro scale and combined with mass spectrometry (MS). The micro dry columns (MDC) are broken into resolved-peak segments after development and these are inserted directly into the instrument. The use of combined MDC/MS

shortens the time of analysis of complex mixtures. The micro dry columns appear to be generally useful adjuncts to other forms of chromatography.

ACKNOWLEDGMENTS

We gratefully acknowledge the design of the microcolumn "scratching table" and the counterbored "breaking collet" (Figs. 3 and 4) by Carl F. Smith. This paper presents the results of one phase of research carried out at Jet Propulsion Laboratory, California Institute of Technology, under Contract No. NAS7-100, sponsored by the National Aeronautics and Space Administration.

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Application of Back Titration of EDTA with Mercury(II) to the Analysis of Ilmenite

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The overall versatility, sensitivity, and general convenience of EDTA or CDTA *(6)* as direct titrimetric reagent or in back titration of their excess, had found widespread applications for successful determination of elements or analysis of their mixtures. The good results which we obtained in analysis of solders, bearing and type metals, stainless steels, bronzes, brasses and their special varieties, lead pigments *(7–9)* and various mixtures including titanium, iron, aluminum, vanadium, and chromium *(10–11)* renewed our interest in applying the same procedure to the analysis of ilmenite. This is the source of titanium metal which finds application in military crafts and steel metallurgy; and its oxide which enters in pigments and porcelain industries.

In our country sources of ilmenite are large deposits of black sands extending along the coast of the mediterranian sea as well as ignious intrusions in the eastern desert near the red sea.

The classical methods of analysis are tedious, time and reagent consuming as they involve: (a) sulfide separation of group (II), mainly Cu; (b) sulfide precipitation of iron(III) from its mixture with Ti, in ammoniacal tartrate medium, after treatment of the silica-free solution with Na_2O_2 to separate Al, Cr, and V as aluminate, chromate, and vanadate; (c) separation of unstable iron(II) sulfide and removing the last traces of tartrate by repeated washing, otherwise it will interfere with the permanganate or dichromate method of determining iron(III); and (d) Kjeldahl digestion of titanium tartrate complex prior to precipitation of Ti as hydroxide in case cupferron is not used for its determination.

Poddar *(13)* determined Ti in ilmenite and other ores by a gravimetric, apparently tedious method using as reagent salicylhydroxamic acid.

From thirty different cations Ti could be extracted with hexyl phosphates *(12)* prior to its determination by back titrating excess of EDTA with zinc acetate using xylenol orange as indicator with an error of 1.7–4%.

Foerster *(4)* determined Al in titaniferous alloys by a method involving separation of silica, subjecting the filtrate to a mercury cathode electrolysis to remove interfering ions and precipitation of Ti with cupferron

prior to back titrating excess of CDTA with $ZnSO_4$. Interfering elements are those not removed by electrolysis and known to chelate with CDTA.

Bruile *(2)* determined Ti and Al simultaneously in ferrotitanium containing Cu and Si. His method involved chelating both cations with EDTA, titrating excess of EDTA with $ZnSO_4$ using xylenol orange, destroying with tartaric acid the Ti–EDTA complex prior to titration of liberated EDTA with $ZnSO_4$ and finally destroying with KF the Al–EDTA, complex prior to titration of liberated EDTA in the same solution with 1.2 and 2.7% error, respectively.

Among the methods cited in the literature for traces of chromium and vanadium in ilmenite is to be mentioned the polarographic method of Branzovsky *(1)* exploiting the two different half-wave potential values of Cr(VI) in NaOH amounting to -0.89 volt and of V(V) in NH_4OH–EDTA amounting to -1.25 volt vs. sat. calomel electrode.

Khalifa determined macro and micro amounts of Fe(III) alone or in binary mixtures with requisite accuracy by back titration of excess EDTA with Hg(II) in ammoniacal buffers *(5)*. He successfully analyzed mixtures of Ti and Fe(III) by the same procedure but in hexamine buffers of pH 6–6.5 for total or of pH 7 for Fe(III) after masking Ti with lactic or HF acid.

EXPERIMENTAL METHODS

The water used was always de-ionized. The chemicals were all of the requisite purity. They were sulfates of Cu(II); vanadate of ammonium; hydroxide and acetate of sodium; bisulfate, dichromate, and fluoride of potassium; zinc oxide; aluminium metal; hydrogen peroxide; cuperferron; mercuric nitrate; EDTA; urotropine sulfuric, hydrofluoric, perchloric, nitric, lactic, and sulfurous acids; H_2S gas; murexide and EBT indicators.

Solutions

The 0.010 and 0.0010 *M* EDTA solutions were prepared in the usual way and standardized with standard zinc solutions prepared from zinc oxide and nitric acid.

The 0.01 *M* aluminium solution was prepared by dissolving the calculated amount of the metal in few ml of conc. H_2SO_4 sufficient to make the final solution 4% in acid. The cool solution was made with water up to the requisite volume.

The 0.01 *M* V(IV) solution was prepared from NH_4VO_3; it was treated with excess H_2SO_3, the excess acid was removed by boiling and

the resulting blue solution was made with water up to the requisite volume.

The 0.01 *M* Cr(III) was prepared by treating the solution obtained by dissolving the calculated amount of potassium dichromate in water with H_2SO_3. The excess acid was removed by boiling and the resulting green solution was made with water up to the requisite volume.

The 0.01 *M* $CuSO_4$ solution was prepared by dissolving the calculated amount of $CuSO_45H_2O$ in the requisite volume of water.

All the above solutions were standaradized both by the ordinary recommended procedures and by the potentiometric back titration of excess EDTA in urotropine buffered media.

The 1% H_2O_2 solution was prepared by ordinary dilution from 100-vol sample.

The titration assembly consisted of a 150-ml beaker; a 1/50 graded microburette; a mechanical stirrer; calomel and silver amalgam electrodes.

The pH and potentiometer were model PYE Cat. No. 11085.

Procedures

Place 1 g of powdered ilmenite into a 500-ml beaker, add 25 ml of conc H_2SO_4, 75 ml of H_2O, cover with a watch glass; boil for 1 hour to dissolve most of the sample, cool, dilute with water, filter into an evaporating dish using 540 Whatman filter; ignite the residue in a platinum crucible, fuse it with $KHSO_4$, repeat fusion once more if necessary; dissolve the melt in 100 ml of 10% H_2SO_4; evaporate in a dish to white fumes of SO_3 to dehydrate silica, cool, add 200 ml of water, boil, filter at once in a 600-ml beaker, wash repeatedly with hot water; ignite the residue involving SiO_2 and some impurities in a platinum crucible; volatilize SiO_2 by aid of HF and few drops of 1:1 H_2SO_4; fuse the residue with $KHSO_4$; dissolve it in 10% H_2SO_4 and add its solution to the main filtrate (SiO_2 is computed by difference). Proceed as A or as B.

(A) With copper sulfide separation. To determine Cu, pass H_2S through the diluted slightly acidic warmed filtrate; coagulate CuS on a water bath; filter through 541 filter; dissolve the residue in 1:4 HNO_3, filter, to the filtrate add 4 ml of 0.01 *M* EDTA, 40 ml of 2% urotropine and back titrate with Hg(II) using silver amalgam as the indicator electrode; to the boiled filtrate from CuS add few drops of conc HNO_3, filter if necessary, make the solution with water up to 500 ml; to a 100-ml aliquot, add solid Na_2O_2 until alkaline; boil and filter; dissolve the residue of iron and titanium hydroxides into 50 ml of 1:4 H_2SO_4 and make with water up to 250 ml.

For iron plus titanium, to a 5-ml aliquot, add 7 ml of 0.01 *M* EDTA,

0.15 ml of 1% H_2O_2, 5 ml of 2% urotropine, water up to 40 ml; adjust the pH to 6–6.5 with few drops of 30% NaAc, and back titrate with Hg(II) as above.

For iron alone, to another 5-ml aliquot add few drops of conc lactic acid to mask Ti *(16)* , let stand for 15 minutes, add 40 ml of 2% urotropine and back titrate with Hg(II) as above. Compute Ti by difference.

For total Al, Cr, and V, make the filtrate from hydroxides of Fe and Ti with water up to 50 ml; acidify a 15-ml aliquot with dil H_2SO_4; boil off excess of H_2O_2 after reduction of Cr(VI) to Cr(III) and V(V) to V(IV); add 5 ml of 0.01 *M* EDTA, boil for 15 minutes to ensure complete complexation of Cr(III) and Al; cool; add 40 ml of 2% urotropine, adjust the pH to 6–6.5, and back titrate with Hg(II) as above.

For Cr + V, treat another 15-ml aliquote as above, add 2 ml of 15% KF to mask Al, 3.5 ml of 0.01 *M* EDTA; boil to ensure complexation of Cr. and V; add 40 ml of 2% urotropine, and back titrate with Hg(II); compute Al by difference.

For Al + V, boil a third 15-ml aliquot in a platinum dish for 30 minutes to remove H_2O_2; acidify with $HClO_4$; heat to white fumes of the acid adding HF to volatilize chromium as chromyl fluoride *(3)*; repeat fuming to expel all chromium; cool, dilute, transfer to the titration beaker; pass SO_2 gas to reduce V(V) to V(IV); boil off SO_2; cool, add 4 ml of 0.01 *M* EDTA; boil for 3 minutes; add 40 ml of 2% urotropine, and back titrate with Hg(II). Compute each of Cr and V by difference.

