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Briefs

Lower Limits of the Potentiometric Titration of Perchlorate Using a Perchlorate Ion-Selective Electrode. WALTER SELIG, *Lawrence Livermore Laboratory, University of California, Livermore, California 94550.*

Three titrants were evaluated for the potentiometric determination of perchlorate. The emf levels were monitored with a perchlorate ion-selectrode indicator electrode and a double-junction reference electrode. The lower limits for the titration at ambient temperature were extended to approximately 0.09 mmol/50 ml ($1.7 \times 10^{-3} N$) from the previous 0.25 mmol/50 ml. If Gran plots are used, they can be further extended to approximately 0.01 mmol/50 ml ($2.1 \times 10^{-4} N$).

Microchem. J. **22**, 1–6 (1977).

Ultramicrodetermination of Nitrogen in Organic Compounds. VIII. Weighing of Hygroscopic and Volatile Samples for the Sealed-Tube Method. KEIKICHI MIYAHARA AND TOMO TAKAOKA, *Shionogi Research Laboratory, Shionogi and Co., Ltd., Fukushima-ku, Osaka 553, Japan.*

A new method for handling hygroscopic and volatile samples for decimilligram determination of organic nitrogen by the sealed-tube method is described. It involves the use of a copper-foil capsule in which hygroscopic and volatile samples can be dried to constant weight and weighed. Satisfactory results were obtained with a number of compounds.

Microchem. J. **22**, 7–14 (1977).

Organoparticulate Analysis of Amine Arenesulfonates. T. D. KACZMAREK, D. C. PHILLIPS, AND J. D. B. SMITH, *Westinghouse Research Laboratories, Pittsburgh, Pennsylvania 15235.*

The new technique of organoparticulate analysis has been used to investigate the thermal decomposition reactions involving arene- and heterocyclic sulfonic acids and their amine salt derivatives. With only a few exceptions, these compounds exhibit strong organoparticulation at temperatures below 200°C as indicated by their effect on the output current of an ion chamber detector.

Microchem. J. **22**, 15–26 (1977).

Solid-State Exchange Colorimetry in the Successive Analysis of Iron(II) and Iron (III) in Solution. M. K. GADIA AND M. C. MEHRA, *Environmental Contaminants Research Group, Chemistry Department, Université de Moncton, Moncton, N.-B., Canada E1A 3E9.*

Use has been made of a stable surface generated by the insoluble coordination compound, mercuric tetrathiocyantocobaltate(II). This compound responds selectively toward iron in solution and a colorimetric method has been developed for the successive analysis of iron(II) and iron (III) in natural systems.

Microchem. J. **22**, 27–33 (1977).

Oxidimetric Determination of Some Compounds with Hexamminecobalt(III) Tricarbonatocobaltate(III). MILOSLAVA VAŠATOVÁ AND JAROSLAV ZÝKA, *Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia.*

The possibilities of utilization of the hexamminecobalt(III)−tricarbonatocobaltate(III) complex for potentiometric determination of some substances are discussed. The reaction scheme for this reagent is proposed for acidic media (the "generation" of Co^{3+}). A microdetermination of iodide has been developed. The oxidation of thiocyanate, thiosulfate, and hydrogen peroxide is briefly discussed.

Microchem. J. **22,** 34–44 (1977).

Ion Chromatographic Determination of Sulfur and Chlorine Using Milligram and Submilligram Sample Weights. F. SMITH, JR.,[1] A. MCMURTRIE,[2] AND H. GALBRAITH,[2] *Dionex Corporation,[1] 1228 Titan Way, Sunnyvale, California 94086 and Galbraith Laboratories,[2] Knoxville, Tennessee 37921.*

Oxygen flask combustion followed by Ion Chromatography was used for the determination of chlorine and sulfur. Determination of chlorine in the presence of large amounts of bromine is possible.

Microchem. J. **22,** 45–49 (1977).

Selective Spectrophotometric Determination of Uranium. B. W. BUDESINSKY, *Phelps Dodge Corporation, Morenci, Arizona 85540.*

Uranium(VI), 10–100 µg, is extracted with 2% dibenzoylmethane in benzene at pH 6–7 while other cations are masked with CDTA (maximum of 8 g/100 ml). Treatment with HCl followed by complexation with Arsenazo III and measurement of the absorbance at 650 nm completes the determination. Iron(III), copper(II), aluminum, and zinc do not interfere.

Microchem. J. **22,** 50–54 (1977).

Determination of Sulfur Dioxide in Air. B. W. BUDESINSKY, *Phelps Dodge Corporation, Morenci, Arizona 85540.*

The determination is based on a preliminary purification with a cellulose filter and 80% isopropanol and absorption of the sulfur dioxide by means of two midget impingers in hydrogen peroxide. The resulting sulfate is titrated with barium perchlorate.

Microchem. J. **22,** 55–59 (1977).

Improvement of Oxygen Determination Method in Organic Compounds through Addition of Chlorohydrocarbon Vapor to Carrier Gas. WOLFGANG J. KIRSTEN, *Department of Chemistry, Agricultural College of Sweden, S-750 07 Uppsala 7, Sweden.*

The addition of chlorohydrocarbon vapor to the carrier gas enhances the quantitative conversion of oxygen to carbon monoxide in the pyrolytic oxygen determination method according to Schütze-Unterzaucher.

Microchem. J. **22**, 60–64 (1977).

Rapid and Simple Determination of Aldose Reductase and Sorbitol Dehydrogenase. B. MATKOVICS, CS. KONCZ, AND L. SZABÓ, *Biological Isotope Laboratory, Institute Biochemistry, "A. J." University Szeged, Szeged, Hungary.*

Aldose reductase activity measurement is based on photometric *o*-toluidine aldose back-measurement. The spectrophotometric method based on the quantitative decrease in NADH proved suitable for the measurement of sorbitol dehydrogenase activity.

Microchem. J. **22**, 65–69 (1977).

Study of the Stability of Pyrimidine Series Cytostatics, Ftorafur and Fluorouracil Injections. HANA TOMÁNKOVÁ AND JAROSLAV ZÝKA, *Institute for Control of Drugs, Prague and Department of Chemistry, Charles University, Prague, Czechoslovakia.*

Ftorafur subjected to elevated temperatures and ultraviolet irradiation first yielded 5-fluorouracil. In the second stage of the decomposition of the compound, the pyrimidine ring opened between N_3 and C_4 and between C_6 and N_1 with the formation of urea. The various compounds were determined using thin-layer chromatography and spectrophotometry in the ultraviolet and visible regions.

Microchem. J. **22**, 70–84 (1977)

Serotonin in Blood Platelets: Studies on a Simple Assay. J. GOLDMAN AND R. J. THIBERT, *Department of Chemistry, University of Windsor, Windsor, Ontario, Canada N9B 3P4.*

A simple, sensitive assay for aqueous solutions of serotonin, especially platelet lysates, is outlined. The precision of the assay is acceptable for routine use, the coefficient of variation being less than 10%. Interferences and the method for eliminating them are discussed.

Microchem. J. **22**, 85–91 (1977)

Separation and Gravimetric Determination of Barium Using Sulfadimethoxin Salicylaldimine. PRABUDDHA JAIN AND KAMAL K. CHATURVEDI, *Department of Chemistry, Holkar Science College, Indore, India.*

Sulfadimethoxin salicylaldimine has been found to be a wide spectrum precipitant of a number of metal ions. The present paper describes the determination of barium in the presence of a number of ions including calcium and strontium.

Microchem. J. **22**, 92–95 (1977)

Microdetermination of α-Amino Acids by Colorimetry and Atomic Absorption Spectrophotometry. Y. A. GAWARGIOUS, AMIR BESADA, AND M. E. M. HASSOUNA, *Microanalytical Research Laboratory, National Research Centre, Dokki, Cairo, Egypt, A. R. E.*

α-Amino acids are determined after conversion to their corresponding Cu(II) chelates. One method, the copper complex is treated with cyanide and phosphomolybdic acid in HCl, followed by colorimetric measurement of the molybdenium blue produced. In the other method, the absorbance of the copper chelate is directly recorded on aspiration in the atomic absorption spectrophotometer.

Microchem. J. **22,** 96−102 (1977).

Lower Limits of the Potentiometric Titration of Perchlorate Using a Perchlorate Ion-Selective Electrode[1]

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Received July 28, 1976

INTRODUCTION

The potentiometric titration of perchlorate with tetraphenylarsonium chloride and a perchlorate ion-selective electrode (ISE) was first reported in 1968 by Baczuk and DuBois *(1)*. Smith and Manahan *(8)* later improved the sensitivity of this titration by operating at 2°C.

We have compared tetraphenylarsonium chloride, tetraphenylphosphonium chloride, and tetra-*n*-pentylammonium bromide as titrants for the potentiometric determination of perchlorate. In addition, we have explored the lower limits of this titration at ambient temperature. An integrated automated titration system controlled by a PDP-8/I minicomputer[2] was used to generate titration curves and to evaluate the experimental results *(4)*.

EXPERIMENTAL

The titrants were approximately 0.05 N aqueous solutions of tetraphenylarsonium chloride (J. T. Baker Chemical Co., "Baker grade"), tetraphenylphosphonium chloride (J. T. Baker Chemical Co., Technical grade), and tetra-*n*-pentylammonium bromide (Eastman white label). Ammonium perchlorate solutions were prepared from "Fisher Certified" reagent. Aliquots were diluted to 50 ml with distilled water. The titration system was controlled by a PDP-8/I minicomputer processor. A summary of its operation and a schematic drawing of the equipment were published elsewhere *(4)*. The emf was monitored by an Orion model 93-81 perchlorate ion-selective electrode and double-junction reference electrode (salt bridge filled with 0.1 N ammonium fluoride).

[2] Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Energy Research and Development Administration to the exclusion of others that may be suitable.

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Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water cooling-plate and an aluminum plate connected to ground.

Titration endpoints were calculated according to Savitsky and Golay *(6)*. A convolute was used for a third-order second derivative using 25 points. The zero-crossing was found by linear interpolation near the sign change. At perchlorate concentrations of less than 0.05 mmol/50 ml, the Gran *(5)* method was used to calculate the endpoints.

All titrations were performed at ambient temperature $(23 \pm 1°C)$.

RESULTS AND DISCUSSION

Baczuk and DuBois in 1968 reported the potentiometric titration of perchlorate with tetraphenylarsonium chloride using a perchlorate ISE *(1)*. The indicating electrode was an Orion model 92-81; the reference electrode was a double-junction model in which the salt bridge was filled with ammonium nitrate solution. The titrant was 0.05 *N*. Their method was used for 1.5 to 2 mmol of perchlorate.

Smith and Manahan *(8)* used the same electrode system and titrant strength. They consider the limit of this method near 0.25 mmol of perchlorate per 50 ml of solution. They were, however, able to lower this limit to 0.05 mmol of perchlorate per 50 ml by operating at 2°C. This decreased the solubility of the tetraphenylarsonium perchlorate precipitate and thus enhanced the steepness of the titration curve.

The 92-series ISE used by the above-mentioned workers has recently been replaced by a 93-series model, which is less sensitive to air bubbles and static electricity, does not require periodic refilling with two filling solutions, and has a longer operating life. We have recently compared the two ISE models in the titration of ammonium perchlorate with tetraphenylarsonium chloride *(7)*. The 93-series electrode yielded more precise results, and the titration curves were less noisy than with the 92-series electrode. Therefore, in the following work the new 93 model was used.

Tetraphenylphosphonium chloride has previously been used for the precipitation of perchlorate *(2)*. This compound can be prepared more easily and at a lower cost than the arsonium analog. (The stibonium analog is much less soluble and is thus of limited usefulness.) The tetraphenylphosphonium chloride has, to our knowledge, not been used in the potentiometric titration of perchlorate. Dosch *(3)* recently reported the precipitation of perchlorate with tetra-*n*-pentylammonium bromide. This reagent has not been used previously in the potentiometric titration of perchlorates. We have compared the three titrants (tetraphenylarsonium chloride, tetraphenylphosphonium chloride, and tetra-*n*-pentylammonium bromide) by replicate titrations of 0.25 mmol of am-

TABLE 1
COMPARISON OF THREE TITRANTS FOR PERCHLORATE[a]

Titrant	Mean normality	No. of runs	Standard deviation	Mode of calculation
Tetraphenylphosphonium chloride	0.04123	5	0.000041	Savitsky and Golay
Tetraphenylarsonium chloride	0.04752	5	0.000067	Savitsky and Golay
Tetrapentylammonium bromide	0.05148	5	0.000245	Gran

[a] Taken: 0.256 mmol ammonium perchlorate per 50 ml.

monium perchlorate. The results are summarized in Table 1. The titrants were of approximately the same strength ($\sim$0.05 N). The phosphonium salt was of technical grade, and this may account for its slightly lower normality. As shown by the standard deviations, the phosphonium and arsonium salts yielded similar precision. The size of the potentiometric breaks and their steepness were almost identical. Hence, only one curve, i.e., that for the phosphonium analog, is presented in Fig. 1. This figure also shows the Gran plots (5), previously discussed (4).

Figure 2 shows the titration curve for ammonium perchlorate with tetra-n-pentylammonium bromide. The break at the endpoint is too shallow for sucessful calculation of the second derivative by the Savitsky and Golay convolute (6). This is because of the fairly large solubility of the
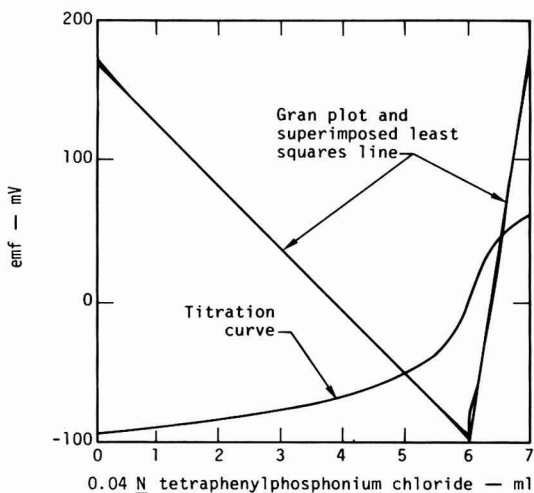
FIG. 1. Titration of 30 mg of ammonium perchlorate with $\sim$0.04 N tetraphenylphosphonium chloride.
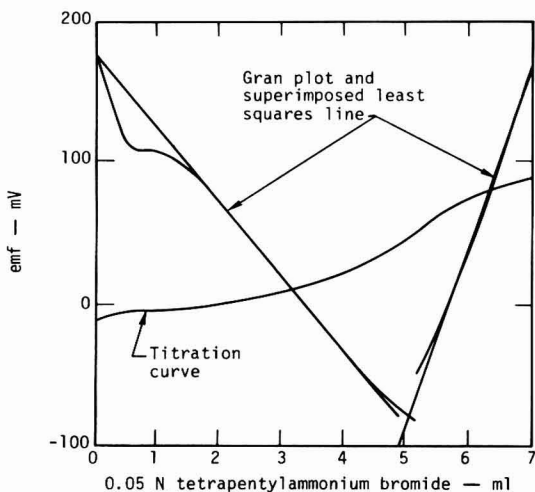
FIG. 2. Titration of 30 mg of ammonium perchlorate (0.255 mmol) with ~0.05 N tetrapentylammonium bromide.

precipitate. The endpoint was therefore calculated by extrapolation of the linearized (Gran plot) data. The precision was considerably lower than for the other titrants. We therefore do not recommend tetra-n-pentylammonium bromide for the potentiometric titration of perchlorates.

Although the phosphonium and arsonium salts serve equally well for the potentiometric titration of perchlorate, we have used only the arsonium analog for the investigation of the lower limits of this titration. A summary of the results is given in Table 2. Down to approximately 0.05 mmol of perchlorate (1.7×10^{-3} N), the titration curves are sufficiently steep to be evaluated by the second derivative method. At lower levels, however, the Gran method must be used. Figure 3 shows the titration curve for 0.0213 mmol of ammonium perchlorate as well as the Gran plot. It also shows least-squares extrapolation of the linear portion of the Gran plot past the endpoint. Because the portion of the curve past the endpoint

TABLE 2

STATISTICS FOR SMALL AMOUNTS OF PERCHLORATE
TITRATED WITH TETRAPHENYLARSONIUM CHLORIDE

Ammonium perchlorate, mmol taken	Molarity of perchlorate	Mean normality	No. of runs	Standard deviation	Mode of calculation
0.0859	1.72×10^{-3}	0.04598	5	0.000104	Savitsky and Golay
0.0435	8.7×10^{-4}	0.04690	5	0.000209	Gran
0.0213	4.3×10^{-4}	0.04809	5	0.0040	Gran
0.0106	2.1×10^{-4}	0.05073	4	0.00194	Gran
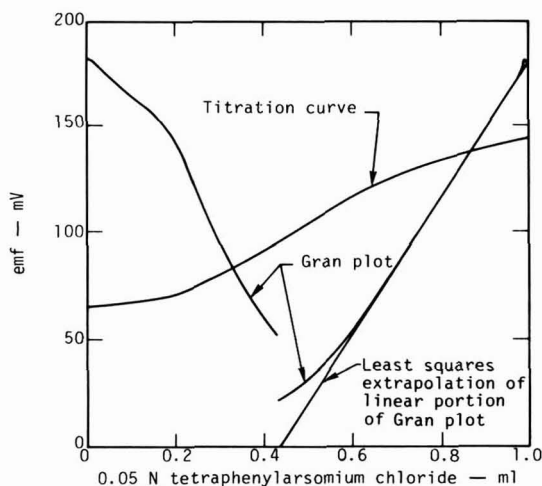
FIG. 3. Titration of 0.0213 mmol of ammonium perchlorate with ~0.05 N tetraphenylarsonium chloride.

is more linear, it is used for the evaluation of endpoints.

It can be seen from Table 2 that as the amount of perchlorate decreases, so does the precision of the results. Also, the apparent normality tends to increase with decreasing amounts of perchlorate. Attempts to make a blank correction did not prove successful. We therefore recommend for the estimation of low levels of perchlorate (in the range of 2×10^{-4} to 9×10^{-4} N), standardization of the titrant vs an amount of perchlorate close to that expected in the unknown.

Further scaling down of this method is no doubt feasible by carrying out the titration below ambient temperature according to the method of Smith and Manahan (8).

SUMMARY

Three titrants (tetraphenylarsonium chloride, tetraphenylphosphonium chloride, and tetra-n-pentylammonium bromide) were evaluated for the potentiometric determination of perchlorate. The emf levels were monitored with a perchlorate ion-selective indicator electrode and a double-junction reference electrode. The tetraphenylonium salts were equivalent, yielding the same precision and magnitude of potentiometric breaks. Considerably smaller breaks were obtained with tetra-n-pentylammonium bromide, which, therefore, is not recommended as titrant.

The lower limits for the potentiometric titration of perchlorate at ambient temperature were extended to ~0.09 mmol per 50 ml (1.7×10^{-3} N) from the previous 0.25 mmol per 50 ml. If Gran plots are used, they can be further extended to ~0.01 mmol per 50 ml (2.1×10^{-4} N).

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Ultramicrodetermination of Nitrogen in Organic Compounds

8. Weighing of Hygroscopic and Volatile Samples for the Sealed-Tube Method

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Several methods have been used for the quantification of hygroscopic and volatile samples prior to analysis *(1–5,7,8,13)*. The use of aluminium foil offers several advantages over other existing techniques due to easy construction and sealing. However, aluminium cannot be used for the sealed-tube method *(6)* in which nitrogen is collected over 50% potassium hydroxide, because aluminium reacts with alkali to generate hydrogen.

In our laboratory, capsules made from copper foil have proved successful for the drying and weighing of decimilligram samples of hygroscopic and volatile solid substances to constant weight before analysis.

HYGROSCOPIC SUBSTANCES

Copper-Foil Capsules

A simple device for making foil capsules is illustrated in Fig. 1. Copper foil was cut into pieces 20 × 20 mm as shown in (a). We used a copper-clad laminate 0.035 mm thick. The cut-out foils were immersed in 40% iron(III) chloride solution for 4 to 5 min at room temperature to erode them to 0.015 to 0.02 mm in thickness, then washed with water, immersed in warm acetic acid, washed with water again, and dried in an oven. This eroding is required to form copper oxide on the inner surface of the capsule to ensure complete combustion when the sample is heated. In this case, two solutions of sulfuric and hydrochloric acid were unsuitable as an eroding solution. Using 25% nitric acid solution for 2 to 3 min at room temperature, the copper foils could easily be thinned to 0.015 to 0.02 mm in thickness, but the capsules made often did not have the copper oxide formation. Such a capsule at times leaves uncombusted carbon after analysis. Capsules are constructed with a tool made from a polished stainless steel rod 2 mm in diameter by rolling the foil and making duplicate folds as shown in (b) and (c). These folds are pressed into a seal with a plier. The capsules are heated in a current of nitrogen at 800°C for 2 hr in

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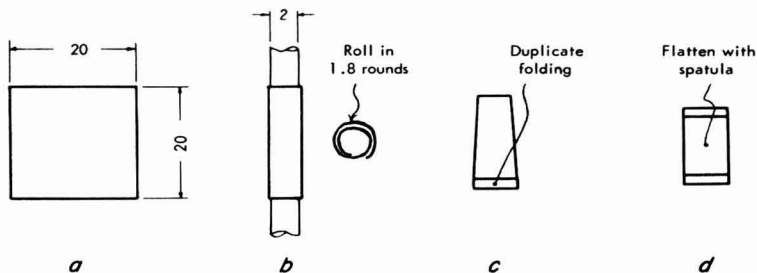
FIG. 1. Foliated copper capsule for hygroscopic and volatile samples.

an electric furnace, and finally cooled under nitrogen. The resulting copper capsule (c) weighs about 15 mg.

Weighing and Drying Samples

Samples (0.3 to 0.5 mg) are added to the weighed capsule, care being taken to avoid leaving the sample around its top, and introduced into the bottom of the capsule by vibrating the tweezers (which hold a folded part of the capsule) very weakly by tapping them with the hand. To close the capsule, the top is folded in duplicate folds with a pair of tweezers on a polished metal plate and the capsule is pressed with a plier as shown (d). The sample is dried to constant weight under the desired conditions of vacuum and temperature. The capsule may be weighed immediately after removal from the drying apparatus.

Results and Discussion

Figures 2 and 3 show the weight increase caused by absorption of moisture, when dried hygroscopic samples are allowed to stand open
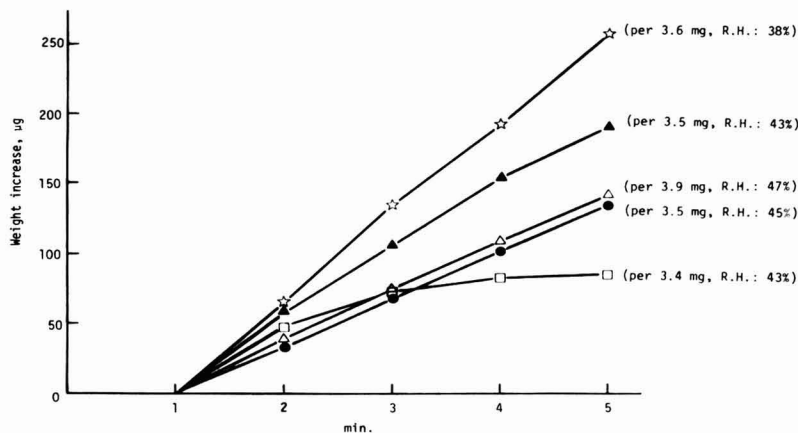
FIG. 2. Weight increase caused by absorption of moisture in milligram scale. □: Tetraalanine methylester hydrochloride; ▲: methylsulfinylethylthiamine hydrochloride; △: cephalexin; ☆: dry magnesium perchlorate; ●: 1-hydroxy-5-NO_2-2-(1H)-pyridone-T.M.A. salt.
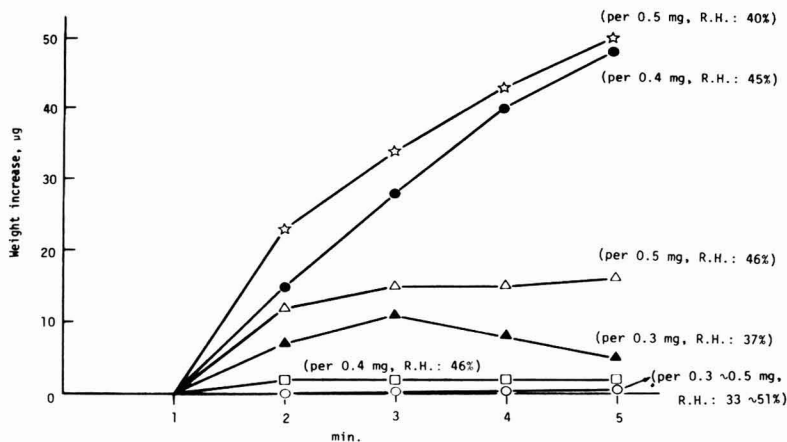
FIG. 3. Weight increase caused by absorption of moisture in decimilligram scale. The symbols ☆, ●, △, ▲, and □ are as described in Fig. 2. Samples were weighed in open copper boats. The mark ○ corresponds to the case when the substance of mark ● was weighed in a copper foil capsule.

under shown conditions. As shown in Fig. 2, the weight increase of milligram samples used is 30 to 60 μg/min in the beginning, but is not always proportional to time. Tetraalanine methylester hydrochloride gives a constant weight after 3 min. But decimilligram samples change 2 to 20 μg/min in the beginning and are liable to give a constant weight for only a short time, as shown in Fig. 3. Such samples, when weighed, seem to be nonhygroscopic if the weighing takes place in 2 to 4 min. The analytical procedure is carried out as described previously (6,10–12). We found a blank value of 0.093 μl and a standard deviation of 0.015 μl for 11 tests. In Table 1, complete combustion of the sample taken in a capsule was confirmed by the analysis of sulfathiazole, which is nonvolatile and is well known as a very difficult sample with which to make complete combustion by the ordinary Dumas method.

Tables 2–4 give analytical results for the wet and completely dried samples. Here the wet sample is obtained by leaving it in a laboratory

TABLE 1
ANALYTICAL RESULTS FOR COPPER-ENCAPSULATED SULFATHIAZOLE[a]

Expt	Sample (μg)	N Found (%)	ΔN (%)
1	276.1	16.52	+0.06
2	330.0	16.58	+0.12
3	364.4	16.50	+0.04
4	344.3	16.56	+0.10
5	367.0	16.51	+0.05

[a] Calcd N%: 16.46.

TABLE 2
ANALYTICAL RESULTS FOR HYGROSCOPIC CEPHALEXIN, $C_{16}H_{17}N_3O_4S^a$

Expt	Copper-encapsulated compound						Noncapsulated compound	
	Wt of wet sample S_1 (μg)	Found NA_1 (%)	Wt loss on drying ΔS (μg)	Wt of dry sample S_2 (μg)	Found N (%)	Error (%)	Wt of wet sample S_3 (μg)	Found NA_3 (%)
1	390.7	11.38	21.8	368.9 (370.2)	12.05 (12.01)	−0.05 (−0.09)	330.9	11.34
2	488.3	11.36	29.8	458.5 (460.3)	12.10 (12.05)	0 (−0.05)	350.7	11.35
3	363.3	11.35	22.2	341.1 (342.4)	12.09 (12.05)	−0.01 (−0.05)	429.1	11.34
4	422.3	11.36	25.6	396.7 (398.0)	12.09 (12.05)	−0.01 (−0.05)	398.1	11.26
5	421.0	11.23	26.8	394.2 (395.5)	11.99 (11.95)	−0.11 (+0.15)	315.3	11.14

[a] Calcd N%: 12.10. ( ): uncorrected value; mean of errors: −0.036% (−0.078%); standard deviation of error: 0.046% (0.044%). S_1: sample allowed to absorb moisture to constant weight and then placed in capsule; S_2: sample dried to constant weight by described method; S_3: sample in the same condition as that of S_1 when placed in the copper boat (9); NA_1, N, and NA_3: results from S_1, S_2, and S_3, respectively.

TABLE 3
ANALYTICAL RESULTS FOR HYGROSCOPIC TETRAALANINE METHYLESTER HYDROCLORIDE, $C_{13}H_{25}N_4O_5Cl^a$

Expt	Copper-encapsulated compound						Noncapsulated compound	
	Wt of wet sample S_1 (μg)	Found NA_1 (%)	Wt loss on drying ΔS (μg)	Wt of dry sample S_2 (μg)	Found N (%)	Error (%)	Wt of wet sample S_3 (μg)	Found NA_3 (%)
1	460.1	15.26	23.3	436.8 (438.1)	16.07 (16.03)	+0.19 (+0.15)	438.4	15.16
2	407.5	15.28	21.4	386.1 (387.0)	16.13 (16.09)	+0.25 (+0.21)	445.0	15.19
3	404.8	15.12	22.0	382.8 (385.0)	15.98 (15.89)	+0.10 (+0.01)	366.6	14.99
4	480.3	15.18	26.8	453.5 (454.3)	16.08 (16.05)	+0.20 (+0.17)	419.8	15.21
5	426.5	15.22	20.8	405.7 (407.0)	16.00 (15.95)	+0.12 (+0.07)	343.1	15.09

[a] Calcd N%: 15.88. ( ): Uncorrected value; mean of errors: +0.172% (+0.122%); standard deviation of error: 0.061% (0.081%). S_1, S_2, S_3, NA_1, and NA_3 are as described in Table 2.

atmosphere, including a suitable water vapor, in order to reach perfect weight equilibrium. Table 5 gives analytical results with wet and dry samples which deliquesce at about 57% relative humidity. A series of NA_1 and NA_3 shows a comparison between the capsule and noncapsule methods (see Tables 2–5).

We found that NA_1 and NA_3 are in fair agreement with each other

TABLE 4

ANALYTICAL RESULTS FOR HYGROSCOPIC METHYLSULFINYLETHYLTHIAMINE HYDROCHLORIDE, $C_{13}H_{20}N_4OS_2Cl_2$[a]

Expt	Copper-encapsulated compound						Noncapsulated compound	
	Wt of wet sample S_1 (μg)	Found NA_1 (%)	Wt loss on drying ΔS (μg)	Wt of dry sample S_2 (μg)	Found N (%)	Error (%)	Wt of wet sample S_3 (μg)	Found NA_3 (%)
1	360.4	13.47	31.0	329.4 (330.2)	14.74 (14.70)	+0.13 (+0.09)	379.5	13.41
2	308.5	13.43	25.8	282.7 (283.9)	14.65 (14.59)	+0.04 (−0.02)	289.4	13.26
3	413.8	13.45	35.7	378.1 (379.0)	14.72 (14.69)	+0.11 (+0.08)	344.0	13.31
4	335.8	13.50	29.9	305.9 (306.3)	14.82 (14.80)	+0.21 (+0.19)	415.3	13.30
5	349.3	13.55	28.2	321.1 (321.5)	14.74 (14.72)	+0.13 (+0.11)	310.7	13.26

[a] Calcd N%: 14.61. ( ): Uncorrected value; mean of errors: +0.124% (+0.09%); standard deviation of error: 0.061% (0.075%). S_1, S_2, S_3, NA_1, and NA_3 are as described in Table 2.

TABLE 5

ANALYTICAL RESULTS FOR HYGROSCOPIC 1-HYDROXY-5-NO_2-2(1H)-PYRIDONE-T.M.A. SALT, $C_9H_{15}N_3O_4$[a]

Expt	Copper-encapsulated compound						Noncapsulated compound	
	Wt of wet sample S_1 (μg)	Found NA_1 (%)	Wt loss on drying ΔS (μg)	Wt of dry sample S_2 (μg)	Found N (%)	Error (%)	Wt of wet sample S_3 (μg)	Found NA_3 (%)
1	617.1	15.42	87.5	529.6 (530.9)	17.97 (17.93)	−0.36 (−0.40)	404.6	15.27
2	371.5	15.44	59.5	312.0 (312.4)	18.38 (18.36)	+0.05 (+0.03)	436.4	15.20
3	410.9	15.29	64.2	346.7 (347.1)	18.12 (18.10)	−0.21 (−0.23)	319.7	15.19
4	414.6	15.23	67.1	347.5 (348.0)	18.18 (18.15)	−0.15 (−0.18)	442.8	15.33
5	430.2	15.41	69.7	360.5 (361.0)	18.39 (18.36)	+0.06 (+0.03)	394.4	15.20

[a] Calcd N%: 18.33. ( ): Uncorrected value; mean of errors: −0.122% (−0.150%); standard deviation of error: 0.179% (0.183%). S_1, S_2, S_3, NA_1, N, and NA_3 are as described in Table 2.

because S_1 and S_3 are wet samples under the same relative humidity. A series of results obtained by our method agreed fairly well with calculated values (see the N% found in Tables 2−5). In our procedure, the weight increase was 0.1 to 0.4 μg/min (see the ○ mark in Fig. 3). Numbers in parentheses are uncorrected values of the weight increases. Our method permits rapid drying for decimilligram quantities under vacuum, with moisture readily eliminated through the folds in the capsule.

VOLATILE SUBSTANCES

Weighing of Samples

Samples are taken into copper foil capsules, as above, and weighed.

Results and Discussion

The analytical procedure is carried out as above. The results for volatile samples by this method are given in Tables 6–8. Table 6 shows the results where volatility was 2 to 3 μg/min for 300 to 600 μg at room temperature. In this case the weight loss was not observed for 5 min. Table 7 shows the results with t-butylaldoxime with a volatility of about 10 μg/min for 400 μg at room temperature. In this case the weight loss was less than 0.1 μg for 7 min and a weight correction was not necessary. Table 8 shows the results with pyrazine with a volatility of 80 μg/min for about 350 μg at room temperature. In this case, the weight loss occurred and a weight correction was needed. The standard deviation of error obtained from Tables 6 to 8 is 0.119% (n = 26). The handling and weighing were quite rapid and simple and required no special attachments.

TABLE 6

ANALYTICAL RESULTS FOR COPPER-ENCAPSULATED VOLATILE COMPOUNDS[a]

Sample and Expt	Wt of sample (μg)	N Found (%)	ΔN (%)
o-Nitrophenol[b]			
1	345.8	10.03	−0.05
2	396.3	9.89	−0.18
3	359.2	9.90	−0.17
4	347.3	9.96	−0.11
5	454.0	9.88	−0.19
4-Cyanopyridine[c]			
6	394.8	26.86	−0.05
7	315.3	26.87	−0.04
8	314.1	27.02	+0.11
9	291.5	26.73	−0.18
10	404.8	26.81	−0.10
p-Toluidine[d]			
11	362.9	12.79	−0.28
12	306.4	12.95	−0.12
13	362.9	13.05	−0.02
14	321.8	12.90	−0.17
15	351.0	12.89	−0.18

[a] Mean of errors: −0.115%; standard deviation of error: 0.0947%.
[b] N% = 10.07; volatility, 2.1 μg/min per 314 mg, at 29°C.
[c] N% = 26.91; volatility, 3.2 μg/min per 320 μg at 29°C.
[d] N% = 13.07; volatility, 3.1 μg/min per 345 μg at 27.8°C.

TABLE 7

ANALYTICAL RESULTS FOR COPPER-ENCAPSULATED t-BUTHYLALDOXIME VOLATILITY[a]

Expt	Loss in quantity (μg/min)	Operating time (min)	Wt. of sample (μg)	N Found (%)	ΔN (%)
1	0.02	6.25	482.4	13.67	−0.18
			(482.5)	(13.67)	(−0.18)
2	0.04	6.75	415.8	13.68	−0.17
			(416.1)	(13.67)	(−0.18)
3	0.08	7.51	379.2	13.74	−0.11
			(379.8)	(13.72)	(−0.13)
4	0.10	7.25	486.8	13.69	−0.16
			(487.4)	(13.67)	(−0.18)
5	0.04	6	594.4	13.67	−0.18
			(594.6)	(13.66)	(−0.19)

[a] 9.9 μg/min per 428 μg, at 29°C; calcd N%: 13.85. ( ): Uncorrected value for the vaporization of sample after weighing; mean of errors: −0.160% (−0.172%); standard deviation of error: 0.0291 (0.0239).

TABLE 8

ANALYTICAL RESULTS FOR COPPER-ENCAPSULATED PYRAZINE VOLATILITY[a]

Expt	Loss in quantity (μg/min)	Operating time (min)	Wt. of sample (μg)	N Found (%)	ΔN (%)
1	0.16	6.0	496.8	35.10	+0.12
			(497.8)	(35.03)	(+0.05)
2	0.16	6.5	342.6	34.92	−0.06
			(343.6)	(34.81)	(−0.17)
3	0.38	7.5	380.9	35.19	+0.21
			(383.8)	(34.93)	(−0.05)
4	0.18	7.0	388.8	34.83	−0.15
			(390.1)	(34.71)	(−0.27)
5	0.48	6.5	513.0	35.08	+0.10
			(516.1)	(34.86)	(−0.12)
6	0.28	6.0	524.7	34.94	−0.04
			(526.4)	(34.83)	(−0.15)

[a] 82.3 μg/min per 359.2 μg, at 29°C; calcd N%: 34.98. ( ): Uncorrected value for vaporization of sample after weighing; mean of errors: +0.030% (−0.118%); standard deviation of error: 0.1348% (0.1092%).

SUMMARY

A new method for handling hygroscopic and volatile samples for decimilligram determination of organic nitrogen by the sealed-tube method was described. It involves the use of a copper-foil capsule in which hygroscopic and volatile samples can be dried to constant weight and weighed. Satisfactory results were obtained with this method.

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Organoparticulate Analysis of Amine Arenesulfonates

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INTRODUCTION

Previous publications in this area have described the adaptation of the new technique of organoparticulate analysis *(6)* (OPA), using ion chamber and condensation nuclei instrumentation, to investigate the thermal decomposition of various malonic acids *(10)* metal acetylacetonates *(7)*, and diazonium compounds *(8,9)*. We would now like to report the investigation of various arene- and heterocyclic sulfonic acids and their amine salt derivatives using the same technique.

EXPERIMENTAL

Four sulfonic acids and 17 amine salt derivatives were investigated in this work. The sulfonic acids thus evaluated were obtained from Aldrich Chemical Company and were used without further purification. These were: benzenesulfonic acid, *p*-toluenesulfonic acid, 3-pyridinesulfonic acid, and 1-naphthalenesulfonic acid.

The amine salt derivatives were all prepared by precipitation from acetone solution, using a slight excess of amine *(2)* with the exception of those derived from 3-pyridinesulfonic acid, which were prepared in aqueous solution. Two commercially available quaternary ammonium arylsulfonates (i.e., tetraethylammonium *p*-toluenesulfonate and trimethylphenylammonium benzenesulfonate), obtained from Pfaltz and Bauer, Inc., were also evaluated in this work.

The sample preparation and organoparticulation test procedures, using an ion chamber detector, were similar to those described previously *(6–8,10)*. The special polymeric epoxy material, which was used in the preparation of the sample, was found to organoparticulate at temperatures well above those shown by the amine sulfonates *(10)*.