(B) Without copper sulfide separation. To a 200-ml aliquot add conc ammonia till the solution is highly ammoniacal; add solid Na_2O_2; boil keeping the solution always ammoniacal; filter; dissolve the residue of iron and titanium hydroxides in 50 ml of 1:4 H_2SO_4, and make with water up to 500 ml.

For iron plus titanium or iron alone follow procedure (A). The filtrate F contains Cu, Al, Cr(VI), and V(V).

Synthetic mixtures containing Cu, Al, Cr(III), and V(IV) were analyzed to investigate the optimum conditions of analysis.

I. for Cu, Al, Cr(III), and V(IV), the total is determined by boiling with excess EDTA for 15 minutes, cooling, buffering with 40 ml 2% urotropine (pH 6.5), and back titrating the excess of EDTA with Hg(II).

II. In another identical mixture Cu(II), Cr(III), and V(IV) are determined by procedure I, after masking Al with KF (2 ml of 15%) prior to the addition of EDTA.

III. In a third identical mixture, Cu(II), Al, and V(IV) are determined by procedure I, after volatilizing chromium as chromyl fluoride;

adding a few drops of sulfurous acid to the diluted solution to reduce V(V) to V(IV), and expelling the excess SO_2 by boiling.

IV. In a fourth identical mixture, Cu is determined alone by direct volumetric titration with EDTA using murexide as indicator at pH 8, after oxidizing V(IV) and Cr(III) to their highest states of valency by fuming with perchloric acid.

Modified procedure applied to the analysis of the filtrate (F). Boil the filtrate to expel H_2O_2, slightly acidify with $HClO_4$, and finally make with water up to 100 ml.

For total, Cu, Al, Cr. and V, dilute a 10-ml aliquot, add few drops of H_2SO_3 to reduce Cr(VI) to Cr(III) and V(V) to V(IV), expell excess of SO_2 by boiling; add 4 ml of 0.010 *M* EDTA and use procedure I.

For Cu, V, and Cr, treat another 10 ml with sulfurous acid expell the excess of SO_2 by boiling, add 4 ml of 0.010 ml of EDTA and proceed as in procedure II prior to the back titration of excess EDTA with Hg(II).

For Cu, Al, and V, a third 10-ml aliquot is treated as in procedure III prior to the back titration of excess EDTA with Hg(II).

For Cu alone, use procedure IV.

Compute each of Al, Cr, and V by difference.

RESULTS AND DISCUSSION

In the present work qualitative analysis of an Egyptian sample of ilmenite revealed the absence of phosphorus, calcium, and magnesium and the presence of manganese in traces (less than 0.1%). In presence of these elements the present procedures are to be slightly modified.

It is noteworthy to remark that treatment of the ore solution, containing Cu, Cr, V, Fe, Ti, and Al, with ammonia prior to its oxidation with Na_2O_2 affords the advantage over classical methods of analysis, of keeping copper in solution with Al, Cr(VI), and V(V) which do not interfere with EDTA titration of copper using murexide *(15)* thus avoiding the copper sulfide precipitation and subsequent steps.

The present procedure for iron and titanium shortens the time of analysis to a great extent as it avoids the iron sulfide separation. The procedure for Al eliminates the difficulties encountered in determining it gravimetrically.

In masking Al with fluoride it is recommended to use KF which forms on cold the potassium hexa fluoro-aluminate which is insoluble in excess KF, dilute acids and alkalies *(14)*, and boiling EDTA.

Trials to mask Cr(III) with citrate and Al with fluoride prior to determining Cu and V(IV) by back titration of excess EDTA with Hg(II) revealed partial masking of Cu and V with citrate.

In determining total Cu(II), Al, Cr(VI), and V(V) in dilute per-

chloric acid, reduction with SO_2 yields Cr(III) and V(IV) (to be chelated with EDTA) and Cu(I) which on boiling to remove excess SO_2—unlike Cr(III) and V(IV)—is oxidized with dilute $HClO_4$ to Cu(II)

It is customary to use as back titrant for excess EDTA, mercury(II) ranging from 0.01 to 0.05 *M*, with corresponding potential end point breaks of 100–200 mV per 0.1 ml of titrant. In a previous communication *(10)* we reported that such a procedure as applied to the determination of titanium alone or in mixtures, gives more precise and accurate results with smaller amounts, a reason why we used 0.01 *M* mercury-(II) with which the potential breaks are still of good order of magnitude amounting to an average of 100 mV/ o.1 ml of o.o1 *M* Hg(II).

Table 1 lists typical results of analysis of five synthetic 4-component mixtures and Table 2 those of analysis of an ilmenite ore with the classical and the present methods.

TABLE 1

ANALYSIS OF QUATERNARY MIXTURES

V (mg)		Al (mg)		Cu (mg)		Cr (mg)	
Taken	Found	Taken	Found	Taken	Found	Taken	Found
0.3872	0.3899	0.2156	0.2155	0.6288	0.6280	0.6922	0.6901
0.3872	0.3883	0.1078	0.1077	0.3144	0.3140	0.6922	0.6912
0.3872	0.3862	0.2156	0.2157	0.3144	0.3140	0.3461	0.3473
0.1936	0.1924	0.2156	0.2156	0.6288	0.6280	0.3461	0.3479
0.1936	0.1950	0.1078	0.1070	0.6288	0.6280	0.6922	0.6280

TABLE 2

ANALYSIS OF ILMENITE

No.[a]	Fe_2O_3 (%)	TiO_2 (%)	CuO (%)	Al_2O_3 (%)	Cr_2O_3 (%)	V_2O_5 (%)
1	52.2	47.82	0.08	1.23	0.18	0.56
2	52.7	48.07	0.10	1.10	0.15	0.47
3	52.7	48.07	0.10	1.10	0.16	0.43

[a] Procedure used: (1) classical methods; (2) present procedures without sulfide separation; and (3) present procedures involving sulfide separation.

SUMMARY

Procedures for the relatively rapid and accurate analysis of ilmenite based on the potentiometric back titration of excess EDTA with mercury(II) are given. By their aid five quaternary mixtures and an ilmenite ore were successfully analyzed. Potential breaks detecting end points in determining total of 2–4 components were

an average of 100 mVolts/0.1 ml of 0.01 *M* mercury(II). Some deviations among results of the classical and the present methods were manifested only with constituents present in traces.

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Vapor Sampling and Gas Liquid Chromatography of Some Volatile Materials in Biological Solutions[1]

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During the past 6 years this laboratory has utilized direct head space gas sampling for GLC in quantitative and qualitative analyses of part per million levels of volatile materials in biological fluids *(1–15)*. Although many other laboratories have used similar methods for qualitative studies, surprisingly few investigators have taken advantage of the quantitative potentialities of head space gas sampling for GLC analysis *(16–19)*. The purpose of the present study was to show the reliability of the method.

MATERIALS AND METHODS

Preparation of standard solutions. Acetaldehyde, methyl sulfide, acetone, butanone, ethyl alcohol, and 2-pentanone were added separately to distilled water, urine, blood and milk to yield, by serial dilution, the ranges of concentration shown in Table 1. In additon, a composite solution containing all components was made up in distilled water and another in homogenized milk at the concentration range shown in Table 1.

Most of the dilutions for the standards were prepared from stock so-

TABLE 1

DATA DESCRIBING STANDARD CURVE REGRESSION LINES OF SOME CHEMICAL COMPOUNDS IN WATER AND BIOLOGICAL FLUIDS

Compound and solvent	No. of conc	Range	Slope[a] $\times 10^{-4}$	Constant	Standard error of estimate
1. Acetaldehyde			(ppm)		
Composite in water	5	0.1–10	6.8	+0.15	0.23
Water	4	0.1–10	8.1	+0.02	0.01
Urine	6	0.1–50	11.0	+0.31	0.27
Cow plasma	5	1.0–50	23.0	+1.2	0.7

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TABLE 1—Continued

Compound and solvent	No. of conc	Range	Slope [a] × 10^{-4}	Constant	Standard error of estimate
2. Methyl sulfide (two instruments A and B)					
Composite in water	5	(A) 0.1–2	1.3	+0.025	0.016
	5	(B) 0.1–2	1.3	+0.025	0.021
Water	6	(A) 0.02–1	1.1	+0.03	0.05
	6	(B) 0.02–1	1.1	+0.06	0.07
Urine	6	(A) 0.02–20	2.0	+0.23	0.34
	6	(B) 0.02–20	1.7	+0.49	0.85
Cow plasma	6	(A) 0.04–1	4.3	+0.05	0.13
	6	(B) 0.04–1	4.4	+0.09	0.15
Milk	9	(A) 0.005–0.4	2.8	+0.005	0.005
	9	(B) 0.005–0.4	3.0	+0.006	0.006
Composite in milk	4	(A) 0.04–10	3.0	+0.008	0.06
	4	(B) 0.04–10	2.8	+0.050	0.06
3. Acetone					
Composite in water	5	0.1–10	5.7	+0.10	0.1
Water	8	0.1–100	5.9	+0.2	0.6
Urine	6	0.1–50	7.0	+0.1	0.1
Cow plasma	4	0.2–10	8.4	−0.07	0.01
Milk	9	0.1–50	7.9	+0.25	0.5
Composite in milk	6	0.1–50	7.7	+0.04	0.4
4. Butanone					
Composite in water	5	0.4–5	3.1	+0.05	0.07
Water	7	0.1–10	3.6	+0.02	0.83
Urine	6	0.08–40	3.7	−0.03	0.86
Cow plasma	7	0.25–25	7.5	−0.10	0.10
Milk	5	0.04–0.4	7.6	+0.04	0.01
Composite in milk	4	0.08–20	8.0	+0.05	0.11
5. Ethanol					
Composite in water	5	1–100	53	+2.5	3.1
Water	5	0.5–25	77	+0.2	0.35
Urine	5	1–50	86	+0.8	1.7
Cow plasma	7	1–100	88	+1.9	5.4
Milk	7	1–200	84	+1.7	2.0
Composite in milk	4	0.2–50	65	+0.2	1.9
6. 2-Pentanone					
Composite in water	5	0.25–5	2.9	+0.04	0.04
Water	6	0.1–2	3.0	−0.00	0.04
Urine	6	0.08–40	3.8	−0.48	0.98
Blood	5	0.2–50	10	−0.35	0.64
Milk	7	0.004–0.4	16	−0.003	0.005
Composite in milk	6	0.04–40	14	+0.21	0.67