As described previously *(6–8,10)*, two temperatures were read from the ion chamber detector thermograms; the *threshold* temperature which corresponded to the onset of organoparticulation (as shown by an initial falloff in amplified ion current) and the temperature which signified a *50% decrease* in the amplified ion current (usually $0.8 \rightarrow 0.4$ mA). These values enabled an "organoparticulation temperature range" (i.e., OPTR) to be determined for each sample. Several measurements with the same

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sample indicated that the reproducibility of the measurements using this technique was of the order of ±2°C.

Characterization of the effluent from some of the amine-*p*-toluenesulfonates and from *p*-toluenesulfonic acid was attempted using mass spectrometry. The experimental procedure, which employed a Perkin–Elmer Model 270 gas chromatograph/mass spectrometer, has been described previously *(6–8,10)*. Although traps for both particulates and vapor effluent were employed as previously described *(10)* only the vapor trap was found to contain a useful analytical signal.

3. RESULTS

Organoparticulation Data

Figures 1−5 show typical organoparticulation responses obtained on the ion chamber detector with a representative group of samples (i.e., *p*-toluenesulfonic acid, 1-naphthalenesulfonic acid, morpholinium *p*-toluenesulfonate, N, N-dimethylanilinium *p*-toluenesulfonate, and ben-
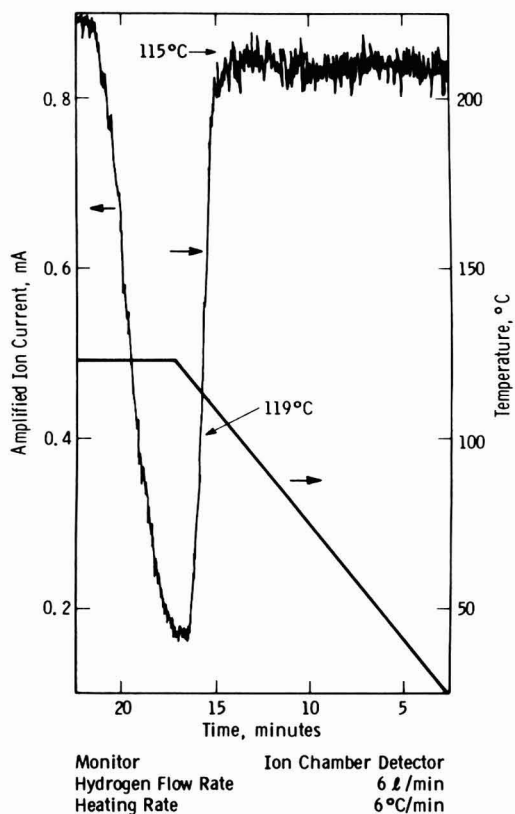

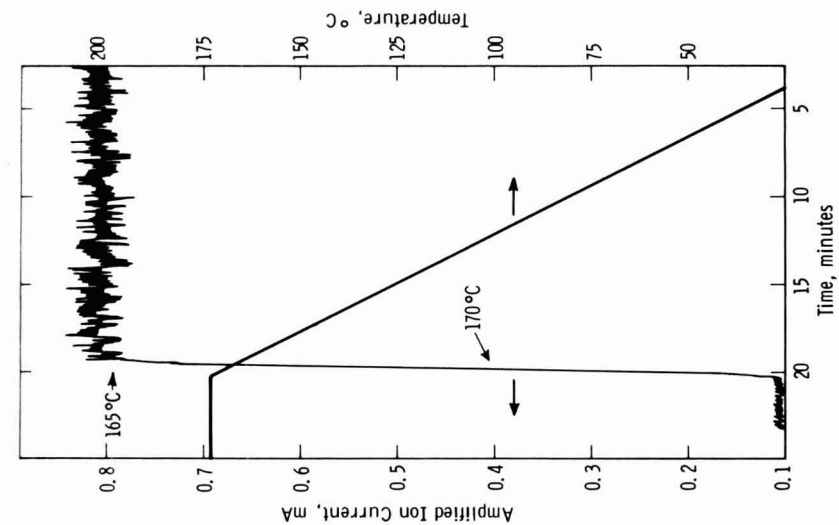FIG. 1. Organoparticulation pattern for *p*-toluenesulfonic acid.

FIG. 3. Organoparticulation pattern for morpholinium p-toluenesulfonate.
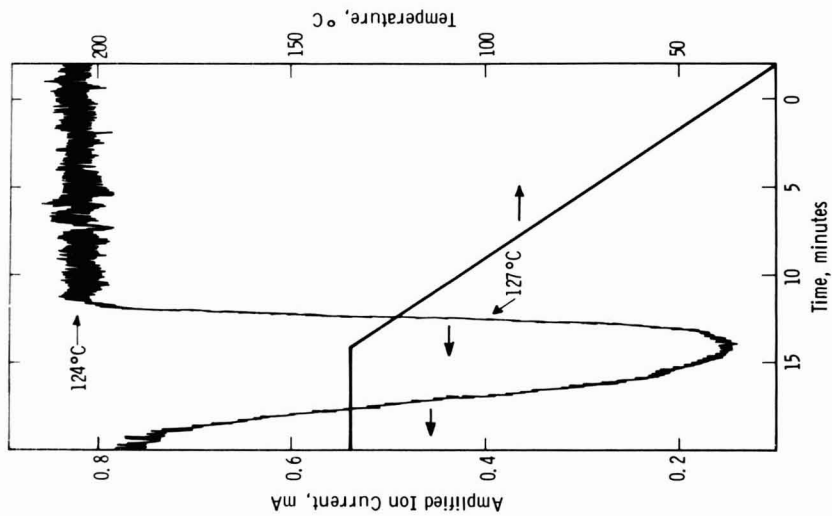

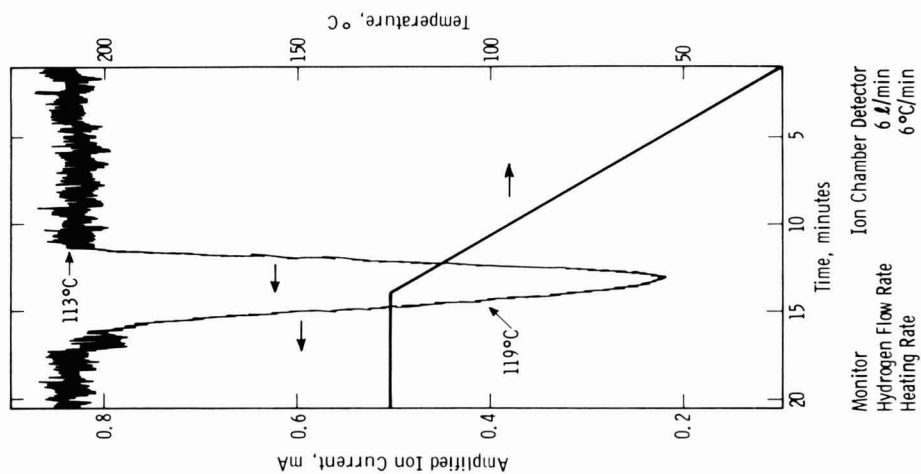FIG. 2. Organoparticulation pattern for 1-naphthalenesulfonic acid.

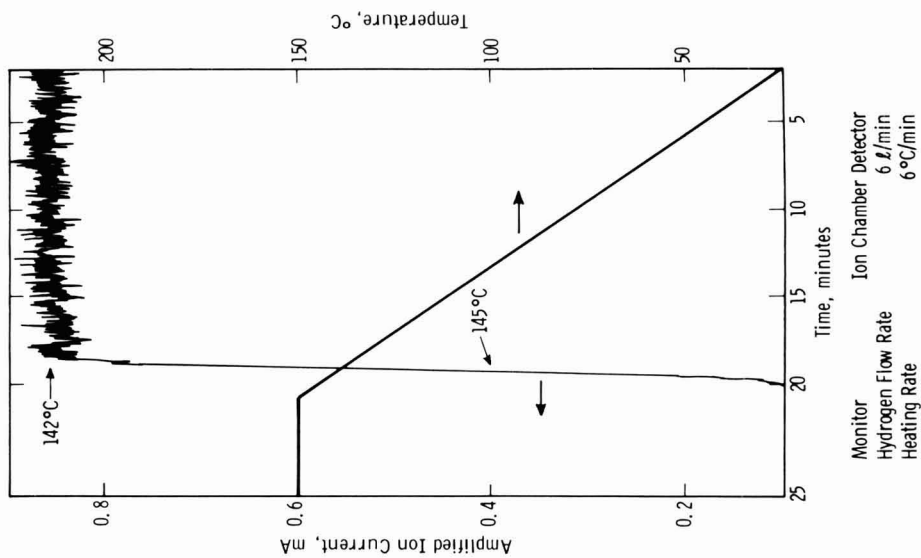Fig. 5. Organoparticulation pattern for benzenesulfonic acid.

Fig. 4. Organoparticulation pattern for N. N-dimethylanilinium-p-toluenesulfonate.

zenesulfonic acid. In each instance, very strong organoparticulation signals are found.

Table I summarizes the OPTR data found with the four sulfonic acids. For comparative purposes, the literature melting points are included. It is noticed that the lowest OPTR values are found with benzene- and p-toluenesulfonic acids, and the highest value is found with 3-pyridinesulfonic acid. Also, there does not appear to be any correlation between the various OPTR values and their literature melting points.

Table 2 gives the OPTR data for the various amine p-toluenesulfonates

TABLE 1
OPTR DATA FOR THE SULFONIC ACIDS

Test sample[a] no.	Sulfonic acid	Organoparticulation temperature range (OPTR) (°C)	Literature melting point (°C)
SC-193/1	p-Toluenesulfonic acid	115−119	103−106
SC-234/1	Benzenesulfonic acid	113−119	44−50
SC-235/1	3-Pyridinesulfonic acid	117−187	>300
SC-236/1	1-Naphthalenesulfonic acid	124−127	77−79

[a] Sample prepared as 66% (w/w) mixture in cured epoxy resin.

TABLE 2
OPTR DATA FOR THE AMINE p-TOLUENESULFONATES

Test sample[a] no.	Amine p-toluenesulfonate	Organoparticulation temperature range (OPTR) (°C)	Melting point (°C)
SC- 63/1	morpholinium p-toluene-sulfonate	165−170	124−126
SC-195/1	benzyldimethylamino p-toluenesulfonate	164−167	185−188
SC-196/1	N,N-dimethylanilinium p-toluenesulfonate	142−145	95−98
SC-197/1	n-propylamino p-toluene-sulfonate	171−174	140−143
SC-198/1	pyridinium p-toluene-sulfonate	153−158	115−120
SC-199/1	1-methylimidazolium p-toluenesulfonate	175−177	92−95
SC-200/1	t-butylamino p-toluene-sulfonate	176−178	223−226
SC-201/1	n-butylamino p-toluene-sulfonate	176−178	122−125
SC- 95/1	tetraethylammonium p-toluenesulfonate	>200	100−105

[a] Sample prepared as a 66% (w/w) mixture in a cured epoxy resin.

evaluated in this work. All of the eight compounds were found to or-ganoparticulate in the temperature range of $142-178°C$. The lowest OPTR value was observed with benzyldimethylamino p-toluenesulfonate and the highest with n-butyl and t-butylamino p-toluenesulfonates. Tet-raethylammonium p-toluenesulfonate was found not to organoparticulate beneath 200°C. Comparison of the literature melting points with the OPTR values once again illustrates a lack of correlation between these two parameters. This effect has been observed with the other groups of or-ganic compounds *(7–9)*.

The OPTR data in Table 3 summarize the values obtained with the remaining amine sulfonates, i.e., those prepared from benzenesulfonic acid, 3-pyridinesulfonic acid, and 1-naphthalenesulfonic acid. It is noticed that, although strong organoparticulation signals were detected with the amine salts prepared from benzenesulfonic acid [i.e., with pyridinium, morpholinium, and n-butylamino benzenesulfonates], none were detected from the 3-pyridinesulfonate salts and only weak ones with their 1-naphthalenesulfonate counterparts. In addition, a sample prepared with trimethylphenylammonium benzenesulfonate did not organoparticulate beneath 200°C.

TABLE 3
OPTR DATA FOR AMINE SALTS OF BENZENE-, 3-PYRIDINE-,
AND 1-NAPHTHALENESULFONIC ACID

Test sample[a] no.	Amine sulfonate	Organoparticulation temperature range (OPTR) (°C)	Melting point (°C)
SC-270/1	Pyridinium benzene sulfonate	$151-154$	$132-134$
SC-271/1	Morpholinium benzene-sulfonate	$184-188$	$110-112$
SC-272/1	n-Butylamino benzene-sulfonate	$187-189$	$108-110$
SC-273/1	Morpholinium 3-pyridine-sulfonate	>200	>230
SC-274/1	n-Butylamino 3-pyridine-sulfonate	>200	>250
SC-275/1	Morpholinium 1-naphthalene-sulfonate	190^b	$193-196$
SC-276/1	n-Butylamino 1-naphthalene-sulfonate	190^b	$252-254$
SC- 94/1	trimethylphenylammonium benzenesulfonate	>200	$192-194$

[a] Sample prepared as a 66% (w/w) mixture in a cured epoxy resin.
[b] Weak signal obtained; not strong enough to reach the "50%" stage on the ion chamber detector.

Mass Spectral Results

The mass spectral data obtained with five of the test samples are shown in Figs. 6–10. Also included in these figures are mass spectra used as aids in the interpretation.

Figure 6, for the vapor effluent from the p-toluenesulfonic acid, reveals that the major constituents of the vapor are phenol and toluene. The presence of toluene is not surprising in that this is the major (if not sole) signal obtained if p-toluenesulfonic acid itself is introduced into the mass spectrometer under the conditions of effluent analysis. The generation of phenol is not as clear, however. Classical chemistry teaches that phenols will be formed from sulfonic acids only by fusion with alkali at 290–340°C; even then the product expected from p-toluenesulfonic acid would be cresol, not phenol (3).

The mass spectra obtained with vapor effluent from pyridinium p-toluenesulfonate are shown in Fig. 7. A comparison of Fig. 7a with 7b indicates that the major component in the vapor phase would appear to be pyridine, shown by strong signals at m/e 79 (the parent ion of pyridine) and at m/e 52. The main fragmentation mode for pyridine in the mass spectrometer is through loss of mass 27 (HCN) (1).
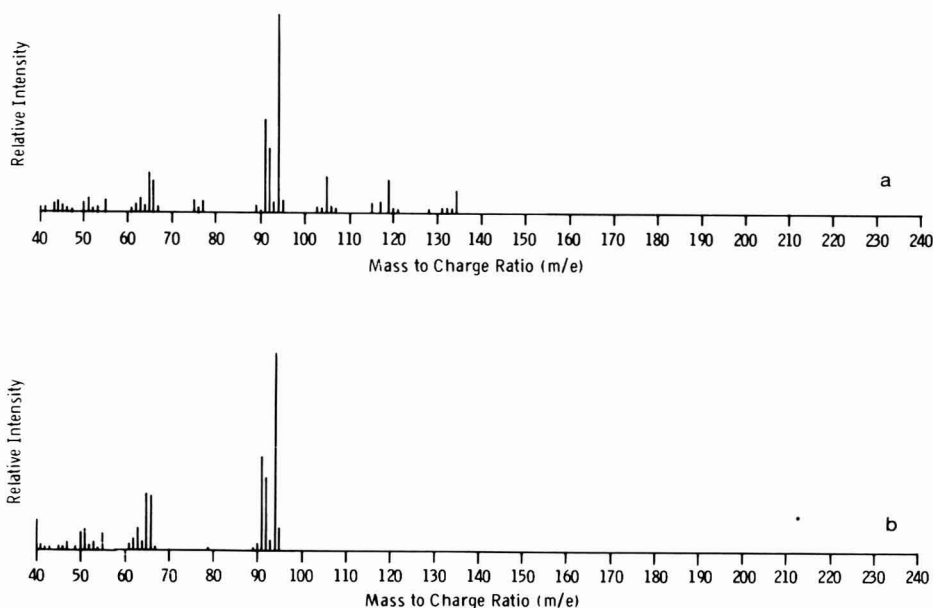

FIG. 6. (a) Mass spectra of vapor effluent from p-toluenesulfonic acid in SC 193/1. (b) Mass spectra of phenol ("67%") and toluene ("33%") as constructed from the literature.
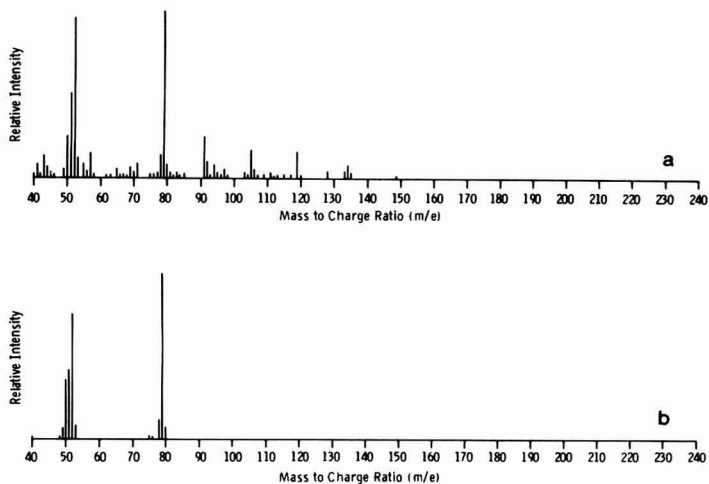
FIG. 7. (a) Mass spectra of vapor effluent from pyridinium-p-toluene sulfonate sample [SC-198/1]. (b) Mass spectra of pyridine as reconstructed from the literature.

With the remaining three samples, i.e., morpholinium p-toluene-p-toluenesulfonate (Fig. 8), n-propylamino-p-toluenesulfonate (Fig. 9), and benzyl dimethylamino p-toluenesulfonate (Fig. 10), no real indication of significant amounts of the amino component was discernible in the vapor phase mass spectra. The major effluent with these latter samples appears to be toluene. It should be particularly noted that the m/e 135 peak of benzyl dimethylamine (in Fig. 10b) is not to be confused with the strong m/e 134 peak due to aromatic hydrocarbon in Fig. 10a. The pattern of peaks occurring at m/e 105, 119, and 134, which are prominent in the amino sulfonate vapor effluent in Figs. 8–10, was subsequently established to be derived from the epoxy resin used in the sample preparation. The most likely compounds responsible for these peaks are diethylbenzene and diethyltoluene. This is shown by the mass spectra of Fig. 9c.

DISCUSSION

Very little information is available in the chemical literature concerning the thermal stabilities of heterocyclic and arenesulfonic acids and their amine salts. However, some arenesulfonic acids are considered thermally unstable although benzenesulfonic acids can be distilled under vacuum (4). The thermal instability of amine benzenesulfonates and p-toluenesulfonates appears not to have been observed to any major degree; the fact that these materials are used in qualitative organic chemistry to characterize and identify amines (5) [because of their ease of synthesis and their sharp melting points] suggests that they are considered to be thermally stable compounds.
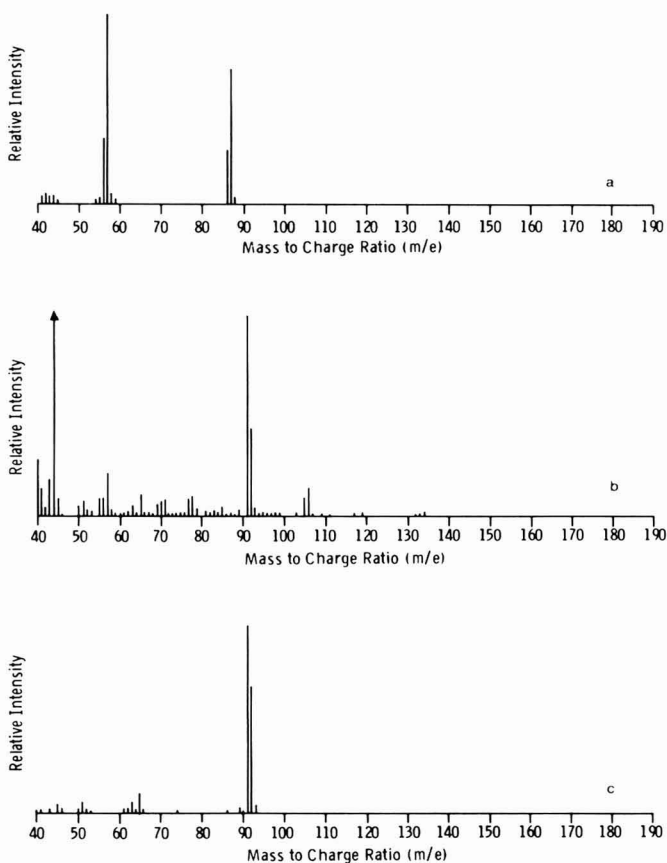
FIG. 8. (a) Mass spectra of morpholine as reconstructed from the literature. (b) Mass spectra of vapor from morpholinium *p*-toluenesulfonate sample [SC-63/2]. (c) Mass spectra of toluene as reconstructed from the literature.

Nevertheless, the mass spectral work done here with *p*-toluenesulfonic acids and their various aminesulfonates indicates that substantial thermal decomposition of these compounds does indeed occur. As mentioned before, the decomposition mechanism probably involves cleavage of the C−S bond followed by evolution of SO_2, aromatic, and amine (from the amine sulfonates) fragments.

As described previously (*6−10*), a critical *minimum size* particulate is required to register a signal on the ion chamber detector. Thus, vapor phase association (possibly by H-bonding) would be required with the end products derived from the thermal decomposition of the arylsulfonic acids and aminesulfonates, to explain the observations reported in this work. Vapor phase association of molecules such as phenols, amines, and SO_2 would be very likely because of the presence of polar groups in these
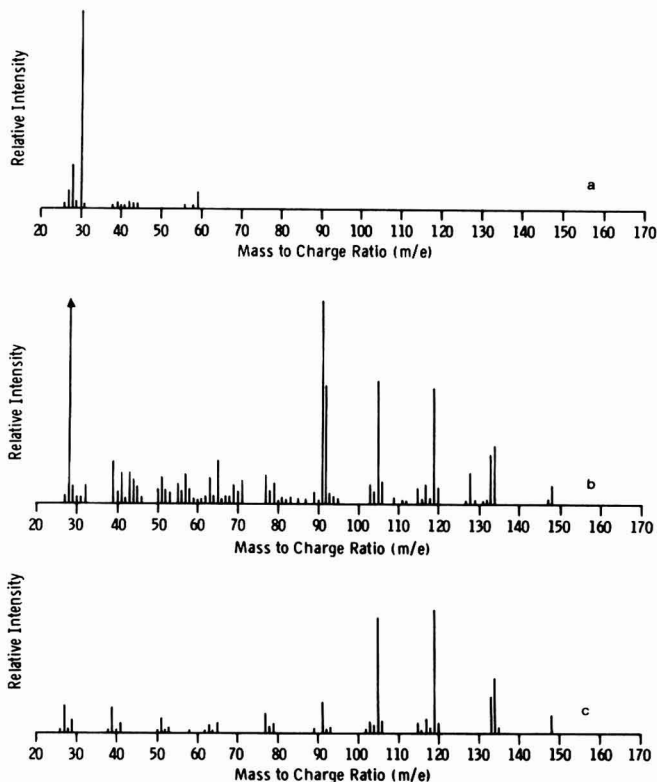
FIG. 9. (a) Mass spectra of *n*-propylamine as reconstructed from the literature. (b) Mass spectra of vapor effluent from *n*-propylamine-*p*-toluenesulfonate sample [SC-197/1]. (c) Mass spectra of 1, 2-diethylbenzene ("75%") and mixed diethyl toluene ("25%") as constructed from the literature.

molecules. The formation of an "aerosol" suspension of organic material in a SO_2 atmosphere could also give rise to the required particulate size.

The formation of phenol/SO_2 vapor phase associated species probably explains why the benzenesulfonic acid sample (SC-234/1) and *p*-toluenesulfonic acid (SC-193/1) exhibit similar OPTR values (i.e., 113–119 and 115–119, respectively). From the mass spectral data discussed previously, it would appear that both compounds, on undergoing thermal decomposition, liberate phenol and SO_2 as major end products. The necessity for vapor phase association of molecular fragments is substantiated by the fact that materials such as phenol, SO_2, toluene, and various amines are incapable *by themselves* of registering a signal on the ion chamber detector.

The explanation just given seems plausible for the particulate formation noted with the sulfonic acids and the amine sulfonates, such as pyridinium *p*-toluenesulfonate (where significant amounts of amine were detected in
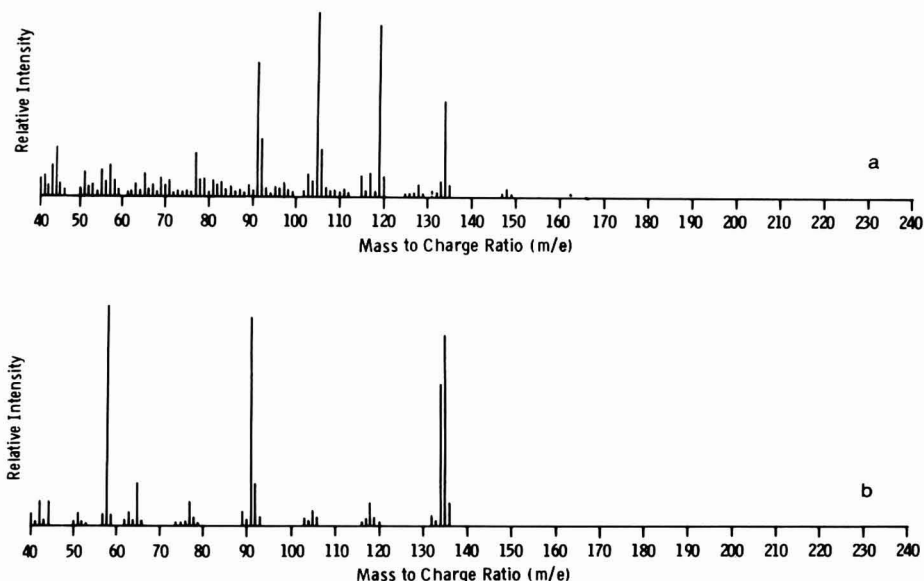
FIG. 10. (a) Mass spectra of vapor effluent from benzyldimethylamino-*p*-toluenesulfonate sample [SC 195/1]. (b) Mass spectra of laboratory sample of benzyldimethylamine.

the vapor phase). However, it is more difficult to rationalize the strong particulate formation observed with the amine sulfonates such as *n*-propylamino, benzyldimethylamino, and morpholinium *p*-toluene-sulfonates. In these cases, the mass spectral studies were unable to identify (other than toluene) any polar molecule which would readily associate in the vapor phase. It is possible that polar molecules may have originally been present and somehow been "lost" during the particulate trapping process. Further work is continuing with these three compounds in an attempt to characterize the nature of the particulates more fully.

One other interesting observation is that the quaternary ammonium benzene- and *p*-toluenesulfonates *do not* exhibit any organoparticulation beneath 200°C (refer to data in Tables 2 and 3). The reason for this may lie in the different structure of these quaternary salts compared to the tertiary and secondary ones.

Tertiary Amine Sulfonate   Quaternary Amine Sulfonate
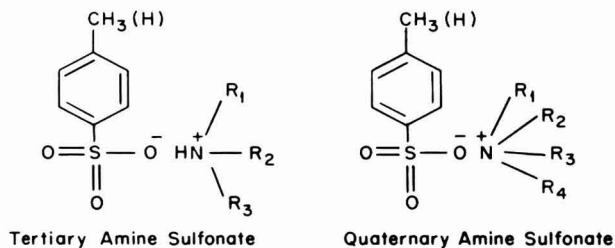
The presence of a labile proton (H^+) on the amine nitrogen, in the case of the tertiary amine sulfonate, might conceivably make it more susceptible to thermal decomposition than the quaternary ammonium salt (because of the increased possibility of proton migration). Then again, the presence of the hydrogen atom may facilitate the formation of H bonding in the vapor phase to form organoparticulates detectable on the ion chamber detector. The absence of this labile hydrogen in the quaternary ammonium compound might possibly prevent any appreciable vapor phase association from occurring.

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Solid-State Exchange Colorimetry in the Successive Analysis of Iron(II) and Iron(III) in Solution

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INTRODUCTION

In recent years there has been considerable interest in the trace analysis of metallic contaminants of ground waters in view of the potential health hazards these elements pose for the public at large. The allowable concentration of an element in water bodies is routinely monitored in the public health laboratories. The national standards for maximum allowable concentrations of trace contaminants in waters are respected as far as possible. The Canadian standards for drinking water have been spelled out in the official document of the Ministry of National Health and Welfare issued in 1969 *(2)*. In the water control laboratories generally the total metal ion concentration is measured using the established instrumental techniques such as spectrophotometry (visible or atomic absorption), or in some specific cases the electrochemical procedures based on the principle of polarography or potentiometry are also employed *(7,8)*. However, very little attention has been paid to the oxidation states of the elements of interest. In order to have a correct picture of the actual state of the species present in an aqueous sample the analytical procedure should have the capability to distinguish and quantitize the various valence states of the element of interest.

In the present communication we report a procedure to characterize the valence states of the element iron in natural waters. The analytical procedure uses the principle of solid-state exchange colorimetry. In the previous communications from the author's laboratory it has already been shown that metallic ions in aqueous samples can be selectively analyzed using the solid-state exchange colorimetric technique *(3–5)*. In the present study use has been made of a stable surface generated by the insoluble coordination compound mercuric tetrathiocyanatocobaltate(II). It has been observed that this compound responds selectively toward iron in solution and on the basis of this observation a colorimetric method has been developed for the successive analysis of iron(II) and iron (III) in natural aqueous systems.

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MATERIALS AND METHODS

All the reagents used in this study were of analytical grade purity. The stock solutions were prepared in deionized distilled water and diluted when necessary. The absorption measurements were made on the Bausch and Lomb Spectronic-70 spectrophotometer. The absorption cell was of 15-ml capacity and had a cylindrical form with quartz windows at both ends. The solid reagent can easily be synthesized in the laboratory using the procedure described by Adams and Raynor (1). In this study the compound used was procured from Eastman Organic Chemicals, New York, and was used without further purification. The acidity or the alkalinity of the medium was adjusted using pure HNO_3 or NaOH only.

PROCEDURE

Initial observations were conducted using the batch equilibrium technique. The solid reagent was made to react with the solution containing iron at an appropriate acidity and the reaction was followed spectrophotometrically. Since the presence of iron in the solution imparted a red color to the supernatant phase (thus making it a selective reagent for iron), it could easily be monitored in the visible range. The reagent has remarkable stability toward decomposition in acidic or faintly alkaline conditions. However, to avoid the formation of the hydrolytic complex products of iron and other metal ions in solution, all the determinations were made in the acidic condition of pH 2 adjusted with HNO_3.

In the actual quantitative runs, iron(III) solutions were diluted from the stock (1000 ppm) to yield a series of solutions varying from 0 to 20 ppm concentration in a 50-ml volumetric flask. The pH of the medium was adjusted prior to making up the final volume. To these prepared standards
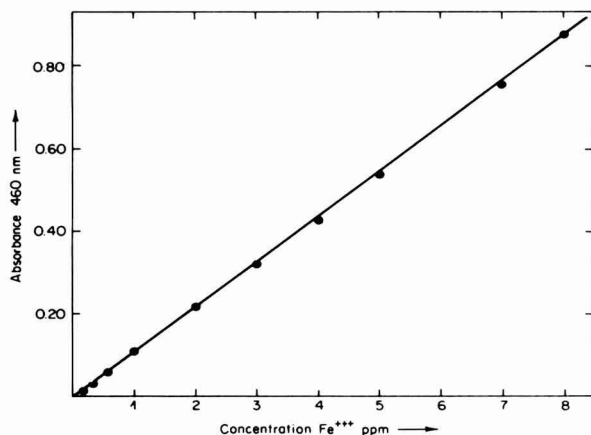

FIG. 1. Spectrophotometric response of the solid-state reagent in aqueous medium.

400–500 mg of the solid reagent Hg[Co(SCN)$_4$] was carefully added. The mixture was mechanically stirred for several minutes and set aside for at least 1 hr for the equilibrium to be attained. In each case there was formation of the red-colored supernatant phase whenever the system had iron(III) present in it. This red-colored solution showed an absorption peak in the visible region at 460 nm, thus indicating that in all probability the known Fe(SCN)$^{2+}$ complex is formed (literature value for the absorption peak of this complex is between 450 and 480 nm) (6). In each experiment a 15-ml volume of the supernatant phase was carefully transferred to the cylinderical absorption cell and the spectrophotometric data were recorded against a blank free of iron(III) concentration. A calibration curve was thus constructed to ascertain the region in which the absorption was linear with the iron(III) concentration.

A similar reaction with iron(II) did not produce the red-colored supernatant phase, as distinct from the reaction with iron(III). However, when 0.5 ml of 30% hydrogen peroxide was added to the iron(II) solution in ppm range and the mixture was equilibrated for 1 hr, the same red-colored phase showed up as in the case of iron(III). To ascertain that iron(II) responds to the solid reagent in the presence of H$_2$O$_2$ a series of quantitative runs was made under similar experimental conditions for varying concentrations of iron(II) standards. In this case as well, a linear curve was obtained in a limited range of iron(II) concentration (cf. Fig. 2).

Apparently iron(III) reacts directly with the solid reagent, while the iron(II) does not do so. The reagent becomes responsive to iron(II) in the presence of the hydrogen peroxide. A distinction between the two valence
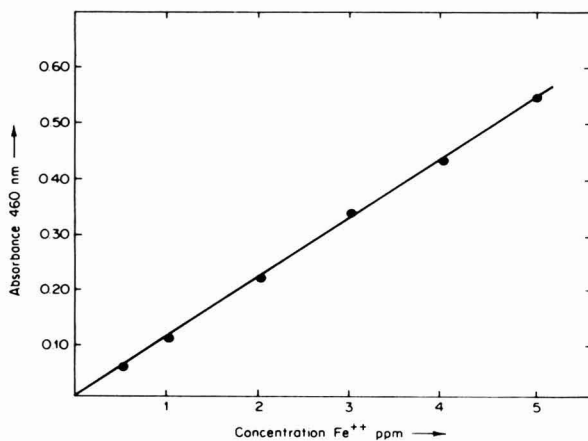

F$_{IG}$. 2. Spectrophotometric response of the solid-state reagent in H$_2$O$_2$ medium (data from Table 2).

states of iron is thus possible and each species can be quantitized as shown earlier at 460 nm, the peak of absorption of the supernatant phase.

The optimal conditions of analysis for the two valence states of iron were established by studying the effect of the equilibration period, acidity or alkalinity, and the presence of diverse ions in the system. Once these conditions were established the analytical procedure was applied to the successive analysis of iron(III) and iron(II) in natural waters such as lake water, river water, and drinking water. The accuracy of the method developed was also compared with the official method of analysis for iron in natural waters (7).

RESULTS AND DISCUSSION

The spectrophotometric response of the solid reagent toward iron(III) was found to be linear in the range 0.1 to 8.0 ppm. The actual experimental data are shown in Table 1 and Fig. 1. It is interesting to note that the lower limit of detection of 100 ppb is comparable or even superior to some other colorimetric methods of analysis for iron(III) (6). At the outset it may be said that the solid-state exchange colorimetric procedure is rapid and relatively free of experimental inconveniences such as the control of acidity or the ionic strength, etc. The reaction proceeds smoothly in range 0–3.5 pH, but as mentioned earlier an acidity of 0.01 M (pH = 2) was thought desirable to avoid the formation of hydrolytic species of iron(III) at the elevated pH. The presence of common cations and anions as encountered in natural waters has no effect on the intensity of the color developed by the interaction of iron(III) with the solid reagent. A 10- to 50-fold excess of foreign ions such as Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Na^+, Cl^-, NO_3^-, SO_4^{2-}, and CH_3COO^- produced no ill effects on the iron determination in the ppm range. However, some interference was noticed if Cu and

TABLE 1

SPECTROPHOTOMETRIC RESPONSE OF IRON(III) AT pH 2.0 (HNO_3) AND 460 nm[a]

Sample no.	Concn Fe^{3+} (ppm)	No. of determinations	Net average absorbance	Range	Standard deviation
1	0.1	6	0.011	0.010–0.012	0.0011
2 ·	0.25	6	0.028	0.027–0.030	0.0016
3	0.5	6	0.064	0.062–0.068	0.0020
4	1.0	6	0.125	0.120–0.127	0.0026
5	2.0	5	0.225	0.223–0.228	0.0029
6	3.0	6	0.330	0.320–0.340	0.0088
7	4.0	5	0.430	0.420–0.433	0.0072
8	5.0	6	0.553	0.550–0.560	0.0063
9	7.0	6	0.765	0.750–0.770	0.0104
10	8.0	4	0.880	0.880–0.900	0.0112

[a] Period of contact: 1 hr.

Zn ions were simultaneously present in the system. Nevertheless in the 1–5-ppm iron(III) concentration range the tolerance limit was 6-fold excess for the interfering ions. The ion Hg^{2+} interfered at all concentrations.

The reaction is fairly rapid and one can observe the formation of the red-colored supernatant phase in a few minutes of contact time. A period of contact of 1 hr was arbitrarily chosen to suit the experimental needs. No color change was observed in a prolonged contact of over 6 hr.

One can visualize the exchange reaction on the basis of ion displacement through exchange at the solid surface as:

$$Hg[Co(SCN)_4] + Fe^{3+} \rightleftharpoons Co^{2+} + Hg(SCN)_2 + Fe(SCN)^{2+} + SCN^-$$
$$\text{(solid)} \qquad\qquad\qquad\qquad\qquad\qquad \text{(colored)}$$
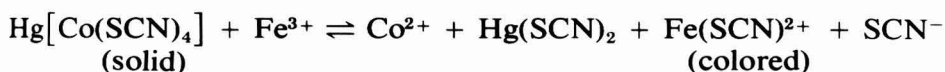
The ferric thiocyanate complex is well known. However, the color intensity of the red complex is governed by the free SCN^- ion concentration in the medium. An uncontrolled large excess of the complexing ligand can lead to the formation of complex species other than those shown in the equation above (6). In this case the ligand concentration is automatically controlled by the solubility of the solid compound at a desired acidity. The solubility product of mercuric tetrathiocyanatocobaltate(II) has been determined in this study and a value of pK_{sp} of 27.56 has been obtained at pH 2. This certainly reflects the low aqueous solubility of the solid compound and thus at any given stage a large free excess of the thiocyanate ions cannot be envisaged. It will be appropriate to assume under these conditions that the reaction expressed in the above equation remains valid and that one is actually measuring the absorption of a reproducible species in the system. It can also be argued that very few free Hg^{2+} ions are present in the system; otherwise, as noted above, their free existence would lead to the diminishing of the color intensity. Fortunately the free cobalt ions have no ill effect on the ferric thiocyanate color under the experimental conditions proposed.