[a] Conc in ppm/peak height. Peak height measured as percentage of full scale deflection × attenuation factor.

lutions of 100 ppm. They were prepared freshly by weighing 10 mg of the chemical compound in a capillary tube drawn to a fine tip. The material and capillary tube were dropped into about 10 ml of the fluid under investigation in a 100-ml volumetric flask, broken up with a glass rod, the rod rinsed with the fluid and the volume adjusted to 100 ml. By that procedure reproducible concentrations in solution of even low boiling volatile compounds such as acetaldehyde and methyl sulfide could be prepared.

The urine used was a fresh composite from human adults. Blood was freshly drawn by venipuncture of individual cows, preserved with 1.0% NaF and the plasma used was separated by centrifugation. Fresh, commercial, pasteurized-homogenized milk containing 3.5% fat was used.

GLC analysis. Two GLC instruments were employed for the analyses. One operator was able to prepare samples and operate the two instruments routinely and simultaneously. At times, two different analysts were used.

Varian-Aerograph gas chromotograph models 600 B (A) and 500-C (B), each with an H_2 flame-ionization detector were employed with 304.8-cm by 0.318-cm o.d. stainless steel columns packed with 20% Carbowax 20M on 60/80-mesh, acid-washed HMDS-treated Chromosorb P. Typical operating conditions follow: column temperature 100°C; nitrogen gas flow in millimeters/minute 15.4 (A) and 17.4 (B); hydrogen gas flow in millimeters/minute 24.4 (A) and 26.0 (B).

Sample preparation. Two ml of the solution to be examined was transferred into a 6.5-ml glass serum vial containing 1.2 g of anhydrous Na_2SO_4. The vial was sealed with a rubber serum cap and placed for 2 minutes in a beaker of 60°C water at a level above that of the vial liquid. It was then shaken for 5 minutes, the serum cap was changed to prevent liquid contamination of the sampling needle, and the vial was returned to the 60°C bath for an additional 8 minutes. The vial (in the beaker) was taken to the instrument and 1 ml of vapor was withdrawn through the serum cap using a 1-ml Hamilton gas-tight syringe with a 2-inch, 25-guage needle and the vapor was injected into the chromatograph.

Calculations. Peak heights (% of full-scale deflection) were recorded from duplicate analyses for each concentration of each chemical compound in the test and standard solutions.

Minor corrections for day-to-day changes in instrument sensitivity were made by assuming a hypothetical response of 1600 (peak height × attenuation) for 1 ppm of acetone. Analysis of a standard 1 ppm of acetone solution (external standard) several times during the day permitted use of an external correction factor for minor changes (e.g., peak height

of a component × 1600/peak height of 1 ppm of acetone = corrected peak height). By comparing the average corrected peak heights of samples with the corresponding known concentrations, regression equations for each chemical and diluent were calculated by the least squares method.

Reproducibility of sampling and GLC analysis. A composite aqueous solution contaiing 1 ppm each of acetone, butanone, and 2-pentanone; and 10 ppm of ethanol was analyzed 12 times by the suggested procedure and quantitative reliability of the analytical procedure determined by statistical analysis of the results.

Analysis of variance among instruments, operators, internal vs. external standards, individual injections, and different days. Variance among the results of GLC analyses by two analysts on two different days using two instruments was analyzed. The same two instruments were employed by each analyst on each day for examination of four replicate samples. The samples were composite mixtures of acetone (1 ppm), acetal (1 ppm), butanone (1 ppm), ethanol (10 ppm), and 2-pentanone (1 ppm). Since the acetone peak was employed as an internal standard, only four component peak heights were included in the statistical study. Peak heights also were adjusted to an external acetone standard as previously described. With the two operators, two instruments, two days, and two standards used in the analysis of four replicates each with four peaks, 256 peak heights were recorded and subjected to analysis of variance.

Results. Table 1 presents data describing the regression equations for data obtained on each of the six test substances and in each of the solutions analyzed. Regression equations are expressed in terms of ppm. It was possible to calculate usable equations for each of these systems except for acetaldehyde in milk. It is suspected that acetaldehyde reacts with the milk protein.

Several factors appear to be related to the overall sensitivity of this method, as solubility characteristics of the solute, boiling point, polarity, interaction of the solute with other components, and response of the constituent in the hydrogen flame. Although each of these factors is involved with each component in solution in an analysis, the influence of one often predominates. For example, the solubility of 2-pentanone in the lipids of milk markedly increases the slope (compare slopes from the regression equations in Table 1 for water and milk).

The low boiling point and insolubility of methyl sulfide in water result in an ideal situation for vapor analysis (slopes $1.1–1.3 \times 10-4$). Although there is an effect of solubility of the methyl sulfide in lipids, it is

small compared with the solubility of 2-pentanone with its higher boiling point.

The low response for ethanol (note the relatively large slope), Table 1 probably is due to a combination of factors. Most significant of these are solubility and polarity. The strong affinity of ethanol for water results in relatively less of it in the vapor phase. This, in combination with a lower response of the short-chain molecule in the flame detector, makes the lower limits of detection of ethenol at about 0.1 ppm, compared with 0.005 ppm for methyl sulfide.

Although the results presented in Table 1 are calculated from duplicate analyses of each sample, they are reported on only one instrument except for methyl sulfide. It is apparent that the regression equations for the two instruments are similar. These data are typical of those observed for the other components on the second instrument.

Reproducibility of sampling procedure and GLC analyses of the same aqueous solution of acetone, butanone, ethanol, and 2-pentanone are illustrated by the mean peak heights and standard deviations for the four-component mixture, Table 2. These data were calculated using both external and internal the correction factors suggested in this paper.

TABLE 2

MEANS AND STANDARD DEVIATIONS FOR 12 GLC ANALYSES OF A FOUR COMPONENT MIXTURE USING ACETONE FOR AN INTERNAL STANDARD AND USING THE CORRECTION FACTOR DESCRIBED ON TWO INSTRUMENTS (A AND B)

		Peak height × attenuation							
		Acetone (1 ppm)		Butanone (1 ppm)		Ethanol (10 ppm)		2-Pentanone (1 ppm)	
		x	σ	x	σ	x	σ	x	σ
External acetone standard	(A)	1599	8	2442	16	1943	13	2500	9
	(B)	1667	3	2414	12	1593	14	2371	9
Internal acetone standard	(A)	—	—	2439	11	1940	9	2501	11
	(B)	—	—	2309	6	1524	8	2276	10

Analysis of variance indicated that results from head space gas sampling with subsequent GLC analysis was dependent upon the instrument used, the operator and the day the analysis was made, in descending order of influence. Neither standard (internal or external) nor individual injections on a given day significantly influenced the results. It was con-

cluded from this study that standard curves must be prepared by the operator for the instrument used on the day that unknowns are to be analyzed to obtain maximum correlation of peak heights to concentration of unknowns.

DISCUSSION

Obviously, many problems of head space gas sampling have not been discussed here. Such problems as relating aroma vapor concentration to sample volatile material concentration or establishing the effect of a large concentration of one volatile on the others can be answered only by fabricating such a mixture in the same solvent system and analyzing it. Standard solutions must be made in the biological fluid under investigation at the concentration ranges of concern in order to relate one with the other. However, with such a comparison, quantitative relationships can be established.

Occasionally, one component in a mixture will exist in such high concentration that it covers a large area of the chromatogram. Such was the case in vapor analyses of beer conducted in this laboratory. The ethanol peak spread over a large portion of the chromatogram. When that occurs, a preliminary fractionation must be made.

When care is given to control the sampling technique and gas-tight syringes and needles are maintained in good condition, it is not difficult to obtain quantitative analyses by head space gas sampling and GLC analysis.

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MICROCHEMICAL JOURNAL **14**, 478–480 (1969)

Simple Spectrophotometric Microassay Method for Cycloalkanol Compounds

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A simple and rapid microassay procedure for cycloalkanol compounds is described in this note. The method is based on the observation that a cycloalkanol mixed with aqueous phenol gives a stable, intensely colored solution upon addition of sulfuric acid. A similar procedure has been described for the assay of carbohydrates *(1)*.

EXPERIMENTAL METHODS

Vacuum-distilled phenol in doubly-distilled water (5% v/v) was kept in a brown glass bottle. Ethanol was of spectroscopic grade.