The iron(II) species do not produce the colored complex species with the thiocyanate ion. This makes it possible to distinguish between the two valence states of iron. In principle one measures iron(III) first and then through an oxidative step transforms the entire iron species to the iron(III) state. The iron(II) concentration is obtained by the difference of the two absorption measurements. This is possible only if the entire iron(II) in the system is quantitatively transformed to iron(III) in the oxidative step. Our results do show that in an HNO_3–H_2O_2 medium iron(II) is in fact quantitatively transformed to the higher oxidation state. The quantitative data in Table 2 show that even at 500 ppb concentration iron(II) can be quantitatively analyzed by this solid-state exchange reac-

tion with a coefficient of variation of 1.8%. The successive analysis of
iron(III) and iron(II) in the same solution is thus possible.

This procedure has been applied to the analysis of iron(III) and iron(II)
concentrations in three varieties of waters as shown in Table 3. The
results have been compared with the official method of analysis, which
uses the ferrous phenanthroline complex and a reductive step in the
analysis (7). The remarkable similarity of the two sets of experimental
results leaves no doubt that one can employ solid-state exchange col-
orimetry with an equal degree of flexibility. The latter has the added

TABLE 2

SUCCESSIVE SPECTROPHOTOMETRIC ANALYSIS OF IRON(III) AND IRON(II) AT pH 2.0 AND 460 nm[a]

Sample no.	Concn Fe^{3+} (ppm)	Concn Fe^{2+} (ppm)	No. of determinations	Net average absorbance	Range	Standard deviation
1	2.0	0.0	6	0.225	0.223–0.228	0.0029
2	2.5	0.0	4	0.272	0.270–0.275	0.0035
3	2.0	0.5	4	0.274	0.270–0.280	0.0051
4	3.0	0.0	6	0.330	0.320–0.340	0.0088
5	2.0	1.0	4	0.331	0.325–0.337	0.0056
6	4.0	0.0	5	0.430	0.420–0.433	0.0072
7	2.0	2.0	4	0.430	0.425–0.433	0.0060
8	5.0	0.0	6	0.553	0.550–0.560	0.0063
9	2.0	3.0	4	0.552	0.550–0.555	0.0043
10	6.0	0.0	4	0.660	0.655–0.665	0.0086
11	2.0	4.0	4	0.660	0.657–0.667	0.0086
12	7.0	0.0	6	0.765	0.750–0.770	0.0104
13	2.0	5.0	4	0.764	0.760–0.767	0.0090

[a] Period of contact: 1 hr.

TABLE 3

SUCCESSIVE DETERMINTION OF IRON(III) AND IRON(II) IN NATURAL WATER SAMPLES
AND ITS COMPARISON WITH THE OFFICIAL METHOD OF ANALYSIS

Sample	Solid state reagent (ppm)[a]		Official method (7) (Fe–ortho) phenanthroline complex) (ppm)	
	Fe^{3+}	Fe^{2+}	Fe^{2+}	Fe^{3+}
Tap water[b] (local)	0.31 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.30 ± 0.01
Turtle Creek Lake (Moncton)	0.20 ± 0.01	Not detected	Not detected	0.20 ± 0.01
Petitcodiac River (New Brunswick)	0.66 ± 0.01	Not detected	Not detected	0.65 ± 0.01

[a] An average of three determinations.
[b] Analysis performed on a running water sample.

advantage that it employs a relatively less expensive compound, which can be stored indefinitely since it is a solid. The exchange reaction is rapid and one can envisage its use in field analysis as well.

ACKNOWLEDGMENT

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Oxidimetric Determination of Some Compounds with Hexamminecobalt(III) Tricarbonatocobaltate(III)

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In our previous works we studied the oxidation of a number of inorganic and organic substances with solutions of tervalent cobalt compounds, especially cobaltic acetate. The results obtained were reviewed in this journal (2). A disadvantage of this reagent, whose high redox potential promised energetic oxidizing effects (the Co(III)/Co(II) couple), is the fact that this effect has been offset to a considerable extent by the presence of complexing agents, especially acetates, with a consequent strong decrease in the redox potential. Compounds with which cobalt can be obtained in dissociated form are very unstable and attempts to generate Co(III) coulometrically have failed.

In 1965 a work by Baur and Bricker (1) appeared describing the use of a complex compound $[Co(NH_3)_6][Co(CO_3)]_3$ as an analytical oxidant, which combines to a certain degree the advantages of complex and noncomplex Co(III) compounds. The substance, solid or dissolved in a saturated $NaHCO_3$ solution, is very stable, but tervalent cobalt in the ionic form with strong oxidizing properties is liberated from the carbonate anion in acidic media. Hence it is possible to "generate" cobaltic ions from the complex during titrations in acidic media.

The complex anion $[Co(CO_3)_3]^{3-}$ was prepared earlier by oxidation of a mixture of $KHCO_3 + [Co(NH_3)_4CO_3]_2SO_4$ by persulfate or by oxidation of $KHCO_3 + CoCl_2$ by hydrogen peroxide. On addition of solid $Co(NH_3)_6(NO_3)_3$ or $Co(NH_3)_6Cl_3$ to a green solution containing the $[Co(CO_3)_3]^{3-}$ anion, green complex salt, $[Co(NH_3)_6][Co(CO_3)_3]$, precipitates. As it is sparingly soluble in water (only 0.038 g of the substance dissolves in 100 g of water) and its aqueous solutions are unstable, it was not used previously for analytical purposes.

Baur and Bricker (1) dissolved $[Co(NH_3)_6][Co(CO_3)_3]$ in an $NaHCO_3$-saturated solution, thus increasing the complex solubility approximately 10 times. The authors studied the dependence of the solution stability on the pH, the CO_3^{2-} concentration, and the time. The medium recommended by them (pH = 7.6) and the excess carbonate prevent separation of $Co(OH)_3$ and dissociation

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$$[Co(CO_3)_3]^{3-} + 6H_2O \rightleftharpoons [Co(H_2O)_6]^{3+} + 3 CO_3^{2-},$$
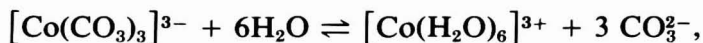
and ensure the reagent stability in solution. The Co(III) liberated from the complex was utilized by the authors for the determination of Fe(II), Ce(III), and V(IV) in a $0.5-5$ M H_2SO_4 medium by direct photometric titration; Fe(II) was also titrated potentiometrically, with Ferroin used as an indicator.

The present paper continues the study described above. The stability of the reagent was studied for dependence on the temperature and the duration of heating, and the conditions were studied for potentiometric titration of iodide, ascorbic acid, some sulfur-containing inorganic substances, and hydrogen peroxide with a $[Co(NH_3)_6][Co(CO_3)_3]$ standard solution in saturated $NaHCO_3$. The oxidation of iodide was studied in detail and a procedure has been developed for its microdetermination in the presence of chloride, bromide, and nitrate.

EXPERIMENTAL

Reagents. Solid $[Co(NH_3)_6][Co(CO_3)_3]$ and its $5 \times 10^{-3} N$ solution were prepared by the procedure described by Baur and Bricker *(1)*. The solution was standardized by titrating an $Fe(NH_4)_2(SO_4)_2$ solution of a known concentration in a 1.8 N H_2SO_4 medium; the titer was checked every 2 weeks. According to the authors *(1)* the complexion, $[Co(NH_3)_6]^{3+}$, is sufficiently stable that it does not dissociate even in strongly acidic media ($K_c' = 2.2 \times 10^{-34}$ mol/liter). On the other hand, anion $[Co(CO_3)_3]^{3-}$ decomposes at pH lower than 7, liberating Co^{3+} ion; at pH higher than 8, brown precipitate of $Co(OH)_3$ separates in aqueous solutions. The authors *(1)* found that the optimum pH from the point of view of the solution stability is the pH of the saturated $NaHCO_3$ solution (7.6), when $Co(OH)_3$ does not separate and the dissociation is suppressed by a high concentration of CO_3^{2-} according to the equation

$$[Co(CO_3)_3]^{3-} + 6H_2O \rightleftharpoons [Co(H_2O)_6]^{3+} + 3 CO_3^{2-}.$$
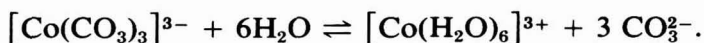
According to the authors *(2)*, the oxidant solutions are more stable in the daylight than very dilute solutions stored in the dark.

The other solutions, e.g., $5 \times 10^{-3} N$ $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 1 M H_2SO_4, $5 \times 10^{-3} N$ $K_2Cr_2O_7$, $5 \times 10^{-3} N$ KI, $5 \times 10^{-3} N$ $KBrO_3$, $5 \times 10^{-3} N$ ascorbic acid, 0.1 N H_2O_2, $0.1 - 10^{-3} N$ $Na_2S_2O_3$, and 0.1 N NH_4SCN, were prepared and standardized using common procedures. Other reagents were prepared from p.a. chemicals. Hydrochloric acid (5 N) employed for the microdetermination of iodide was prepared by diluting concd HCl with doubly distilled water.

Apparatus. A Multoscop V millivoltmeter (Laboratorní Přístroje, Czechoslovakia) was employed for the potentiometric measurements.

Formal redox potentials were measured on a PHM-64 pH-meter (Radiometer, Denmark); this instrument was also used for titrations in an inert atmosphere. A platinum wire indicator and a saturated calomel reference electrodes were used.

The solutions for the microdetermination of KI were measured by syringe (Interchangeable) and the standard titrant solution was added from a microburette (Agla, England).

RESULTS

Dependence of the Standard Solution Stability on the Temperature

This dependence has not yet been studied; we dealt with it in order to find out whether the reagent could be used in determinations of some organic compounds, where the substance has to be oxidized with excess reagent at an elevated temperature. A $[Co(NH_3)_6][Co(CO_3)_3]$ solution standardized at 20°C was heated to 40, 60, 80 and 100°C; after a certain time it was cooled to the original temperature and the change in the titer was determined by titration of a solution of Fe(II) with a known concentration. The concentration dependence on the duration of heating was plotted for each temperature (Fig. 1).

It has been found that the solution concentration decreases by 1.16% after 10 min of heating at 40°C; it virtually does not change on further heating (a decrease of 0.87% was found after an additional 4 hr). At higher
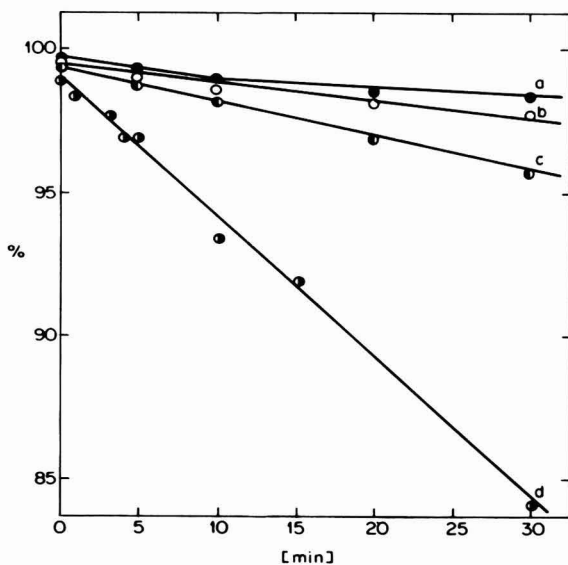

FIG. 1. Dependence of the stability of a $[Co(NH_3)_6][Co(CO_3)_3]$ solution in saturated NaHCO$_3$ on the heating time. (a) $t = 40°C$; (b) $t = 60°C$; (c) $t = 80°C$; (d) $t = 100°C$.

temperatures the titer decreases approximately linearly and the decrease is faster at higher temperatures (see Fig. 1).

Reactions of the Titrant in an Acidic Medium

As has already been mentioned, the solid complex cation $[Co(NH_3)_6]^{3+}$ virtually does not dissociate and Co(III) is liberated only by dissociation of the anion:

$$[Co(CO_3)_3]^{3-} + 6\ H_2O \rightleftharpoons [Co(H_2O)_6]^{3+} + 3\ CO_3^{2-}. \tag{1}$$
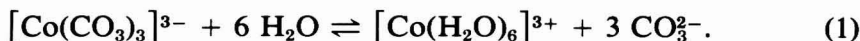
Sodium hydrocarbonate, in the saturated aqueous solution of which the reagent is dissolved, simultaneously undergoes hydrolysis

$$NaHCO_3 + H_2O \rightleftharpoons NaOH + H_2CO_3. \tag{2}$$
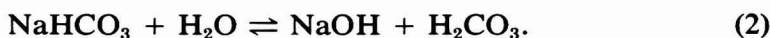
The hydrolysis products are further dissociated,

$$NaOH \rightarrow Na^+ + OH^- \tag{3}$$

$$H_2CO_3 \overset{K_1}{\rightleftharpoons} H^+ + HCO_3^-, \tag{4a}$$

$$HCO_3^- \overset{K_2}{\rightleftharpoons} H^+ + CO_3^{2-}. \tag{4b}$$
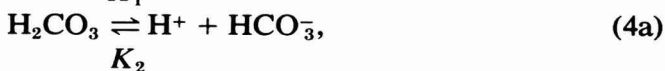
The concentration of CO_3^{2-} formed by reaction (4b), determined by dissociation constant $K_2 = 5.2 \times 10^{-11}$ mol/liter, suffices for suppressing the dissociation according to Eq. (1) and consequently Co(III) is quantitatively complexed.

If H^+ ions are added to a solution of $[Co(NH_3)_6][Co(CO_3)_3]$ in saturated $NaHCO_3$, the hydroxide formed by the hydrolysis is first neutralized

$$OH^- + H^+ \rightleftharpoons H_2O. \tag{5}$$

The concentration of water thus increases, enhancing the hydrolysis of $NaHCO_3$ (Eq.(2)) and, to a small degree, the dissociation of the reagent (Eq.(1)). After complete hydrolysis of $NaHCO_3$, further addition of H^+ ions suppresses the dissociation of H_2CO_3 (Eqs. (4a, 4b)) and the concentration of CO_3^{2-} in the solution decreases. This effect, together with increasing concentration of water (Eq.(5)), disturbs dissociation equilibrium (1). This equilibrium can be reestablished only through dissociation of the complex anion

$$[Co(CO_3)_3]^{3-} + 6\ H_2O \rightarrow [Co(H_2O)_6]^{3+} + 3\ CO_3^{2-}. \tag{6}$$

During titrations with a $[Co(NH_3)_6][Co(CO_3)_3]$ standard solution in satu-

rated NaHCO₃ in an acidic medium with a high concentration of H⁺ ions, Eqs. (5) and (6) proceed simultaneously and Co(III) is liberated in ionic form.

On the basis of this mechanism it is possible to explain anomalous shapes of potentiometric titration curves obtained in attempts to determine iodide indirectly, and the fluctuation and decrease in the potentials during direct titrations of I⁻ in 0.1−0.9 N HCl media, as will be shown below.

Determination of Iodide

The effect of the medium. The following titration procedure was employed: 5 ml of a 5×10^{-3} N KI solution, whose factor was determined by titration with KBrO₃, was diluted to 50 ml with the appropriate electrolyte and potentiometrically titrated with a [Co(NH₃)₆][Co(CO₃)₃] standard solution; 0.02-ml portions of the titrant were added in the vicinity of the equivalence point.

The iodide was oxidized only to iodine in an H₂SO₄ medium, but the potential stabilization was slow and the values were quite irreproducible.

If the titration is performed in HCl, the iodide is first oxidized to iodine and on further addition of the titrant to I⁺, as indicated by discoloration of starch temporarily colored blue. As the titration was sufficiently fast in HCl media, the effect of the acid concentration on the titration course was followed in a range of 0.1−9 N HCl. Only one break appeared on the potentiometric curves in media of 0.1−0.3 N HCl, corresponding to the reaction $2I^- \rightarrow I_2 + 2e^-$; however, the break is sufficiently high only in 0.1 N acid. Immediately after reaching the equivalence point the potential begins to decrease to the value corresponding to Co(III) bound in the complex and thus this oxidation reaction cannot be utilized for the determination of iodide. This effect is caused by depletion of H⁺ ions in neutralization of the NaOH formed by hydrolysis of the hydrocarbonate and the dissociation of $[Co(CO_3)_3]^{3-}$ stops. In solutions 0.3−0.9 N in HCl another potential break occurs, corresponding to the oxidation $I_2 \rightarrow 2I^+ + 2e^-$. However, the potential stabilization in the vicinity of the equivalence point is slow and the potential fluctuates and decreases after the equivalence; the potential decrease becomes smaller with increasing HCl concentration. In media at least 1.5 N in HCl, a sharp and high potential increase occurs, corresponding to the reaction

$$I^- + 2\,Co^{3+} \rightarrow I^+ + 2\,Co^{2+}.$$

On the basis of these experiments, media 4−5 N in HCl were selected as the most suitable for the titration of iodide with a [Co(NH₃)₆][Co(CO₃)₃]

standard solution. As a well-developed potential break occurs at the equivalence point, the potential values stabilize virtually instantaneously and are stable for a long time.

The Effect of Chloride, Bromide and Nitrate

From the results obtained it was evident that the chloride ion does not interfere in the determination of iodide even in the medium of 9 N HCl where the Cl$^-$ concentration is so high that solid NaCl separates during the titration. Therefore the effect of chloride was not followed further.

In the study of the effect of bromide and nitrate, solid KBr or KNO$_3$ was added to 45 ml of 5 N HCl and the mixture was stirred until the substance dissolved. Then 5 ml of 5×10^{-3} N KI solution of a known titer was added and the solution was titrated with a [Co(NH$_3$)$_6$][Co(CO$_3$)$_3$] standard solution, standardized by titration of a Mohr salt solution of a known concentration. The titrant was added in 0.02-ml portions in the vicinity of the equivalence point.

In the presence of KNO$_3$ the solution must be titrated as fast as possible, as otherwise the oxidizing properties of the nitrate will become perceptible; the I$^-$ is oxidized to I$_2$ and the iodine volatilizes, resulting in a lower titrant consumption.

The results obtained are summarized in Tables 1 and 2. It can be seen that bromide does not seriously interfere up to an iodide-to-bromide ratio of 1:5000; an excess of nitrate higher than 1:2500 leads to rapid oxidation $2I^- \rightarrow I_2 + 2e^-$, the iodine volatilization, and consequently to lower results.

Visual Determination of Iodide

Solutions of starch, chloroform, and tetrachloromethane were employed as indicators in these titrations. The visual determinations are

TABLE 1

THE EFFECT OF THE PRESENCE OF BROMIDE ON THE DETERMINATION OF IODIDE

KI:KBr	Taken (mg KI)	Found (mg KI)	Error	
			d (mg)	e (%)
1:0	2.0750	2.0638	−0.0112	−0.54
1:100	2.0750	2.0389	−0.0361	−1.74
1:500	2.0750	2.0493	−0.0357	−1.20
1:1000	2.0750	2.0930	+0.0180	+0.87
1:2500	2.0750	2.1192	+0.0442	+2.12
1:5000	2.0750	2.1132	+0.0882	+4.24
1:7500	2.0750	2.2380	+0.1630	+7.93

based on changes in the coloration of the solution or an organic solvent layer in the presence of iodine liberated during gradual oxidation,

$$2I^- \rightarrow I_2 + 2e^- \rightarrow 2I^+ + 2e^-.$$

Procedure. A solution of 5 or 10 ml of 5×10^{-3} N KI was diluted to 50 ml with 5 N HCl, 1 ml of an indicator added, and then titrated with a $[Co(NH_3)_6][Co(CO_3)_3]$ standard solution to disappearance of the temporary coloration of the solution (the organic solvent layer); 0.05-ml titrant portions were added in the vicinity of the equivalence point.

The visual determinations are summarized and compared with potentiometric titrations in Table 3. From the results it follows that the visual titration employing starch or CCl_4 as indicators can also be applied to the determination of iodide. Chloroform layers are discolored very slowly and the error of the determination is large.

Microdetermination of Iodide

Procedure. The standardized 5×10^{-3} N KI solution was accurately diluted and 1 ml of the dilute solution was added by a syringe to 10 ml of 5 N HCl in redistilled water. The titrant was added from an Agla mi-

TABLE 2
THE EFFECT OF THE PRESENCE OF NITRATE ON THE DETERMINATION OF IODIDE

KI:KNO₃	Taken (mg KI)	Found (mg KI)	Error d(mg)	e(%)
1:0	2.0750	2.0638	−0.0112	−0.54
1:100	2.0750	2.0644	−0.0106	−0.51
1:500	2.0750	2.0670	−0.0080	−0.37
1:1000	2.0750	2.0562	−0.0188	−0.90
1:2500	2.0750	2.0465	−0.0285	−1.37

TABLE 3
COMPARISON OF VARIOUS INDICATION METHODS IN THE DETERMINATION OF IODIDE

Indication	Taken (mg KI)	Found (mg KI)	Error d(mg)	e(%)
CHCl₃	2.0750	2.1852	+0.1102	+5.31
	4.1500	4.3560	+0.2060	+4.94
CCl₄	2.0750	2.0204	−0.546	−2.63
	4.1500	4.0785	−0.0715	−1.94
Starch	2.0750	2.0250	−0.0500	−2.41
	4.1500	2.0250	−0.0500	−2.41
	4.1500	4.0837	−0.0663	−1.59
Potentiometry	2.0750	2.0649	−0.0101	−0.48

croburette, in 5-μl portions in the vicinity of the equivalence point. The blank determination was performed in parallel.

The results obtained are given in Table 4; it is evident that down to 8.3 μg KI can be determined in a volume of 10 ml with good precision. The interferences of other ions were studied in the determination of 8.3 μg KI, by adding solid substances (KCl, KBr, KNO_3). It has been found that chloride does not interfere in the determination; bromide does not interfere up to a ratio of 1:500. As the microtitrations are slower than the macrotitration, the oxidizing effect of KNO_3 is perceptible at its 10-fold excess.

Indirect Determination of Iodide

Back-titration of excess reagent is often employed to check the quantitativeness of the direct determination; this procedure was employed for the determination of iodide.

To 5 ml of 5×10^{-3} N KI was added 5 ml of 7.75×10^{-3} N reagent solution in saturated $NaHCO_3$, the mixture was diluted with water to 25 ml and back-titrated with a 5×10^{-3} N $Fe(NH_4)_2(SO_4)_2$ solution in 2 N H_2SO_4, standardized by titration of a 5×10^{-3} N $K_2Cr_2O_7$ standard solution.

The initial low potential of the complexed Co(III) increased very slowly up to ca. 2.5 ml of Fe(II) in 2 N H_2SO_4, added when most of the NaOH formed by hydrolysis of $NaHCO_3$ was neutralized (the saturated aqueous solution of $NaHCO_3$ is approximately 1 N). Then dissociation of $[Co(CO_3)_3]^{3-}$ started and the potential sharply increased owing to the presence of cobaltic ions in the solution. The redox reaction,

$$Co^{3+} + Fe^{2+} \rightarrow Co^{2+} + Fe^{3+},$$

took place, accompanied by a decrease in the potential, which does not

TABLE 4
MICRODETERMINATION OF IODIDE

Taken (μg KI)	Found (μg KI)	Error e (%)	ΔmV/5 ul (eq)	$t_1{}^a$ (min)	$t_2{}^b$ (min)	Note^c
830	815.23	-1.78	155	0.5	5	
83	82.66	-0.41	135	1–2	5	A
8.3	8.09	-2.58	45	1.5–2	5	A,B
0.83			35		0.5	A,C,D

^{a} t_1, time of potential stabilization at the endpoint.
^{b} t_2, stability of the endpoint potential.
^{c} (A) 5 NHCl in redistilled water. (B) 10^{-3} N $[Co(NH_3)_6]$ $[Co(CO_3)_3]$ in saturated $NaHCO_3$. (C) $5 = 10^{-4}$ N $[Co(NH_3)_6][Co(CO_3)_3]$ in saturated $NaHCO_3$ (D) Potential stabilizes very slowly.

correspond to the equivalence point of any redox reaction which may possibly occur in the system. This can be explained by the fact that part of the Co(III) was previously liberated from the complex during the neutralization of NaOH and reacts with the present reductants.

The oxidation of the iodide cannot be detected in this way; its presence causes only a decrease in the potential maximum, but not a change in the position of the potential break on the potentiometric titration curve. This can be seen from the potentiometric titration curves given in Fig. 2, corresponding to titration of one-half of the amount of I^- with 5 ml of $7.75 \times 10^{-3} N$ reagent and to the determination of 10 ml of $5 \times 10^{-3} N$ KI with 10 ml of $7.75 \times 10^{-3} N$ reagent and the corresponding blanks.

Determination of Ascorbic Acid

We assumed that the oxidation of ascorbic acid by a strong oxidant, such as the generated Co^{3+} ion, will be connected with a greater change in the acid molecule. However, it has been found that the oxidation is analogous to that attained with common oxidants; i.e., dehydroascorbic acid is formed with an exchange of two electrons. It is necessary to titrate in an inert atmosphere, otherwise negative errors arise, owing to the oxidation of ascorbic acid by atmospheric oxygen. On the basis of the
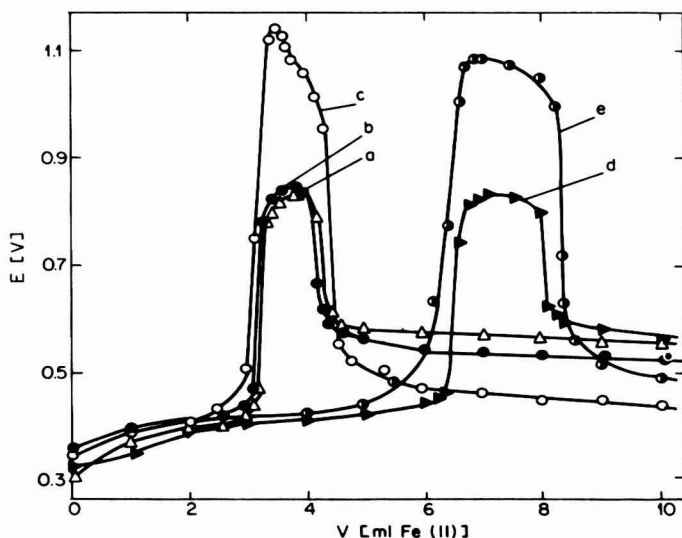
FIG. 2. Potentiometric curves for back-titration of $[Co(HN_3)_6][Co(CO_3)_3]$. (a) 5 ml 5×10^{-3} N KI + 5 ml $7.75 \times 10^{-3} N$ Co(III); (b) 5 ml $2.5 \times 10^{-3} N$ KI + 5 ml $7.75 \times 10^{-3} N$ Co(III); (c) 5 ml $7.75 \times 10^{-3} N$ Co(III); (d) 10 ml $5 \times 10^{-3} N$ KI + 10 ml $7.75 \times 10^{-3} N$ Co(III); (e) 10 ml $7.75 \times 10^{-3} N$ Co(III). Co(III) = $[Co(NH_3)_6][Co(CO_3)_3]$ in saturated NaHCO$_3$; Fe(II) = Fe(NH$_4$)$_2$(SO$_4$)$_2$ in 2 N H$_2$SO$_4$.

experiments following the effect of the medium acidity, the following procedure has been developed.

Forty milliters of 5 N HCl are deaerated by the passage of nitrogen for 15 min, a 10-ml sample of ascorbic acid is added, and the solution is potentiometrically titrated with the reagent with continuous passage of nitrogen. The titrant is added in 0.02-ml portions in the vicinity of the equivalence point.

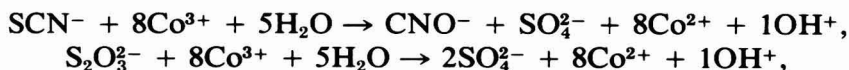The results are given in Table 5.

Determination of Thiocyanate, Thiosulfate, and Peroxide

Attempts to utilize these oxidations quantitatively were unsuccessful. The potentiometric titrations are slow; the most suitable media are 9 N HCl (thiocyanate and thiosulfate) and 3–5 N HCl (H_2O_2). The potential stabilization took 10 min after each titrant addition. The oxidation of thiocyanate and thiosulfate involved eight electrons; i.e., sulfate was the final product. The results were subject to a negative error, amounting to -7% with thiocyanate, which was caused by volatilization of temporarily formed sulfur oxides; approximately the same error for thiosulfate was caused by volatilization of the hydrogen sulfide formed. It is nevertheless interesting that the oxidation leads as far as sulfate in these media. The reactions

$$SCN^- + 8Co^{3+} + 5H_2O \rightarrow CNO^- + SO_4^{2-} + 8Co^{2+} + 10H^+,$$
$$S_2O_3^{2-} + 8Co^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Co^{2+} + 10H^+,$$

can be assumed.

The oxidation of hydrogen peroxide (the formation of O_2) exhibited no advantages over titrations with other reagents because it took a long time, although it was quantitative and its precision attained the level common for oxidimetric determinations.

TABLE 5
DETERMINATION OF ASCORBIC ACID IN AN INERT ATMOSPHERE

t^a (min)	Taken (mg)	Found (mg)	Error	
			mg	e (%)
5	4.6689	4.5810	−0.0879	−1.87
10	4.6689	4.6293	−0.0396	−0.84
15	4.5584	4.5848	+0.0264	+0.56
30	4.5584	4.6055	+0.0469	+0.102

^{a} t. time of passing nitrogen through the titrand solution.

SUMMARY

The possibilities of utilization of the hexamminecobalt(III) tricarbonatocobaltate(III) complex for potentiometric determination of some substances are discussed. The reaction scheme for this reagent is proposed for acidic media (the "generation" of Co^{3+}). A microdetermination of iodide has been developed. The oxidation of thiocyanate, thiosulfate and hydrogen peroxide is briefly discussed.

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Ion Chromatographic Determination of Sulfur and Chlorine Using Milligram and Submilligram Sample Weights[1]

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INTRODUCTION

The simplicity and accuracy of the Schöniger oxidation techniques *(1)* has led to its wide application in routine elemental analysis. However, small sample sizes, interferences, and low concentration levels of heteroatoms in the sample cause the greatest problems in the analysis of scrubbing solutions. Ion Chromatography *(2)* (IC) can specifically analyze multiple trace inorganic anions in a single run by conductimetric detection. Thus it offered the possibility of rapid analysis of heteroatoms in organic compounds. This report summarizes our initial findings for the analysis of chlorine and sulfur by IC. Sample weights ranged from 0.5 to 150 mg. Chlorine percentages in the samples were from 0.4 to 50% and sulfur concentrations were from 0.015 to 33%.

MATERIALS AND METHODS

Equipment and Reagents

A Dionex Model 10 Ion Chromatograph was used for the chlorine and sulfur analysis with these instrumental conditions:

Eluent:	0.0030 M NaHCO$_3$/0.0024 M Na$_2$CO$_3$
Flow rate:	138 ml/hr for chlorine, 230 ml/hr for sulfur
Sample loop:	200 μl
Separator column:	3 mm I.D. × 500 mm
Separator resin:	Dionex low-capacity anion exchange resin
Suppressor column:	6 mm I.D. × 250 mm
Suppressor resin:	Dionex high-capacity cation exchange resin
Detection:	Ion Chromatograph conductivity cell and meter with strip chart output

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A Mettler mechanical balance for weights of 1–150 mg.

A Perkin–Elmer electronic microbalance for weights from 50 μg to 1 mg.

A 500-ml Schöniger apparatus.

Sodium carbonate, sodium bicarbonate, and 30% hydrogen peroxide from J. T. Baker.

Whatman 42 filter paper flags by Arthur H. Thomas Co.

Procedure

The weighed samples were combusted and scrubbed into a mixture of 3% H_2O_2/0.0030 M $NaHCO_3$/0.0024 M Na_2CO_3. After a 30-min equilibration period, the samples were loaded into the Ion Chromatograph sample loop from plastic syringes. Syringe filters were used when visible ash was present.

RESULTS AND DISCUSSION

Calibration curves (Fig. 1) for chlorine and sulfur were obtained by combustion of S-benzyl thiuronium chloride (17.49% Cl, 15.82% S). Sample weights ranged from 50 μg to 6 mg. The calibration curves were linear from 0.25 to 10 mg. For sample weights less than 250 μg, a very slight curvature in the line is noted when the scale response is magnified. Results by IC and classical methods for samples submitted to Galbraith Laboratories for routine sulfur and chlorine analyses are shown in Tables 1 and 2.

Excellent agreement between IC and classical methods was obtained for most samples. The IC chlorine analyses for Samples 70 and 71 show deviation from titration values possibly due to homogeneity problems compounded by the low sample weight used for IC analysis. Sample 74
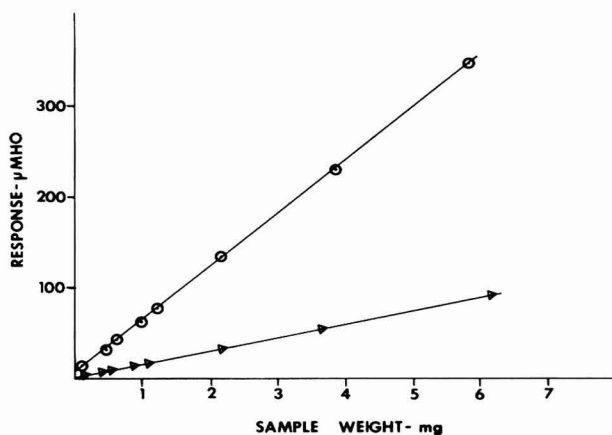

FIG. 1. Chlorine and sulfur calibration curves; ⊙, chlorine; △, sulfur.

TABLE 1
COMPARISON OF CHLORINE PERCENTAGES BY ION CHROMATOGRAPHY
(IC) AND ARGENTIMETRIC TITRATION (AT)

| Sample | Weight for IC analysis (mg) | Chlorine percent | |
		IC	AT
56	2.272	40.47	39.90
57	10.000	2.28	2.39
58	10.984	2.35	2.43
59	4.095	15.44	15.44
60	2.140	1.65	1.71
70	0.532	49.20	47.38
71	0.526	34.22	31.60
72	1.185	11.64	13.20
73	1.056	7.04	6-7
74	16.921	0.54	0.25
	16.201	0.52	0.63
75	16.804	0.41	0.45

TABLE 2
COMPARISON OF SULFUR PERCENTAGES BY ION CHROMATOGRAPHY (IC) TO
KNOWN VALUES (KV) AND BARIUM PRECIPITATION TECHNIQUES (BPT)

| Sample | Weight for IC analysis (mg) | IC | Sulfur percent | |
			BPT	KV
NBS Coal A	22.420	0.546		0.546
NBS Coal B	10.812	1.97		2.02
NBS Coal C	8.279	3.09		3.02
Sulfamic acid	7.469	32.95		33.03
	0.501	32.94		33.03
80	3.988	7.29	7.25	
81	10.900	1.52	1.46	
82	1.560	12.59	11.52	
	0.522	12.49		
84	43.6	0.0150	0.0240	
85	96	0.0326	0.0297	
86	159	0.0258	0.0296	
	29	0.0239	0.0331	
87	110	0.0259	0.0288	

showed very poor reproducibility for the argentimetric titration because of the presence of a large amount of bromine, whereas duplicate analyses by IC showed excellent agreement for chlorine. Sample 73, which showed a large fluoride peak by IC, also gave poor titration precision.

We thought that measurement of trace chlorine, such as that found in the combusted flag, might be hindered by the close elution of the carbo-

nate peak (Fig. 2). To check peak overlap between the CO_3^{2-} and Cl^-, the eluent was changed to 0.0015 M NaHCO$_3$ (Fig. 3). No changes in the chlorine values were observed for any samples.

Sulfur analyses using both barium turbidimetric and gravimetric techniques were compared to IC. The scrubbing solution is the same for both the chlorine and sulfur analyses. The eluent flow rate was raised to shorten the SO_4^{2-} elution time to 7 min. Excellent agreement between IC and known values for the three NBS coal samples and for sulfamic acid
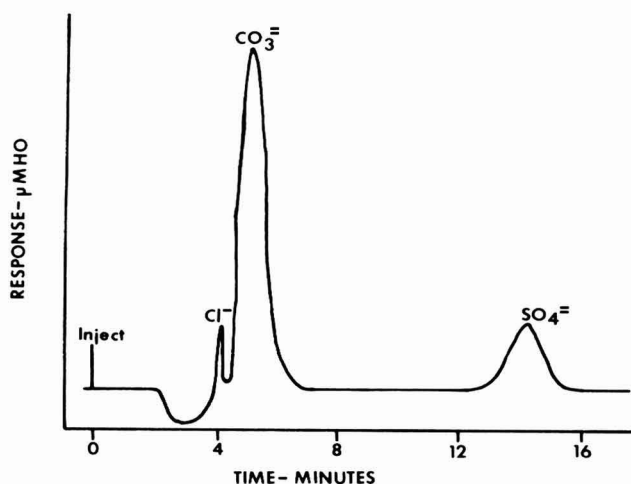

FIG. 2. Ion chromatogram of Schöniger-combusted sample using 0.0030 M NaHCO$_3$/0.0024 M Na$_2$CO$_3$ eluent.
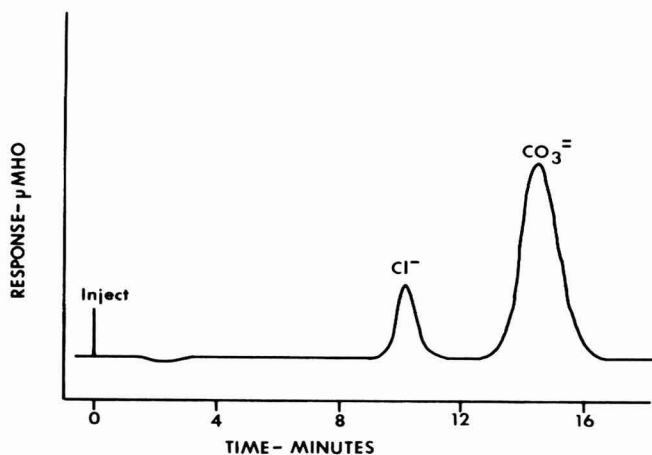

FIG. 3. Ion chromatogram of Schöniger-combusted sample using 0.0015 M NaHCO$_3$.

are shown in Table 2. Comparison of IC values for both the sulfamic acid and for Sample 82, where both submilligram and milligram samples were combusted, shows that if sample homogeneity is not a problem, submilligram sample weights can be accurately analyzed for sulfur. Also, Sample 86 is of particular note because of the good agreement when 159- and 29-mg samples were combusted. For reproducible turbidimetric analyses, 3–5 g of sample must be burned.

SUMMARY

Initial results for Ion Chromatographic analyses of chlorine and sulfur in organic compounds combusted by the Schöniger technique show excellent agreement with analyses using standard methods. Samples weighing less than 1 mg can be analyzed if homogeneity is not a problem. Determination of chlorine in the presence of large amounts of bromine and the analysis of other heteroatoms is also possible by IC. The present limiting factor in the microdetermination of chlorine and sulfur by IC is the presence of these species in the filter paper flag.