General procedure. To 1 ml of a solution containing 10μg to 1 mg of cycloalkanol compound (see Table 1) in a large test tube (16 × 150 mm) add 1 ml of aqueous phenol (5% v/v) and mix. Add rapidly and directly to the surface of the aqueous solution 5 ml of concentrated sulfuric acid (sp gr 1.84). Cool the resulting colored solution in tap water

TABLE 1

DATA FOR SPECTROPHOTOMETRIC DETERMINATION OF CYCLOALKANOL COMPOUNDS

Compound	λ max (mμ)	Absorptivity [a]	Beer's law conformity limit (μg/ml) [a]
Cyclopentanol [d]	414	16	50
Cyclohexanol	425	12	50
Cycloheptanol [d]	426	16	40
Cyclooctanol [d]	432	9	40
2-Methylcyclohexanol [b]	425	16	50
Benzylcyclohexanol [b]	505	1.3	1200
D-Borneol [c]	420	2.9	150
Isoborneol [b]	420	3.5	150
Terpinol [c]	425	2.7	600
Menthol [d]	425	2.0	250

[a] Based on cycloalkanol concentration in 1-ml test solutions.
[b] Dissolved in 25% ethanol.
[c] Dissolved in 50% ethanol.
[d] Dissolved in 100% ethanol.

after 15 minutes or allow it to cool in air for a longer time. Measure absorbance at an appropriate wavelength (see Table 1) in comparison to that of a blank solution which lacks only the cycloalkanol compound.

Procedure for difficultly soluble cycloalkanols. If the cycloalkanol compound is difficult to dissolve in water, solubilize it by addition of up to 1 ml of ethanol and make up volume to 1 ml with water. Proceed as in "General procedure" (above). Prepare a blank containing an appropriate volume of ethanol.

RESULTS AND DISCUSSION

Figure 1 is the visible spectrum of the colored solution obtained by treatment of 2-methylcyclohexanol in the manner described above. Table 1 gives the wavelength of maximum absorbance, the absorptivity based on cycloalkanol concentration and the range of adherence to Beer's law for a number of cycloalkanol compounds treated as described

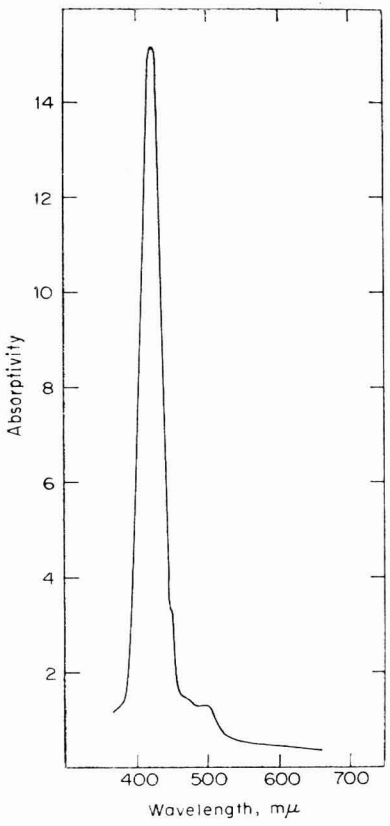

FIG. 1. Visible spectrum of 2-methylcyclohexanol treated with phenol and sulfuric acid.

above. The unsubstituted cycloalkanols have absorptivities between 9 and 16 and λ max for these compounds is between 414 and 432 mμ with a trend to higher wavelength as ring size increases. A small ring-substituent, such as a methyl group, hardly affects the intensity or the position of maximum absorbance. Complex cycloalkanols, however, have relatively low absorptivity. In the case of benzylcyclohexanol, there is also a large shift in the wavelength of maximum absorbance.

Cyclohexane derivatives which give very faint colors are 4-methylcyclohexene, 4-methylcyclohexanone, and cyclohexylamine.

The high absorptivity and the adherence to Beer's law of cycloalkanol compounds of widely differing structure indicates that the method described in this note is useful for determination of less than 100 μg of most cycloalkanol compounds. The method is simple and rapid and requires no special equipment or reagents.

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Labeling of ^{131}I-Hippuran by Ultraviolet Excitation

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The successful application of ultraviolet irradiation of rose bengal *(1)* with radioiodine encouraged us to investigate the possibility of labeling hippuran. The spectrophotometric study of hippuran solution before and after ultraviolet irradiation for 6 hours at 25°C revealed that the two spectra are identical and show maximum absorption at 273 mμ.

EXPERIMENTAL METHODS

The labeling of hippuran was studied using two media, one aqueous and the other ethereal.

(a) Labeling in aqueous medium. A 2-ml portion of $\simeq$0.14 *M* hippuran solution in double distilled water with trace H_2O_2 (30% w/v) (2 lambdas) and 0.1 mCi of $Na^{131}I$ was irradiated by ultraviolet radiation in a quartz transparent tube (0.8 cm diam and 4 cm length). The tube was closed by a ground stopper and placed at 2 cm distance from a Hanovia high pressure quartz mercury lamp. A fan was used to lower the temperature to 24°C. The yield of exchange was determined by paper chromatography using *n*-butanol:acetic acid:water (4:1:1) as solvent. The effect of time of irradiation on the yield of exchange and the change in pH of the aqueous solution during irradiation were investigated.

(b) Labeling in organic solvent. It was found that ether can extract both iodine and *ortho*-iodohippuric acid. A hippuran solution adjusted to pH 3 with 1 *N* HCl can be extracted by ether (80%) due to the conversion of the sodium hippurate to *ortho*-iodohippuric acid.

The labeling procedure consists in adding to the aqueous solution (1 ml of 0.1 mCi of $Na^{131}I$, 1 mg of KI, and 0.5 of KIO_3) 2 ml of ether and acidifying with few drops 1 *N* HCl, whereas all activity is extracted in the ether layer. The 2 ml of ether containing ^{131}I is mixed with 8 ml of ether containing 40 mg of *ortho*-iodohippuric acid in a quartz tube which is closed with a cooling condenser, then we proceed as previously.

(c) The light source. The UV lamp used for irradiation is the Han-

ovia utility quartz lamp SH 616A.[1] It is a 100 W high pressure Hg vapor lamp operating on 220 V, 50 cycle AC through a reactive transformer. The arc lamp generates a high UV over a broad spectral range. In fact the complete spectrum is transmitted : 1894–2800Å (far), 2800–3200Å (middle) and 3200–4000Å (near). Some of the principal lines with their quantum energy are given in Table 1. The short wavelength lines are absorbed by the air gap by the quartz.

(d) Purification of the final product. The ether layer is evaporated and the residue is dissolved in dilute alkali whereas the irradiated aqueous solution is adjusted to pH 8. Both solutions could be purified from unreacted iodide and iodine atoms by passing either on a column of silver chloride adosrbed on silica gel (0.5 ϕ × 10 cm) or on a small

TABLE 1

SOME PRINCIPAL RESONANCE LINES WITH THEIR QUANTUM ENERGY OF THE SH.616A Hg LAMP

Line (A):	3660	3130	2967	2752	2571	2482
Quantum (kcal)	78.11	91.34	96.35	103.52	111.2	115.19
Energy (eV)	3.48	3.97	4.19	4.5	4.83	5

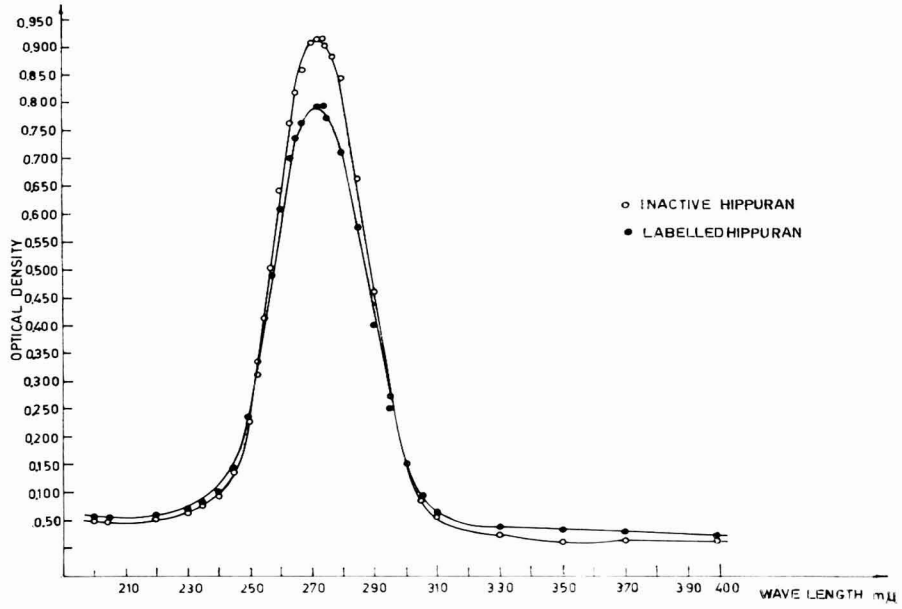

FIG. 1. Ultraviolet spectrum of inactive and labeled hippuran.

[1] Hanovia Inc. Lamp Division Newark 5, New Jersey Special Energy Distribution of quartz Hg-vapor Arc tube 616A-13 type SH.

column (0.2 ϕ × 2 cm) of Dowex 1-X8 in the chloride form. Elution of adsorbed labeled hippuran could be achieved by eluting with 0.09% saline. The radiochemical purity of the final product exceeds 99%, it was checked by spectrophotometry and paper chromatography. A maximum absorption spectra is attained at 273 mμ which agrees with that of the inactive hippuran of same concentration (Figs. 1 and 2).

RESULTS AND DISCUSSION

By ultraviolet irradiation of the two hippuran solutions, aqueous and ethereal, at 24°C the yield of exchange at different time intervals of irradiation is illustrated in Table 2. A blank solution of 0.14 *M* hippuran,

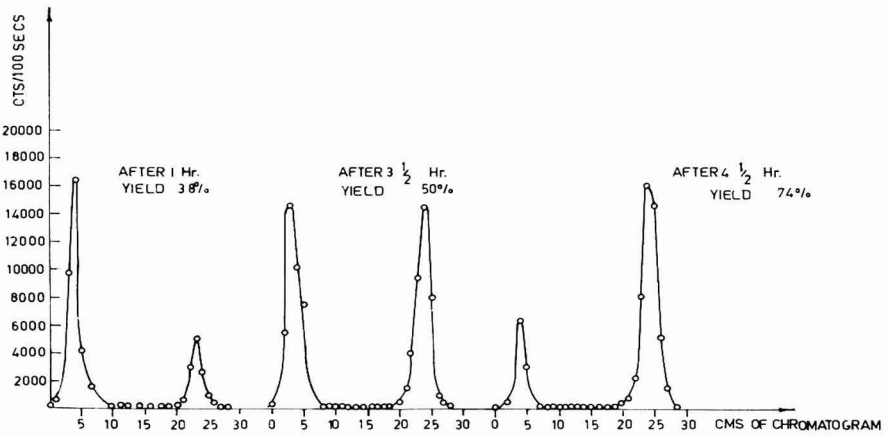

FIG. 2. Paper chromatogram of irradiated *ortho*-oiodohippuric acid with radioiodine in ether.

TABLE 2

EFFECT OF IRRADIATION TIME BY UV ON THE YIELD OF EXCHANGE AND pH OF SOLUTION

~0.14 *M* Aqueous solution				~0.01 *M* Ethereal solution		
Time (min)	pH	Yield (%) of labeled hippuran	Observation	Time (min)	Yield (%) of labeled hippuran	Observation
15	6.3	22		30	32	
30	6	28	Slight yellow coloration	60	38	Faint yellow coloration
60	5.7	44		210	50	
120	5.4	57		270	74	
180	5.3	66				
240	5.25	70				

containing all chemicals and radioactivity, but not irradiated, contained 5% of labeled hippuran after 300 minutes. The final labeled hippuran has a similar spectrum as the inactive one which means that no side-products were detected by either spectrophotometry or chromatography. The ethereal solution of hippuran which is 10 times less concentrated gives the same yield of exchange as the aqueous one, with very faint coloration meaning less photolysis and higher purity of the final product.

A weaker solution of hippuran than 0.14 *M* gave a lower yield of exchange. Hippuran is a very stable organic compound with a high m.p. 172°C. The iodine content is about 35% existing in a stable organically bound state. The faint yellow coloration obtained by UV irradiation of the exchange mixture may be mainly due to slight photolysis on its lowest energy bond, viz, the C–I bond *(2)* forming a dissociated radical and an excited iodine atom as:

$$RI + h\nu \rightleftharpoons R^{\cdot} + I^{\cdot}.$$

This has been checked by irradiating a blank hippuran solution of same concentration without additives, whereas the same yellow coloration as well as decrease of pH are noticed. The quantum yield of the photolysis step is only about 10^{-2} ($\phi = 0.02$). This low yield in a liquid is due in that radicals formed in a dissociation reaction are surrounded by a cage of closely packed molecules. They recombine within a time not much greater than 10^{-13} of a second, a typical vibration period, even if they may diffuse away from each other the chance is high that the same pair soon meet again and recombine (geminative recombination).

The presence of a trace of an oxidizing agent catalyzed the labeling reaction. In a previous study *(1)* of labeling rose bengal with ^{131}I by UV, absence of H_2O_2 lowered the yield from 70 to 17% during 4-hours irradiation time.

The process resulting from the absorption of UV radiation by iodide ions may be described, similarly to Hayon *(3)*, as follows:

$$(I^{-}H_2O) \xrightarrow{h\nu} (I^{-}H_2O)^{\cdot}, \quad (1)$$

$$(I^{-}H_2O)^{\cdot} \longrightarrow (I^{-}H_2O) + \text{heat}, \quad (2)$$

$$(I^{-}H_2O)^{\cdot} \longrightarrow I + H_2O^{-}, \quad (3)$$

$$(I^{-}H_2O)^{\cdot} \longrightarrow I + H + OH^{-}. \quad (4)$$

This means that atomic radioiodine is formed as well as H+ and HI, as found by McDonald *(5)*, on irradiating iodobenzene. Trace iodide solution under UV irradiation does not show any coloration nor decrease in

pH due to the very low concentration of the iodide ion. The decrease in pH may be mainly due to the photolysis of hippuran where the liberated iodine atom can undergo the radiolytic steps described by Hayon.

Therefore, we can summarize the overall exchange mechanism as:

$$I_2^* \xrightarrow{h\nu} 2I^*, \quad (1)$$

$$RI + I^* \longrightarrow RI^* + I, \quad (2)$$

$$I + I^* \longrightarrow I + I^*, \quad (3)$$

$$I^* + I^* \longrightarrow I_2^*. \quad (4)$$

Noyes *(5)* proposed 2 alternative mechanisms for such exchange reactions thermodynamically similar: one involves a direct substitution by an iodine atom and the other involves the formation of a carbonium radical.

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MICROCHEMICAL JOURNAL **14,** 486–489 (1969)

The Rapid and Direct Determination of Thiosulfate in the Presence of Sulfite Assayed as Barium Salts

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The thiosulfate ion may be determined by a variety of methods-*(2)–(8)* but its measurement in the presence of sulfite *(9,10)* has not been directly accomplished. The methods which allow for the presence of sulfite are indirect and those performed on the milligram level will result in considerable error because of the alkalinity of soluble sulfites. The reaction between thiosulfate and hydrogen peroxide appeared promising, however, and the reaction may be represented as follows:

$$Na_2S_2O_3 + 4H_2O_2 \longrightarrow Na_2SO_4 + 3H_2O + H_2SO_4. \quad (1)$$

One mole of sulfuric acid is liberated per mole of thiosulfate. The same reaction with sodium sulfite can be represented as follows:

$$Na_2SO_3 + H_2O_2 \longrightarrow Na_2SO_4 + H_2O. \quad (2)$$

This reaction yields neutral products, but sodium sulfite is alkaline enough to seriously interfere with reaction (1).

In the particular case at hand, both compounds were in the form of their barium salts and the peroxide reaction was shown to be as follows:

$$BaSO_3 + H_2O_2 \longrightarrow BaSO_4 + H_2O. \quad (3)$$

$$BaS_2O_3 + 4H_2O_2 \longrightarrow BaSO_4 + 3H_2O + H_2SO_4. \quad (4)$$

Samples of pure barium thiosulfate and barium sulfite were prepared. The barium thiosulfate crystallized as the monohydrate and after drying to constant weight was weighed as the anhydrous salt. The peroxide reaction was applied to both compounds and the sulfuric acid was determined alkalimetrically and by titration with barium perchlorate using thorin as indicator. The results are shown in Tables 1–3.

Several mixtures of barium thiosulfate monohydrate and anhydrous barium sulfite were prepared. The concentration of the thiosulfate in the mixtures was adjusted to give 80, 50, 20, 10, and 0%, respectively as the anhydrous form. The first sample in Table 4 represents only the pure monohydrate of barium thiosulfate, with no sulfite added.

TABLE 1

TITRATION OF ANHYDROUS BaS_2O_3 USING NaOH

Sample wt	Vol 0.01 NaOH	(%)
12.130	9.74	100.1
5.625	4.50	99.7
6.877	5.50	99.8

TABLE 2

TITRATION OF ANHYDROUS BaS_2O_3 USING $Ba(ClO_4)_2$

Sample wt	Vol 0.01 *N* $Ba(ClO_4)_2$	(%)
5.575	4.45	99.5
8.343	6.68	99.8
4.038	3.25	100.3

TABLE 3

TITRATION OF $BaSO_3$

Sample wt	Titer	BaS_2O_3 (%)
12.14	0.05[a]	0.4
10.78	0.04[b]	0.4
11.45	0.03[a]	0.3
10.41	0.03[b]	0.3

[a] Titration with NaOH.
[b] Titration with $Ba(ClO_4)_2$.

TABLE 4

ANALYSIS OF MIXTURES

		As anhydrous BaS_2O_3 (%)	
Sample wt	Titer	Found	Calculated
10.59[a]	7.90	93.0	93.3
11.72	7.55	80.3	80.0
10.46	4.22	50.3	50.0
9.414[b]	3.80	50.3	50.0
19.87	3.25	20.4	20.0
10.25	0.80	10.4	10.0
14.15	0.05	0.4	0.0

[a] Barium thiosulfate monohydrate.
[b] Barium thiosulfate, barium sulfite, and barium sulfate.

PROCEDURE

Weigh out 10–20 mg of sample into a 250-ml flask. Add 10–20 ml of water and 5 ml of 10% H^2O^2 and shake the flask. Allow to boil 5–10 minutes on a hot plate. Cool, and (a) titrate with 0.01 *N* NaOH to the phenolphthalein end point, or (b) add about 50–60 ml of 95% ethanol and titrate with 0.01 *N* barium standard solution using a few drops of the thorin indicator and add the titrant slowly near the end point *(1)*. The use of a magnetic stirrer is advocated especially because of the increased formation of barium sulfate during titration.

$$\frac{\text{Vol. titer} \times 1.247 \times 100}{\text{Wt (mg)}} = \% \text{ anhydrous barium thiosulfate,}$$

$$\frac{\text{Vol. titer} \times 1.337 \times 100}{\text{Wt (mg)}} = \text{hydrated } BaS_2O_3.$$

Reagents and Apparatus

10% H^2O^2 solution made by dilution of reagent grade 30% hydrogen peroxide.