ACKNOWLEDGMENTS

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Selective Spectrophotometric Determination of Uranium

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INTRODUCTION

Spectrophotometry is still the main method for determination of trace amounts of uranium due to the poor sensitivity of atomic absorption and plasma emission spectrometry for this element. There are described about 300 methods of spectrophotometric uranium determination; however, none of them is suitable for routine analysis of concentrations ≤1 ppm of uranium in the presence of huge amounts (15–19 mmol) of iron or copper. Doubtlessly, Arsenazo III, that is, 3, 6-bis (*o*-arsonophenylazo) chromotropic acid, is the most sensitive reagent for spectrophotometric uranium determination *(8)*. Common elements (iron, aluminum, copper, zinc, and calcium) interfere however, if present in higher excess (>100-fold) and must be separated. Triisooctylamine *(5)* and tri-*n*-octylphosphine oxide *(6)* have the reputation of most efficient extractants for uranium in comparison with older tri-*n*-butyl phosphate *(2)* and tetrapropylammonium nitrate *(4)*. The action of both latter reagents is based on the salting effect of aluminum nitrate which is an obvious drawback of both methods. The extraction with triisooctylamine and tri-*n*-octylphosphine oxide occurs in 6–7 *M* hydrochloric acid and 1–4 *M* nitric acid, respectively. In spite of a high efficiency of that extraction, its selectivity is not good since considerable amounts of iron(III), thorium, titanium, vanadium(V), chromium(VI) and molybdenum(VI) are coextracted. On the other hand, phosphate, fluoride, and oxalate decrease the extraction. The use of EDTA (ethylenedinitrilotetraacetic acid) or CDTA (1,2-cyclohexylenedinitrilotetraacetic acid) as masking reagents has little effect even at pH 0.5–0.1 (tri-*n*-octylphosphine oxide) due to the blocking effect of hydrogen ions and small solubility of reagents within those conditions *(3)*.

Most selective separation of uranium(VI) from other elements is achieved by extraction with dibenzoylmethane in benzene employing EDTA *(7)* or better CDTA *(9)* as masking reagent at pH 6–7. That separation in combination with final Arsenazo III spectrophotometry represents a very economical and sensitive determination of uranium and is described in this paper.

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MATERIALS AND METHODS

Reagents. Aqueous solution of uranyl acetate containing 10.0 ppm (w/v) of uranium, 2.0% solution of dibenzoylmethane in benzene, 0.05% aqueous solution of Arsenazo III, aqueous stock solutions of beryllium chloride, magnesium sulfate, aluminum chloride, calcium chloride, titanium(IV) chloride, ammonium metavanadate, chromium(III) chloride, zinc sulfate, yttrium nitrate, cobalt(II) chloride, copper(II) chloride, zirconium nitrate, sodium niobate, ammonium molybdate, silver nitrate, cadmium chloride, tin(IV) chloride, lanthanum nitrate, mercury(II) nitrate, thallium(III) nitrate, lead(II) nitrate, bismuth nitrate, and thorium nitrate contained 1.00% (w/v) of the corresponding metal; 14% (w/v) aqueous ammonia and CDTA. Baker's analyzed reagents had been used throughout.

Instruments. Photometric measurements had been made with a double-beam Cary 118C spectrophotometer (Varian Assoc., Palo Alto, Calif.) and 1-cm quartz cells. Measurements of pH had been performed with Model 801A pH-meter and combined glass electrode 91-01 (both Orion Corp., Cambridge, Mass.).

Procedure. Pipet 100 ml of a sample solution containing 10–100 μg of uranium(VI) into a 250 ml beaker and add 420 mg of CDTA for each millimole of the metal ion present. Stir and neutralize dropwise with 14% aqueous ammonia until the pH of 6–7 is reached (pH-meter). At this point the solution should be free of any metal hydroxide precipitate. If this does not take place add more CDTA and repeat the ammonia neutralization. In that way the total amount of 8 g of CDTA can be consumed. It is not advisable to use more CDTA because of solubility limits. If more CDTA is needed, the sample solution should be diluted with water and the sensitivity of determination is decreased accordingly. After the neutralization is made properly, transfer the solution into a 250-ml separatory funnel and shake mechanically with three 25-ml portions of dibenzoylmethane solution for 5 min each. Collect the upper organic layers in another 250-ml separatory funnel, shake with 50 ml of 0.01 M CDTA–diNa then with two 50-ml portions of 6 M hydrochloric acid and collect that acidic solution in another 250-ml separatory funnel and wash by shaking with 50 ml of benzene. Time of shaking is again 5 min in each case. Transfer the acidic aqueous solution to a 250-ml beaker and evaporate to dryness at 105°. Add 10 ml of 0.1 M hydrochloric acid and 25 ml of water and boil for 2–3 min, cool and transfer into a 50-ml volumetric flask. Add 2.0 ml of 0.05% Arsenazo III, dilute with water to mark, mix well and, after 5 min, measure the absorbance in 1-cm cells at 650 nm against a reagent and buffer (HCl) blank. Calculate the uranium concentration by means of a set of standards containing 20, 40, 60, 80, and 100 μg of uranium(VI) by means

of a calculator with a linear regression program or using a graph. Usually the average deviation does not exceed ±0.1 μg of uranium. The absorbance of solutions is stable at least 24 hr.

The used solution of dibenzoylmethane can be shaken with equal portions of saturated aqueous solution of sodium tetraborate until the aqueous phase has a pH greater than 6. Then the solution is ready for another extraction. In that way, it is possible to use the same solution at least three times.

RESULTS AND DISCUSSION

If the extraction of uranium(VI) described above is performed at shorter intervals of shaking, the relationship between a single period of shaking (in minutes) and the percent of total extracted uranium (in parentheses) is as follows: 1 (52), 2 (77), 3 (91), 4 (99), 5 (100). Theoretical pH of optimum uranium(VI) extraction is 4.3 for 0.1 M CDTA (1), however, in the presence of interfering cations that value must be increased to 6–7 for better masking of those cations by CDTA.

Several typical results of uranium(VI) determination and the effect of 31 various interfering ions are shown in Table 1. From those ions only the

TABLE 1
DETERMINATION OF URANIUM

Uranium (μg)	Foreign ion (mg)		Absorbance at 650 nm	Uranium (μg)	Foreign ion (mg)		Absorbance at 650 nm
15		I	0.048	30	100	Pb(II)	0.097
30		—	0.097	30	200		0.099
45		—	0.145	30	300		0.096
30	100	Fe(III)	0.098	45	0.04	Be(II)	0.146
30	300		0.097	45	0.08		0.165
30	600		0.100	45	0.12		0.233
30	200	Cu(II)	0.096	30	1	V(V)	0.098
30	400		0.096	30	10		0.110
30	800		0.098	30	100		0.145
15	100		0.047	30	1	Nb(V)	0.097
30	300		0.098	30	10		0.099
30	600		0.099	30	100		0.120
15	50	Al(III)	0.047	45	1	Mo(VI)	0.146
30	100		0.098	45	10		0.155
45	300		0.147	45	100		0.178
30	100	Zn(II)	0.096	30	1	Zr(IV)	0.098
30	300		0.096	30	10		0.101
15	600		0.049	30	100		0.167
30	100	Th(IV)	0.098	30	50	Mg(II)	0.098
30	300		0.099	30	100		0.097
45	600		0.144	30	150		0.097
30	100	Si(IV)	0.097	45	1	P(V)	0.142

TABLE 1 (continued)

Uranium (μg)	Foreign ion (mg)		Absorbance at 650 nm	Uranium (μg)	Foreign ion (mg)		Absorbance at 650 nm
15	200		0.046	45	10		0.140
45	300		0.146	45	100		0.110
30	100	Ni(II)	0.099	45	1	As(V)	0.145
30	200		0.097	45	10		0.140
30	500		0.098	45	100		0.130
15	100	Bi(III)	0.047	30	1	Sc(III)	0.097
30	200		0.098	30	10		0.099
45	300		0.146	30	100		0.098
30	1	Ti(IV)	0.098	45	100	Hg(II)	0.146
30	10		0.102	45	200		0.147
30	100		0.151	45	300		0.148
45	1	Cr(III)	0.144	30	1	Ag(I)	0.097
45	10		0.145	30	10		0.099
45	100		0.148	30	100		0.120
45	1	Mn(II)	0.145	30	100	Cd(II)	0.096
45	10		0.147	30	200		0.098
45	100		0.148	30	300		0.098
15	50	Y(III)	0.047	30	1	Sn(IV)	0.097
15	100		0.047	30	10		0.110
15	150		0.049	30	100		0.157
30	100	Co(II)	0.097	45	50	Tl(III)	0.145
30	200		0.098	45	100		0.148
30	300		0.098	45	150		0.149
30	100	La(III)	0.098	30	1	Sb(V)	0.098
30	200		0.097	30	10		0.115
30	300		0.099	30	50		0.145

following amounts (in mg) affect the absorbance more than 5%: Beryllium(II), 0.06; vanadium(V), 10; niobium(V), 100; molybdenum(VI), 10; zirconium(IV), 50; phosphorus(V), 50; arsenic(V), 100; titanium(IV), 50; silver(I), 100; tin(IV), 10; and antimony(V), 10. Most of common ions such as iron(III), copper(II), aluminum(III), and zinc(II) do not interfere even if present in the amount of 15–19 mmol. Common anions (chloride, sulfate, nitrate, perchlorate) do not interfere in amounts of 100 mmol.

The determination based on complexation of uranium(IV) with Arsenazo III is almost twice as sensitive (6) as the method described. However, its selectivity and reproductibility is inferior. Many ions regularly coextracted with uranium [vanadium(V), niobium(V), molybdenum(VI), titanium(IV), tin(IV)] are reduced simultaneously during the transformation uranium(VI) → uranium (IV) and their reduced form bleaches the Arsenazo III.

The use of the calcium complex of EDTA or CDTA *(7)* instead of the noncomplexed form is more detrimental than advantageous due to the blocking of masking activity of those reagents by calcium.

SUMMARY

Uranium(VI) (10–100 μg) is extracted with 2% dibenzoylmethane in benzene at pH 6–7 while other cations are masked with CDTA (maximum of 8 g/100 ml). Shaking with 6 M hydrochloric acid reintroduces uranium(VI) into the aqueous phase, where after evaporation and complexation with Arsenazo III in 0.02 M hydrochloric acid the final measurement of absorbance at 650 nm is performed. Iron(III), copper(II), aluminum, and zinc do not interfere even if present in amounts of 15–19 mmol.

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Determination of Sulfur Dioxide in Air

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INTRODUCTION

During the last two decades the toxic effects of sulfur dioxide in air have spurred the efforts of analytical chemists to discover a convenient method of its determination. Individual methods are well described in a recent monograph *(6)*. The method based on absorption and oxidation of sulfur dioxide by hydrogen peroxide and subsequent barium perchlorate titration of formed sulfate ion found the widest acceptance in industry and at controlling agencies *(7)*. The titration is usually performed with a visual indiciation.

There are numerous indicators, in general containing some oxygen donors in a proper combination with an organic chromogenic system of π-electrons, that give color reaction with barium. From that number, there are about 30 arsono phenyl derivatives; their proper evaluation has never been performed, and from them the 1-(*o*-arsonophenylazo)-2-hydroxynaphthalene-3, 6-disulfonic acid (Thoron, Thorin, Naphtharsen, APANS), introduced by Kuznetsov *(5)*, is the most frequently used indicator for that titration *(4)*.

During the last decade the bis(phenylazo) derivatives of chromotropic acid (containing the sulfo, phosphono, or carboxyl group in the ortho position to the azo group) gained some prominence as indicators of barium titration of sulfate *(1,2)*. Chlorophosphonazo(III) (CPA), that is, 3, 6-bis(*p*-chloro-*o*-phosphonophenylazo)-chromotropic acid, is the most sensitive known colorimetric reagent for barium; the absolute molar absorptivity of barium complex BaH_2L is 52,000 $cm^2/mmol$ at 660 nm and pH 6.8–7.1 *(1)*. Since that reagent is available commercially,[1] during the last years we have investigated its possible application to the determination of sulfur dioxide in air and have compared it with Thorin.

MATERIALS AND METHODS

Reagents. A solution of 0.005 M barium perchlorate was prepared by dissolution of 1.95 g of barium perchlorate (trihydrate) in water and dilution to 1 liter. That solution was standardized by means of anhydrous

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[1] Dojindo, Kumamoto-shi, Japan; ICN–K&K Laboratories, Irvine Calif.; Tridom (Fluka A. G.), Hauppauge, N.Y.

sodium sulfate via titration with CPA (see below). A solution of 0.3 N hydrogen peroxide was prepared by dilution of 17 ml of 30% aqueous hydrogen peroxide to 1 liter with water; 0.1% CPA and 0.1% Thorin, both in aqueous solutions, were purified by passing through a column of resin, dimensions 20×250 mm, filled with cation exchanger CGC-240, $100-200$ mesh, in H-form, to remove the interfering ions; 1.0 M sodium hydroxide. 1.0 M perchloric acid, acetone, p-dioxane, and isopropanol. The CPA and Thorin were the products of ICN$-$K&K Laboratories (Irvine, Calif.). All other chemicals were Baker's analyzed reagents.

Instruments. Standard apparatus *(8)* was used for sulfur dioxide absorption. The pH-checking was done with a Mini-pH-Meter (Gallard$-$Schlesinger Chemical Co., Carle Place, N.Y.).

Procedure. Employing the standard apparatus, dust and sulfuric acid contamination was removed with a 0.8-μm cellulose filter and by means of a midget bubbler filled with 15 ml of 80% aqueous isopropanol. The absorption of sulfur dioxide was accomplished in two midget impingers filled with 15 ml of 0.3 N hydrogen peroxide each. The bubbler and impingers were cooled to 0°C with water and ice. The air flow rate was kept in the range of $1-2$ liters/min. In each case, a sample of 50 liters was taken. Then, the content of the impingers was quantitatively transferred to a 50-ml volumetric flask, the impingers were rinsed with water, the solution was diluted to mark and mixed well. A 10-ml aliquot was pipetted for each titration, 20 ml of acetone or p-dioxane and 5 drops of 0.1% CPA were added, and the pH of solution was adusted to $3-4$ by means of 1 M perchloric acid and 1 M sodium hydroxide. The color of the solution must be vine red or violet. A blue color indicates an inadequate pH adjustment or contamination by some metal ions (Cu^{2+}, Fe^{3+}, Zn^{2+}, Al^{3+}, etc.). The solution was stirred magnetically and titrated with 0.005 M barium perchlorate until its color was clean blue. The color change between vine red and blue was at least one drop sharp. The deterioration of the color change sharpness occurs if the consumption of barium perchlorate solution is higher than 12 ml since the solution becomes too water-diluted and the barium sulfate precipitation is too slow. One milliliter of 0.005 M barium perchlorate corresponds to 0.3203 mg of sulfur dioxide.

The final result was obtained by using the equations

$$\text{ppm (v/v) } SO_2 = 31.162V(237.15 + t)/p, \tag{1}$$

$$\text{mg/m}^3 \text{ } SO_2 \text{ at } 25°C = 81.645V(237.15 + t)/p, \tag{2}$$

where t and p are the ambient temperature (°C) and pressure (in millimeters of mercury); V are milliliters of exactly 0.005 M barium perchlorate. If

the sample and aliquot volumes are different, Eqs. (1) and (2) should be multiplied by factors of $50/v_g$ and $10/v_a$, respectively where v_g and v_a are the actual volumes of sample (in liters) and aliquot (in milliliters).

RESULTS AND DISCUSSION

A comparison was made between the method described above and the classical gravimetric determination of sulfur dioxide via the precipitation of barium sulfate. The results obtained are collected in Table 1. A very good agreement between the two methods can be seen.

Another comparison was made between the above method and the Thorin method (7). A sample corresponding to the aliquot of 1.60 mg of sulfur dioxide was titrated 10 times by each method and the standard deviation of that titration was ±0.02 ml for the above method and ±0.08 ml for the Thorin method. It is very difficult, perhaps impossible, to titrate by means of Thorin the samples with the consumption below 1.00 ml of 0.005 M barium perchlorate. For that reason some authors (7,8) are assuming a blank for this method. However, if a reagent blank is run with the CPA method the color change takes place at 0.02−0.04 ml of 0.005 M barium perchlorate, which is an obvious indication that the consumption is just due to the indicator color change.

We investigated the differences between the Thorin and CPA methods more closely. The spectra of both indicators and their barium complexes are shown in Fig. 1. Employing the continuous variation method and the equimolar dilution method (3), we determined the composition and the effective (conditional) stability constants and collected our results in Table 2. It can be seen from that table that to get the end-point final color (complexed indicator; therefore the subscripts C) it is necessary to add 0.03 and 1.12 ml of 0.005 M barium perchlorate for CPA and Thorin, respectively. Those values are in good agreement with experiment and explain why Thorin is an inadequate indicator for barium titration.

In order to get [Ba] = [SO$_4$] at the end point, a good sulfate titration

TABLE 1
DETERMINATION OF SULFUR DIOXIDE IN AIR

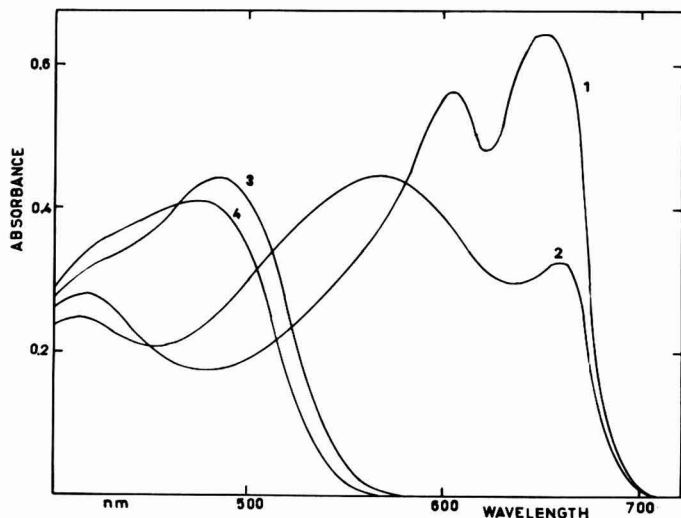| ppm of SO$_2$ found	
Gravimetry	CPA titration
3.7	3.8
6.2	6.3
11.2	11.1
14.3	14.3
18.8	18.6
25.5	25.4

FIG. 1. Spectrum of Ba−CPA(1) and CPA(2) (c_{Ba} = 2.00 × 10^{-4} M, c_L = 1.24 × 10^{-5} M), Ba−Thorin (3) and Thorin (4) (c_{Ba} = 1.00 × 10^{-3} M, c_L = 2.50 × 10^{-5} M); pH 4.01±0.02 throughout.

TABLE 2

SOME PARAMETERS OF CPA AND THORIN COMPLEXATION WITH BARIUM[a]

Indicator	Ionic strength	Temperature (°C)	pH	log K_{11}	pBa$_F$	pBa$_C$	ml$_C$
CPA	0.001 N	25	4.01	6.24±0.03	7.24	5.24	0.03
Thorin	0.001 N	25	4.04	4.73±0.05	5.73	3.73	1.12

[a] K_{11} = [BaL] (c_{Ba} − [BaL])$^{-1}$ (c_L − [BaL])$^{-1}$, pBa$_F$ = log K_{11} + 1, pBa$_C$ = log K_{11} − 1; ml$_C$ are milliliters of 0.005 M Ba(ClO$_4$)$_2$ corresponding to the particular value of pBa$_C$ for the total titrate volume of 30 ml; K_{11} is the effective constant of the complex with molar ratio 1/1 between barium and indicator; [BaL] is the actual concentration of that complex and c_{Ba} and c_L are the total concentrations of barium and indicator, respectively.

requires 2 pBa$_c$ ≈ pK_{SP}, where K_{SP} is the solubility product of barium sulfate. We found pK_{SP} = 10.6 for 65% acetone and K_{SP} = 10.1 for 65% isopropanol, both at 25°C and an ionic strength of 0.001 N. Even from that viewpoint the CPA indication is better.