250-ml glass stoppered flasks.

Standard 0.01 *N* NaOH.

Standard 0.01 *N* Ba $(ClO_4)_2$ [or $BaCl_2$].

Thorin 0.02% in water [2- (2-hydroxy-3, 6-disulfo-1-naphthylazo) benzene arsonic acid].

DISCUSSION

The proposed method holds for the mixture of any proportions of barium sulfite and barium thiosulfate. Even in the presence of barium sulfate, this method is still valid since no soluble sulfate can be formed therefrom. In the case of the soluble sodium salts, the estimation of alkalimetry can be difficult because of the alkalinity of the sulfite, particularly in the milligram range. Attempts to apply this technique directly to sodium salts *(4, 6)* were abandoned when unsatisfactory results were obtained. It is quite possible, however, that when dealing with soluble sulfites and thiosulfates, one may quantitatively form barium precipitates of these anions in perhaps an 80% ethanol solution and, after separation, perform the peroxide reaction. Techniques for the thiosulfate determination *(3, 9)* using an alkaline ferricyanide oxidation with osmium tetroxide as catalyst obviously cannot be used in the presence of sulfites. Iodometric methods are also not applicable because both substances react with iodine.

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MICROCHEMICAL JOURNAL **14**, 490–499 (1969)

Determination of Trace Cobalt in High-Purity Nickel

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1-Pyridylazo-2-naphthol (PAN) since its introduction as a photometric reagent by Cheng and Bray *(1)* has found widespread use in the determination of metal ions. The field has been reviewed by several authors *(2, 7, 8,)* and a most exhaustive evaluation was made by H. Shibata *(9)*. Although, per se, not a specific or even reasonably selective agent, PAN, when applied under appropriate conditions and in combination with masking agents, can be made to react quite selectively. This approach has been used to resolve some tricky analytical problems, including the determination of zinc traces in cadmium and its salts *(6)*.

Almost all metal–PAN complexes exhibit red colors, only palladium and cobalt(III) form green complexes. Inasmuch as palladium is only present in special materials, one may expect to employ the green coloration of the cobalt(III)–PAN complex for some highly selective or even specific tests and determinations. Such expectation is strengthened by the fact that the green complex is extremely robust, and once formed, resists attack by even highly concentrated acids, under which conditions all the red PAN complexes are completely destroyed.

Unfortunately, on a second look the situation is not entirely simple. The green complex forms only via oxidation of the cobalt(II)–PAN complex. This complex is red, not robust and of moderate stability like most of the other metal PAN complexes. Consequently, it will form in alkaline, neutral, or at best, slightly acidic solution under which conditions other metals present will successfully compete with the cobalt(II) for possession of the PAN. Furthermore, the oxidation is quite slow and, thus, kinetic aspects enter and complicate the picture. Application of masking agents seems, therefore, mandatory to improve the selectivity. Unfortunately, most masking agents, when applied in the conventional manner, fail because either the masking of the interferences is inadequate or the cobalt is masked, too.

When, however, a new masking approach was employed *(3, 4)* it became possible to use PAN for the determination of cobalt in nickel, up to a nickel-to-cobalt molar ratio of 20,000. This new technique, called

substoichiometric masking, in brief, operates on the following premise. An interfering species causes unacceptable analytical results only if it is present in amounts exceeding a certain ratio to the species determined, that is, exceeding the interference threshold. If that threshold is overstepped, exclusion of the interference becomes necessary and masking may be considered a possibility. However, execution in the conventional manner, that is, with the masking agent added in excess, often fails because the ion to be determined is also masked. But in many cases it is feasible to add the masking agent in an amount less than equivalent to the amount of interfering material and thereby to reduce the ratio of "free" interfering species to sought-for substance below the interference threshold. A determination then becomes possible. Such a case is the cobalt–nickel system with EDTA as the masking agent.

Substoichiometric masking worked very well with this system but by necessity there was a certain limit in the ratios of nickel to cobalt that could be handled and for the following reasons. The interference threshold for the determination of cobalt in the presence of nickel is Ni:Co about 200. For substoichiometric masking, it is necessary to add EDTA in an amount that complexes enough nickel to reduce the ratio of free nickel to cobalt below the threshold value. This amount of EDTA is conveniently determined by an EDTA titration. This titration can be considered to be reliable to 0.5% relative. The interference ratio in the sample should be within twice that amount, that is, within 1% to assure that neither too much EDTA has been added leading to masking of the cobalt nor too little has been added causing too much nickel to be free. From this 1% margin, the limit for Ni:Co is seen to be 20,000:1. The improvement, though impressive, falls far short of utilizing the sensitivity of the analytical reaction between PAN and cobalt. It seemed, therefore, to be of value to apply the knowledge gained in the previous investigations to expand the study of the system and to possibly develop a method that would allow much higher ratios. The initial reasoning was based on the following facts.

The EDTA complexes of cobalt and nickel, although of nearly identical stability, differ markedly in their kinetic behavior. Nickel EDTA forms slowly and once formed, is robust. This property has been employed as the basis for a determination of nickel in the presence of cobalt *(5)*. The principle of this titration was to add EDTA in excess, next to cool the solution with ice to zero degrees and then to back-titrate with a bismuth solution. The bismuth reacts rapidly with the free EDTA and almost instantly expels the cobalt from its EDTA complex. The nickel remains "frozen in" the complex and even after prolonged standing with excess bismuth, only minute amounts are released. The nickel

determination can be performed in the presence of some other metals, too. Lead, zinc, cadmium, e.g., are also immediately expelled. Copper(II) reacts only slowly. Ions that form strong EDTA complexes, like zirconium(IV) or iron(III), are not replaced by bismuth and are co-titrated. These facts suggested the following approach. To the solution containing the nickel salt is added EDTA and in slight excess, next the solution is cooled, and then enough bismuth solution is added to be more than equivalent to the EDTA that remained free plus those metals that can be displaced from the EDTA complexes. These metals including, of course, the cobalt and any remaining free bismuth, can now be reacted with PAN, the formation of the green cobalt(III)–PAN complex is accomplished, and the determination is finished photometrically.

Preliminary experiments indicated the feasibility of the approach and most importantly, showed that cooling of the solution to zero degrees is not necessary. Even at room temperature, the release of nickel is sufficiently slow to permit completion of the formation of the green cobalt complex before interference due to nickel occurs.

EXPERIMENTAL METHODS

Reagents

Nickel solution 2.0 *F* was prepared by dissolving 59.6 g of J. T. Baker nickel shot (99.9% pure) in a minimum amount of 1:1 nitric acid and diluting to 500 ml.

Cobalt solution 0.100 *F* was prepared by dissolving 1.473 g of cobalt metal (99.9% pure) in a minimum amount of 1:1 nitric acid and diluting to 250 ml. A 2.00×10^{-4} *F* cobalt solution was obtained by appropriate dilution of this stock solution.

Bismuth solution 0.10 *F* was prepared by dissolving 48.3 g of bismuth nitrate in water to which several ml of concentrated nitric acid had been added, and diluting to 1 liter.

The EDTA solution 0.15 *F* was prepared by dissolving 40.5 g of the disodium salt dihydrate in water and diluting to 1 liter.

The PAN solution 0.010 *F* was prepared by dissolving 0.622 g of the solid reagent grade indicator in 95% ethanol and diluting with ethanol to 250 ml.

Murexide indicator powder was prepared by finely grinding 2 g of murexide with 100 g of sucrose.

Buffer, pH 10, was prepared by dissolving 70 g of reagent grade ammonium chloride in 570 ml of concentrated aqueous ammonia and sufficient water to give a final volume of 1 liter.

Procedure

(i) Dissolve sufficient sample in 1:1 nitric acid to provide 20 to 100 μg of cobalt. About 20 ml of 1:1 nitric acid is required for each gram of metallic nickel dissolved.

(ii) Add aquenous ammonia until all the nickel has been converted to the amine complex, then add 10–15 ml of buffer pH 10.

(iii) Add approximately 95% of the stoichiometrically required quantity of solid disodium EDTA. The stoichiometric requirement is 6.34 g of $Na_2H_2Y \cdot 2H_2O$/g of nickel.) Heat the solution and add water until all the EDTA salt is dissolved. The total volume should not exceed 100 ml.

(iv) To the hot solution add a spatula tip of murexide indicator and titrate with 0.15 *F* EDTA until a permanent violet end-point color is reached. Add 1 ml excess EDTA.

(v) Add about 1 g of tartaric acid and allow the solution to cool to room temperature.

(vi) Add any additional masking agents intended and adjust the "pH" to about 2.5. To mask iron(III), add 1 ml of 85% phosphoric acid; to mask copper(II) (if present at a level above 0.2%) and palladium, add 1–3 ml of a saturated aqueous solution of thiourea. The concept of pH as defined in aqueous solution is not intended. Rather the term "pH" is used to indicate the reading of the Beckman Zeromatic II obtained when a glass electrode and a calomel electrode are used with the meter and are immersed in the alcoholic test solution.

(vii) Add 2 ml of 0.1 *F* bismuth nitrate and adjust the "pH" to a value between 1.8 and 2.0. Complete this step and proceed to the next one without delay.

(viii) Add sufficient ethanol to make the solution 50% in that solvent and readjust the "pH" to a value of 2.0. Add the ethanol within 1 minute after the bismuth addition in the preceding step.

(ix) Add 2 ml of 0.01 *F* PAN and allow to stand for 1 hour.

(x) Estimate the volume of the sample preparation at hand in Step ix and place that number of milliliters of water into a separatory funnel; next add 10 ml of 2 *F* HC1 per 100 ml of sample preparation, and then the sample preparation itself.

(xi) Extract with four 5-ml portions of chloroform and collect the extracts in a 50-ml volumetric flask. The solution may be stored, at this point in the procedure, for at least 24 hours without deterioration. If desired, the volume may be reduced by evaporation at this point.

(xii) Add 2 ml of concentrated HCl and dilute to the mark with 95% ethanol.

(xiii) Measure the absorbance of the solution at 625 nm against a blank. After addition of acid the absorbance of the solution should be measured within 1 hour.

Calibration Curve

Prepare a calibration curve by following the above procedure from Step ii for solutions containing known quantities of cobalt nitrate. The cobalt content of these standard solutions should be from 10 to 100 μg in about 50 ml.

DISCUSSION

The procedure described above involves initially complexing both cobalt and nickel with EDTA. Inasmuch as an uncontrolled excess of EDTA is undesirable, the formation of the complexes is conducted as a titration. In contrast to substoichiometric masking *(4)*, the present method is not limited by the precision of the titration, and normally a small excess of EDTA is deliberately added. At a pH of about 2, cobalt(II) is displaced rapidly from its EDTA complex by bismuth. Nickel reacts only slowly. The amount of bismuth added should be in excess of the amount required to complex the excess of EDTA added and to quantitatively displace cobalt. The bismuth excess is deactivated by the addition of alcohol, and thereby the nickel displacement reaction is retarded.

After the nickel is thus masked by the EDTA and the cobalt is freed, PAN is added to the solution and complexation with cobalt proceeds rapidly. Very large excesses of bismuth are, of course, not desirable because of the competition with cobalt for the PAN. Once the cobalt(III)–PAN complex is completely formed (approx 15 minutes) it can be extracted into chloroform and its absorbance measured at 625 nm.

Some other metal–PAN complexes will be co-extracted. The displacement of nickel by bismuth from its EDTA complex although extremely slow proceeds to a certain extent. Consequently, when the PAN is added some nickel–PAN complex will form. The excess bismuth will also form a PAN complex. These species are decomposed by treating the solution before extraction with a few millimeters of concentrated HCl or saturated EDTA solution; the cobalt(III)–PAN complex is not affected.

The extraction of the cobalt(III)–PAN complex proceeds smoothly from the acidic aqueous solution into chloroform. Quantitative recovery of cobalt is achieved by three extractions from an aqueous phase about 0.1 *F* in HCl. The combined extracts are treated directly with concentrated HCl to destroy any remaining traces of potentially interfering

metal panates, and the extract is diluted to volume with ethanol to achieve phase homogeneity.

Since the masking of nickel is based on a kinetic phenomenon, some care must be exercised with regard to the passage of time. In particular, the solution must not be allowed to stand after the addition of excess bismuth at Step vii of the procedure. The maximum time before the ethanol addition varies somewhat with temperature, but is about 1 minute at 20°C. This time can be lengthened by cooling the solution before the addition of bismuth. After addition of PAN, sufficient time should be allowed for complete formation of the cobalt(III)–PAN complex. Normally about 1 hour is required. Once formed and extracted, the green complex is stable for at least 1 hour and the absorbance can be measured at any time during this period.

The feasibility of lowering the amount of cobalt required for the determination was demonstrated. A volume reduction step was added in which the regular 50-ml volume of the final extract was reduced to 25 ml. Specifically constructed 25-ml volumetric with 25-ml bulbs in the flask neck were used. A ® Teflon ring hanging by a glass fiber was inserted in the flask to serve as a "boiling stone," and the flask was heated in a warm air current. The solvent stripping had to be accomplished before the addition of concentrated hydrochloride acid in the final step of the procedure; then full retention of the cobalt(III)–PAN absorbance was observed. Increased pathlength for the photometric measurement would further increase sensitivity.

INTERFERENCES

One of the most attractive features of the method here described is its insensitivity to the presence of foreign ions. Only very few metals are able to react with PAN under the conditions established in the procedure. The PAN complexes of most of these metals are unstable in the presence of strong acid and are destroyed in the acidification step. Palladium(II) is the only exception; however, this metal can be effectively masked at levels up to at least 0.5% (based on nickel) by the addition of thiourea. About 2% copper can be masked by the same reagent. At least 0.3% iron can be masked by the addition of phosphate (as phosphoric acid). The presence of Sn(IV) causes low results. The mechanism is not clear, but indications are that the efficiency of the extraction process is affected. The interference was negligible for tin contents below 0.1%. Other metal ions require no special consideration if present in moderate amounts that is, below 0.5%.

Some reducing agents were found to interfere. Mild reducing agents apparently prevent the complete oxidation of cobalt to the tervalent

state, while strong reducing agents destroy PAN. In either case, the interference can be eliminated by boiling the sample solution with nitric acid before starting the analytical procedure. Other species likely to be present in the solution including chloride, nitrate, and sulfate did not interfere even when present in abnormally high concentrations. Large excesses of PAN produce high blanks leading to loss of precision.

RESULTS

The results of several photometric determinations of cobalt, alone and in the presence of nickel, are presented in Table 1. The value of "cobalt taken" was corrected in cases were some cobalt was introduced as impurity from the nickel stock solution. The nickel salt used to prepare the stock solution was analyzed by the method here presented. The result was 5.4 ppm of cobalt with the analyses showing a standard deviation of 0.16 ppm. This result is typical of the method, and the value is in close agreement with that obtained by polarographic analysis of the nickel. Further confirmation of the accuracy of these determinations was achieved by comparison of these results with those obtained using a synthetic sample prepared by addition of cobalt to a "cobalt free" nickel prepared for the purpose.

TABLE 1

REPRESENTATIVE RESULTS OF THE PHOTOMETRIC DETERMINATION OF COBALT IN NICKEL

Cobalt (μg)			
Taken	Found	Difference	Ni:Co molar ratio
11.0	10.8	−0.2	—
11.6	11.6	0.0	—
14.5	14.7	+0.2	—
22.0	21.7	−0.3	—
23.2	23.3	+0.1	—
66.0	66.1	+0.1	—
32.8	33.0	+0.2	22,500
1.4	1.4	0.0	180,000
2.7	2.6	−0.1	180,000
4.0	4.0	0.0	180,000
5.3	4.9	−0.4	180,000
5.3	4.6	−0.7	200,000
2.7	2.7	0.0	240,000
1.4	1.5	+0.1	290,000
5.3	5.6	+0.3	420,000
1.4	1.5	+0.1	670,000
0.7	0.9	+0.2	1,170,000
0.4	0.3	−0.1	2,040,000

Table 2 contains typical results of cobalt determinations when other elements were present in addition to the nickel. The level of foreign ion in the synthetic sample analyzed is indicated as a percentage based on nickel.

TABLE 2

REPRESENTATIVE RESULTS OF DETERMINATION OF COBALT IN PRESENCE OF FOREIGN IONS IN NICKEL (1-g sample)

Cobalt (μg)			Foreign ion (%)	
Taken	Found	Difference	Symbol	Level
32.8	33.0	+0.2	Zn	0.09
30.9	31.5	+0.6	Zn	0.36
25.7	25.5	−0.2	Mn	0.05
25.7	25.3	−0.4	Pb	0.18
25.7	25.3	−0.4	Pb	0.22
25.7	24.7	−1.0	Pb	0.54
25.7	25.3	−0.4	Pb	0.66
25.7	25.0	−0.7	In	0.97
25.7	25.3	−0.4	Tl	1.70
37.3	32.9	+0.2	Cu	0.13
37.3	37.9	+0.2	Cu	0.27[a]
37.3	36.2	−1.1	Cu	1.3[a]
23.2	23.1	−0.1	Pd	0.04[a]
25.7	24.8	+0.9	Pd	0.45[a]
25.7	25.3	−0.4	V	0.02
25.7	24.1	−1.6	V	0.02
37.3	37.5	+0.2	Fe	0.35[b]
53.9	53.0	−0.9	Fe	0.05[b]
25.0	25.0	+1.0	Fe	0.33[b]
25.7	24.2	−1.5	Sn(IV)	0.12
25.7	24.6	−1.1	Sn(IV)	0.12
25.7	22.0	−3.7	Sn(IV)	0.50

[a] Thiourea masking.
[b] Phosphate masking.

Preparation of Cobalt-Free Nickel

An exceptionally pure sample of nickel was desired for use when evaluating the analytical method here described. It was concluded that the analytical procedure could be adapted to obtain such a material. Using a large amount of reagent-grade nickel, the procedure was followed through Step xi. At this point, the aqueous layer containing nickel—EDTA was separated from the chloroform extracts and concentrated by evaporation. The first solid to appear was crystalline ammonium chloride. On further evaporation, nickel–EDTA crystals began to form.

These were collected, and recrystallized several times from a minimum amount of water. The EDTA was then destroyed by heating with concentrated sulfuric acid, and metallic nickel was recovered by electrodeposition from an ammonium tartrate solution. No traces of cobalt could be detected in the metallic nickel. The nickel so prepared was used in the preparation of synthetic samples of high nickel to cobalt ratios. The results of the analysis of some of these samples are included in the data of Table 1.

CONCLUSIONS

The method here described has been demonstrated to be highly accurate and reliable for the determination of cobalt in high-purity nickel. The method is highly sensitive, allowing the determination of cobalt in the part per million range in nickel and in the sub-part per million range if a simple concentration step is included. Perhaps the most attractive feature of the method described is its insensitivity to foreign substances, at least as long as they are at the level to be expected in pure nickel.

SUMMARY

Cobalt in trace amounts in nickel was determined photometrically. The metals were initially converted to the EDTA complexes. Cobalt was then displaced from its EDTA complex by bismuth and extracted into chloroform as the cobalt(III)–PAN complex. The nickel was masked kinetically as the EDTA complex. In the extract, small amounts of the PAN complexes of nickel and bismuth were destroyed by an acid treatment and cobalt was determined photometrically. The method is highly accurate, reliable, and applicable to parts per million of cobalt in nickel or, with a concentration step included, to parts per 10 million. No interferences were encountered from foreign ions at the levels expected in high-purity nickel. Several characteristics of the analytical system have been exploited to attain the selectivity and sensitivity: the high stability, robust nature, large absorptivity, and the wavelength of the absorbance mixiumum of the cobalt(III)–PAN complex, and the low reaction rate of the nickel–EDTA complex at room temperature.

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Book Reviews

Survey of Analytical Chemistry. By SIDNEY SIGGIA, McGraw-Hill, New York, 1968. xiii + 304 pp. $9.95.

At first glance one wonders how such an ambitious title can be achieved in so short a book. As the reader studies the text, it becomes evident that almost every phase of this ever expanding field is touched upon briefly and that recourse to the literature, text books and monographs listed after each Chapter must be made.

A great deal of emphasis is placed on definition of the problem and the plan of action required to solve the problem. This point of emphasis is well taken and is lacking in many books of this type. Specific examples of analytical problems are cited and discussed and their resolution described. Throughout most of the Chapters are tables of summary evaluation of a wide range of methods. These tables include such practical matters such as accuracy and precision of the concentration ranges, length of analyses, cost of equipment, sample limitations, interferences, etc. By such devices and by other information supplied by the author and his associates, the interrelation of the many different approaches are elucidated.

This book approaches the enormous task of summarizing the vast amount of information involved in this constantly changing field from the standpoint of problem solving rather than the specific techniques which are available in great abundance today.

The final chapter on automated analysis is much too short and too general to serve the purpose it was intended to serve. Some information as to the tremendous use these instruments have been put to, as for example in the medical and diagnostic area, where speed is of paramount importance could have been detailed. In this chapter also could have been discussed the now widely accepted use of elemental analyzers for CHN, which subject was merely mentioned under the elemental analysis chapter.

With only a few minor omissions evident, the author has done a creditable job in tackling such a formidable task and which can benefit not only the student but can be of help to an investigator who is not an analytical chemist but who in the pursuit of a problem needs analytical support. By the use of such a book he can make a reasonable decision as to what method or methods to use.

JOSEPH F. ALICINO, *Squibb Institute for Medical Research, New Brunswick, New Jersey 08902*

Analytical Serology of Microorganisms. Vol. 1. Edited by J. B. G. KWAPINSKI. Wiley, New York, 1969. x + 681 pp. $25.00.

This is the first of two volumes describing the use of serology in the study of microorganisms. Protozoa, fungi, actinomycetes, mycobacteria, spirochetes, mycoplasma, rickettsia, chlamydia, and viruses are included in Vol. 1. The second volume will cover 17 groups of gram-positive and gram-negative bacteria. The editor has asked each contributor to (a) describe serological characteristics of his particular microbial group, (b) indicate the present status of the application of serology in identification and classification of the group, and (c) predict areas of future development. In general, the contributors have fulfilled their mission

competently; especially valuable are the bibliographies (usually through 1967) for each chapter.

The use in the title of the term 'analytical' is puzzling; the text is descriptive rather than analytical but perhaps the word was used to mean "diagnostic." Oddly, "analytical" is to be used for some of the groups in Vol. 2 (e. g., *Shigella*), but not for others (e. g., *Brucella*); yet the use of diagnostic serology is as important in the study of *Brucella* as in that of *Shigella*. The indices for the various chapters vary in quantity and utility; for example, one column of entries is given for every 6 pages of material on mycobacteria and nocardia but only for every 28 pages on spirochetes and for every 40 pages on rickettsia. The single entry for infectious mononucleosis refers to a fragmentary discussion on p. 508, but omitted entirely is any entry to a much more useful section on this disease on pp. 458–460.

Volume 1 contains little overlap among the various chapters except for one annoying case: the chlamydia have their own chapter but also are redescribed in several pages of the chapter on animal viruses. Although it has been apparent for 20 years that the chlamydia are *not* viruses, older virologists seem to have a sentimental attachment for the group; in the present case, the organisms are referred to as viruses (without the use of quotation marks!) 21 times. I found no typographical errors in the text but the publishers are somewhat vague as to the location of their editor; on the bookjacket, he is at Mississippi State; on the title page, in Microbiology at Manitoba; and on p. 1, in Bacteriology and Immunology at Manitoba.

Aside from these minor lapses, the volume is well written and well edited. It will be of considerable usefulness to research and diagnostic microbiologists both in applied and nonapplied fields.

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Reagent Grade Water: How, When, and Why? By MARTHA WINSTEAD. Am. Soc. Med. Technologists, Austin, Texas, 1967. 142 pp.

Water is the most used liquid chemical in the laboratory! However, high-quality water, unlike most laboratory-use chemicals, is obtained usually by on-site purification. A well-managed laboratory should have at least one person either on its staff or that of a support group who has some knowledge of how "pure" water can be obtained, how it can be stored and transported, what the effects of source water are on equipment and effluent, what tests can be performed, and what constitutes a minimum quality control program. The monograph under review, at some installations, might well be required reading for personnel expected to have answers to these questions.

The reviewer recommends addition of this monograph to the book collections of laboratories, whether analytical, chemical, physical, biochemical, or clinical.

Miss Winstead's rapport with the subject stems from the "water problem" faced during 1962–1966 by the South Bend Medical Foundation: Differences for control sera values developed between the seven laboratory installations maintained by that organization. Reagent solutions formulated centrally were sent to the other laboratories, but each used its own water in the analytical procedures. Each hospital's still was found different either in structure or the kind of feed water. Much of the information presented in this monograph was developed during the course of resolving this problem.

The writing at times is personal with the use of "we" or "our experience" in stating the findings and views of the staff of the South Bend Medical Foundation. The reviewer found this style refreshing and commends its use of monographs of this type.

The content of the work is suggested by the three chapter headings: How Can Pure Water be Obtained? When Does Reagent Grade Water Meet the Specifications? Why Test Reagent Grade Water? The first chapter considers the various approaches to the purifications of water and provides short descriptions of some stills and de-ionizers commercially available in the United States. The second chapter presents test procedures for water applicable to many laboratory situations and defines a minimum testing program. The third chapter includes most instructive discussions of the effects of water quality on the performance and results obtained with many common clinical laboratory procedures. Over 150 references are cited in the bibliography.

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The Particle Analyst. Compiled by WALTER C. MCCRONE, RONALD G. DRAFTZ, AND JOHN G. DELLY. Ann Arbor Humphrey Science Publishers, Inc. Company, Ann Arbor, Michigan 1968. (Loose-leaf). $75.00

This book started as a journal issued twice a month but at the end of the first year it was discontinued and the publisher decided to present the published issues as a book. Accordingly the 24 issues were brought together in a handsome loose-leaf binder and a table of contents and an index were added. Covering the "fine points" of microscopy, it serves as a supplement to "The Particle Atlas" by the same authors (reviewed in *Microchem. J.* **13,** 347, 1968) and every owner of the Atlas will want it.

The chapters cover a variety of subjects in the microscopy laboratory. The illustrations, mostly in color but some in black and white, are all on a par with the magnificent reproductions in the Atlas. Several chapters stand out—Problems in Particle Color Photomicrography, Contamination Analysis, Ultramicroanalysis, Measuring the Refraction Indices of Subnanogram Particles, X-Ray Diffraction of Small Particles—to name a few without detriment to the others. There is also an excellent chapter on clean room microscopy and one on portable and pocket microscopes.

The loose-leaf binding feature is a drawback because of the hard use the book is bound to receive. Yet this factor makes it easy to remove the Michel Lévy Birefringence Chart to frame and hang on the wall for ready reference. Three full chapters are devoted entirely to expanded and revised tables for the determination of unknowns by dispersion staining—a very valuable contribution.

An interesting feature is the "Consultant's Quiz." Two full chapters and a section at the end of most of the others consist of answers to questions submitted to Dr. McCrone and his associates. They cover a wide range of subjects and contain much useful information.

All chapters are authoritative and well written, and the references are adequate. The book is unique and even by itself, is worthwhile. Independently, or as a supplement to "The Particle Atlas," microscopists, especially those involved in atmospheric pollution control, will find it extremely valuable.

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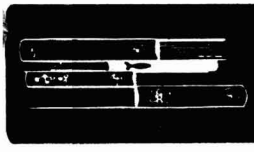

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