The essential role of an organic solvent during the titration is to accelerate the precipitation. Obviously a more aprotic solvent like acetone or p-dioxane has a better effect than isopropanol or ethanol. Thorin-indicated titration can also be performed in aqueous acetone or p-dioxane medium; a minor improvement is achieved in that way. On the other hand, if the CPA-indicated titration should be performed with higher consumption of 0.005 M barium perchlorate than 12 ml it is advantageous to

work with 65% aqueous acetone or *p*-dioxane solutions of barium perchlorate.

As was already reported *(1)*, the CPA indication is also better than Sulfonazo III or Dimethylsulfonazo III. The only drawback of CPA in comparison with those indicators is its higher sensitivity toward interfering ions.

SUMMARY

The determination of sulfur dioxide in air is based on a preliminary purification with a cellulose filter and 80% isopropanol and absorption of sulfur dioxide by means of two midget impingers in 0.3 N hydrogen peroxide. The formed sulfate is titrated in an aliquot with 0.005 M barium perchlorate employing Chlorophosphonazo III (CPA) as an indicator. The method is suitable for 0.3 – 19.0 mg of sulfur dioxide per 50 liters of air. The standard deviation of the titration is ±0.02 ml per consumption of 5.00 ml and is four times smaller than that of the Thorin method.

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Improvement of Oxygen Determination Method in Organic Compounds through Addition of Chlorohydrocarbon Vapor to Carrier Gas

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INTRODUCTION

In spite of much research work which has been done to elucidate the reactions in the pyrolytic oxygen determination method of Schütze-Unterzaucher, the determination of oxygen has been one of the most difficult methods used in microanalytical laboratories.

Since about one year ago we have been working with a Carlo Erba automatic CHN+O elemental analyzer. Some minor troubles encountered in the CHN determination method could be overcome with small modifications which were reported to the manufacturer. It was difficult, however, to obtain correct oxygen results with the methods reported in the literature. It was found finally that the addition of chlorohydrocarbon vapor to the carrier gas enhances the decomposition of the organic oxygen compounds so that the pyrolytic reaction can be carried out at 1020°C, and correct results are obtained also with halogen-containing compounds.

EXPERIMENTAL

Reagents used: 1-chloropentane, 1-chlorododecane, nickelized carbon, 20% Ni, nickel wire 0.07 mm.

Apparatus used: Carlo Erba CHN+O elemental analyzer, model 1104 (*1,2*). Pyrolysis tube and vaporization vessel are as shown in Fig. 1. The stainless steel separation column was replaced with a 230 × 4-mm glass column with the same molecular sieve filling placed outside the column oven, but the original column can surely be used. The restriction steel capillary was placed after the halogen absorption tube from its place before the latter to avoid clogging.

Adjustment of apparatus. Assemble the apparatus according to the manual, but adjust the temperature of the pyrolysis furnace to 1020°C, and fill the pyrolysis tube according to the figure. Adjust the gas flow rate to 10 ml/20 sec. Use fine grain ascarite and phosphorus pentoxide drying agent with indicator (Merck, Darmstadt) in the halogen absorption tube.
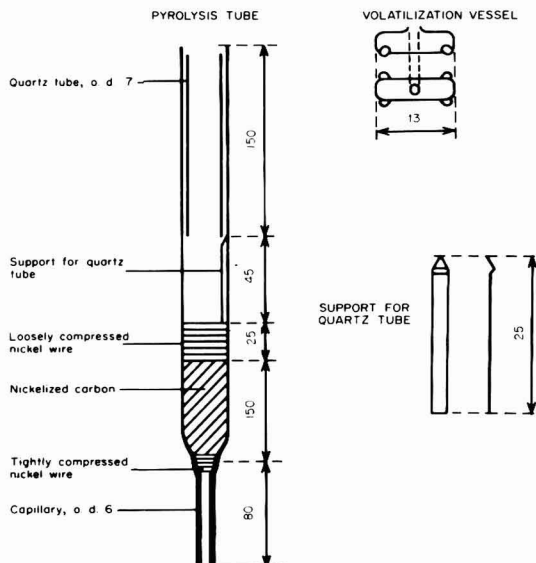
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FIG. 1. Pyrolysis tube and volatilization vessel. The support, $0.3 \times 3 \times 25$ mm, is cut from nickel plate and placed standing along the wall in the pyrolysis tube. Volatilization vessel: volume about 0.3 ml, diameter of orifice about 1.2 mm. The rate of volatilization can be regulated within wide limits by varying the size of the orifice.

Use silver capsules for the samples. Heat the capsules under hydrogen to 750°C and let them cool under hydrogen before use.

Procedure. Pipet about 5 μl of 1-chlorododecane or an equal amount of some other oxygen-free halogenated hydrocarbon into the first capsule of the drum and weigh about 500 μg of the other samples and test compounds into the other capsules. Use dinitrobenzoic acid or a similar compound high in oxygen and nitrogen for the determination of the oxygen factor. When traces of oxygen are to be determined run a blank between the samples and subtract the blank counts from the sample counts. The composition of the blank substance should be similar to that of the sample except, of course, for the oxygen content. The influence of the blank value upon the oxygen factor can usually be neglected.

Fill the vaporization vessel with 0.2 ml of 1-chloropentane and place it upon the drum into the sampler. When the gas flow through the system has become steady, drop the first capsule into the pyrolysis tube with the Autostart switch. Turn off the switch again when the sample has fallen. Allow the base line to stabilize and start analyzing according to the manual.

When the space for sample decomposition in the pyrolysis tube is filled with residues, take out the tube, scratch out the residues with a steel hook, and replace it into the apparatus.

RESULTS

The results obtained with a drum of samples are reported in Table 1. The running conditions reported are slightly different from those given above. Those discussed above are being used in our routine work with good results.

TABLE 1

DETERMINATION OF OXYGEN, ONE DRUM OF SAMPLES[a]

No.	Substance	Weight of sample (μg)	Counts	Factor	Oxygen (4) Calcd.	Found
1	3,5-Dinitrobenzoic acid	577.3	128,359	0.2035	45.3	45.9
2	3,5-Dinitrobenzoic acid	544.2	119,426	0.2062	45.3	45.3
3	3,5-Dinitrobenzoic acid	545.1	119,758	0.2060	45.3	45.4
4	Acetanilide	527.5	29,891		11.8	11.7
5	Sucrose[c]	573.7	140,756		51.4	50.7
6	Bromothiophenic acid	529.7	40,458		15.5	15.8
7	Liquid paraffin[b]	561.0	-		0.0	-
8	Silver nitrate	457.1	61,886		28.3	28.0
9	N-Butyloctadecanamide	514.3	11,518		4.6	4.6
10	3,5-Dinitrobenzoic acid	439.2	95,931	0.2072	45.3	45.1
11	p-Chlorobenzoic acid	503.1	49,675		20.4	20.4
12	Anthracene	548.7	124		0.0	0.04
13	Sucrose[c]	438.3	110,840		51.4	52.2
14	Benzoic acid	562.6	71,515		26.2	26.2
15	p-Bromobenzoic acid	550.6	42,384		15.9	15.9
16	Silver nitrate	573.6	78,370		28.3	28.2
17	o-Iodobenzoic acid	601.1	37,212		12.9	12.8
18	3,5-Dinitrobenzoic acid	576.8	126,187	0.2068	45.3	45.2
19	N-Butyloctadecanamide	551.8	12,433		4.6	4.7
20	Liquid paraffin[b]	467.6	55		0.0	0.02
21	Benzoic acid	473.1	59,582		26.2	26.0
22	Bromothiophenic acid	454.0	33,406		15.5	15.2
23	3,5-Dinitrobenzoic acid	488.3	106,929	0.2066	45.3	45.2

[a] Running conditions: Gas flow rate 10 ml/12.5 sec, 5.8 min × 1, 6.2 min × 4.

[b] Merck Uvasol, Art. 7161. Analysis 7: Sample container jammed, stayed in drum.

[c] The different results of analyses 5 and 13 were caused by varying moisture contents of the samples.

DISCUSSION

When different test substances such as sucrose, acetanilide, and n-butyloctadecanamide were analyzed with methods described in the literature, the yields of carbon monoxide from the samples were much higher with the samples with low oxygen contents than with samples with high oxygen contents. It was supposed that the oxygen was not com-

pletely converted to carbon monoxide, but that carbon dioxide was eluted from the pyrolysis tube. Therefore, larger carbon fillings and highly activated carbon specimens were tried, but without success. Walisch and Marks (3) use a rather high temperature in the zone of the pyrolysis tube where the sample is decomposed, and they add 1% of hydrogen to the carrier gas. We tried that, but had to raise the temperature to 1120°C, as proposed by Unterzaucher (4), in order to obtain equal yields of carbon monoxide from the oxygen of sucrose and that of n-butyloctadecanamide. Only a few analyses could be carried out at this temperature because the quartz tube was quickly deformed under its high inner pressure, and it cracked. Therefore lower temperatures were tried again. Low yields were obtained also with very small samples of sucrose, and high yields with large samples of n-butyloctadecanamide. Hence, the absolute quantity of oxygen was not the critical factor. Blank runs with paraffins were particularly high. It was supposed, therefore, that the substances were not quantitatively decomposed, but that some oxygen remained in the graphitic carbon which was formed, and that this oxygen somehow was extracted by the cracking products of substances low in oxygen, particularly with substances containing saturated aliphatic carbon chains. If this is the case, one could expect that the addition of some hydrocarbon vapor to the carrier gas would solve the problem. The decomposition products of the vapor would extract the oxygen from the precipitating carbon and elute it together with the other oxygen of the sample. A vaporization vessel was made, filled with heptane and put into the drum, so that the heptane was slowly volatilized in the course of the analyses. Also, a drop of heptane was pipetted into the pyrolysis tube before the lid of the sampler was closed.

The method gave immediately good results with substances containing only carbon, hydrogen, nitrogen, and oxygen. Halogen-containing compounds still gave quite high oxygen results. Experimenting then went on according to the same principle: 5% by volume of carbon tetrachloride was added to the heptane, and good results also were obtained immediately with halogen-containing compounds.

The addition of the organic vapor made it necessary to introduce a loosely compressed layer of nickel wire into the zone of the pyrolysis tube where the vapors are decomposed and graphitic carbon is precipitated. This prevents clogging of the tube. If necessary, this layer can be removed and renewed when the sample residues are taken out from the tube.

The molecular sieve column was placed outside the oven because it is more efficient at lower temperatures. Also, the short glass column is much cheaper and easier to change than the steel column. There is much more space in the oven now, and we intend to replace the steel CHN-column there also with a glass column.

We have provided our Carlo Erba analyzer with a timer, which turns off the recorder and the Autostart at the end of the run of a drum. This makes it possible to run two drums with 23 analyses each during the working day, and one drum overnight.

A few experiments that were made in order to shorten the time of analysis by using shorter carbon fillings and shorter separation columns were essentially unsuccessful. In the pyrolysis of most nitrogen-containing compounds, nitrogen-containing graphitic carbon is formed. This formation is, however, not instantaneous, but during a period of 1 or 2 min small amounts of nitrogen are still liberated. The chromatographic system must, therefore, provide for this nitrogen to leave the column before the carbon monoxide is eluted. This necessarily requires a rather long retention time for the latter.

SUMMARY

The addition of chlorohydrocarbon vapor to the carrier gas enhances the quantitative conversion of oxygen to carbon monoxide in the pyrolytic oxygen determination method.

Note added in proof: At the time of printing the following further experiences had been made with the method:

1. A volatilization rate of 1-chloropentane of 18 μl/h was found very appropriate and gave good results and no interference. A filling of 0.15 ml per drum is sufficient.

2. It is not necessary to use chlorododecane or similar compounds in the first place of the drum to condition the pyrolysis tube. The tube is sufficiently conditioned by the chloropentane vapor.

3. With the described method the separation column deteriorates slowly, probably because volatile nickel compounds pass through the halogen absorption tube and are adsorbed in the column. This is avoided by filling the halogen absorption tube in the following manner: 4 cm of silica gel, 70-230 mesh, containing 5% of moisture; 2 cm of lithium hydroxide; 6 cm of dehydrite. When this filling is used the separation column retains a very high efficiency. With a gas flow rate of 10 ml/12 sec excellent separation of the peaks is obtained.

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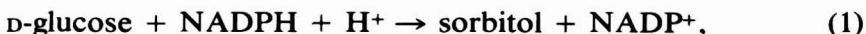
Rapid and Simple Determination of Aldose Reductase and Sorbitol Dehydrogenase

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An ever increasing number of experimental data indicate that the intracellular reaction[1]

$$\text{d-glucose} + NADPH + H^+ \rightarrow \text{sorbitol} + NADP^+, \tag{1}$$

which is catalyzed by aldose reductase (EC 1.1.1.21) and sorbitol dehydrogenase (EC 1.1.1.14), but primarily the activity changes of the former, plays an important role in the development of the secondary symptoms (neuropathy, nephropathy, retinopathy, cataract, etc.) of human diabetes *(2,6)*.

In our view SD, which catalyzes the reaction

$$\text{sorbitol} + NAD^+ \rightarrow \text{d-fructose} + NADH, \tag{2}$$

inhibits the intracellular accumulation of sorbitol, which is harmful to cells.

Sorbitol accumulation might be eliminated by either the inhibition of AR or the activation of SD, among others. Prevention of intracellular sorbitol accumulation by AR inhibition is the more developed *(1)*.

Diabetes was induced experimentally in rats with streptozotocin, and investigations were carried out with regard to how the AR inhibitor AY-22,284 (Alrestatin; Ayerst, New York) influences the reaction catalyzed by the two enzymes measured *in vitro* with and without *in vivo* pretreatment. A study was also made of how the SD activity varies in human diabetics treated with per oral antidiabetics, in comparison with the enzymatic activities measured in normal blood *(5)*.

In the present paper we describe the simple and rapid determination of the above-mentioned two enzymes.

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[1] Abbreviations used: AR = aldose reductase; SD = sorbitol dehydrogenase; NADP = oxidized nicotine adenine di- or trinucleotide; NADPH = reduced nicotine adenine di- or trinucleotide; EC = enzyme catalogue; TRIS = tris (hydroxymethyl) aminoethane hydrochloride.

MATERIALS AND METHODS

Aldose reductase activity determination:

Solutions necessary:

1.0×10^{-2} M glucose solution;

0.33×10^{-3} M glucose-6-phosphate solution;

3.1×10^{-5} M NADPH solution;

2.5 mM MgSO$_4$ solution;

20 mM TRIS buffer of pH 8.0.

In the case of 0.5 ml homogenizate, the concentrations of the compo-nents in the total volume of 3 ml were 3×10^{-5} M glucose, 0.1×10^{-5} M glucose-6-phosphate, 9.3×10^{-8} M NADPH, and 2.5 mM MgSO$_4$; 20 mM TRIS buffer of pH 8.0 was used to make up the volume.

Every solution was prepared from water doubly distilled from a glass vessel.

The tissues examined with respect to enzymatic activity were homogenized in 0.005 M phosphate buffer of pH 7.0. In general the total volume of the measurement mixture was 3 ml, 0.5 ml tissue homogenizate in phosphate buffer being added to the mixture. Measurements were made at 25°C.

Before the enzymatic activity measurements the free glucose contents of the homogenizates were determined. The enzymatic activities were determined by back-measurement of the residual glucose content of the incubation mixture after a given time. The quantitative measurement of the glucose was performed using the o-toluidine method (3). The mea-surement mixture was preincubated for 20 min in the presence of NADPH, but without glucose and glucose-6-phosphate substrate (elimi-nation of nonspecific NADPH consumption). This was followed by the addition of the substrate to the mixture and the actual measurement, which lasted 10 min. After this, the enzymatic action in the measurement mixture was stopped by the addition of some drops of the concentrated trichloroacetic acid solution to an aliquot. The glucose changes due to the AR were calculated from the changes of the initial glucose values after 10 min, and from the original glucose content of the homogenizates tissue. The enzymatic activities were measured in 1-cm glass cells at 630 nm.

In the AR measurement, tissue homogenizate was not added to the control, and the glucose and glucose-6-phosphate mixture was not added to the blank.

The AR activity unit was taken as that amount of enzyme calculated on 1 g of wet tissue which was able to transform 10^{-6} mM glucose and glucose-6-phosphate in 10 min.

In general, the AR activities measured this way in the tissue homogeni-zates lay in the range of $2.38 - 26.42$ U/g wet tissue.

The scatter in the measurements was $\pm 0.1 - 10\%$.

The sorbitol dehydrogenase measurements were carried out as follows:
Solutions used:

0.48 M D-fructose solution;

5.2×10^{-5} NADH solution;

20 mM TRIS buffer of pH 8.0.

In the case of 0.1 ml of homogenizate in 2.5 ml of measurement solution, the concentrations of the components were 1.2×10^{-3} M D-fructose and 13.0×10^{-8} M NADH.

The volume was always made up to 2.5 ml with 20 mM TRIS buffer of pH 8.0.

Here, too, the measurement was preceded by a 30-min preincubation to eliminate nonspecfic NADH consumption, and this was followed by measurement for 10 min.

Measurements were made in 1-cm quartz cells at 340 nm at 25°C.

In the measurements, the 0.1-ml sample was not added to the control, and NADH was not added to the blank.

The unit activity here was that of the amount of enzyme (calculated on 1 g of wet tissue) which could transform 1 mM NADH in 10 min.

The SD activities of the tissue homogenizates measured fell in the interval $0.18-40.87$ U/g of wet tissue. The scatter in the measurements was also between $\pm 0.1-10\%$.

Ammonium sulfate fractionation also was carried out for AR and SD, and it was found that the bulk of both enzymes is precipitated in the 40–50% ammonium sulfate saturation.

In the interest of determining the specific activities of the two enzymes, we measured the protein contents of the tissue homogenizates by the method in (4).

In this case the specific AR activities of the tissue homogenizates lay in the range $0.09-1.40$ U/mmol aldose/10 min/μg of protein.

The measured SD specific activities were $1.26-39.9$ U/mmol NADH/10 min/g of protein.

RESULTS AND DISCUSSION

A rapid and well-reproducible quantitative method was developed for the determination of the AR activities of the tissue homogenizates. The measurement is based on the combined back-measurement of the tissue homogenizate glucose and glucose and glucose-6-phosphate added to the homogenizate; the free aldose contents of the tissue homogenizates were first measured by the earlier-mentioned o-toluidine photometric method. In general the amount of free aldose in the tissue homogenizates (including normal and diabetic liver homogenizates, which contain the most free aldose) lies in the range $3.8-259.7$ mg% (calculated on wet tissue weight). The glucose and glucose-6-phosphate substrate given above is added to

aliquots of the homogenizates, and after removal of the protein the aldose content of the mixture is also determined on a sample taken at the starting point of the measurement. An aldose determination is similarly carried out on a protein-freed mixture (with trichloroacetic acid) after a 10-min incubation, and this value is subtracted from the initial aldose values; this yields the quantitative aldose values, which are utilized to give the AR activities of the homogenizates.

In these cases a calibration curve suitable for the reading-off of aldose changes is prepared by the addition of glucose (3).

The calibration curve used for the determination of SD activities is shown in Fig. 1. The calibration curves serves for the back-measurement of the NADH consumption, and is linear for the NADH values of $1.0-4.0 \times 10^{-7}$ mol plotted on the curve.

The extinction changes were measured at 340 nm. The value 0.001 E corresponds to 2.25×10^{-9} mol NADH.
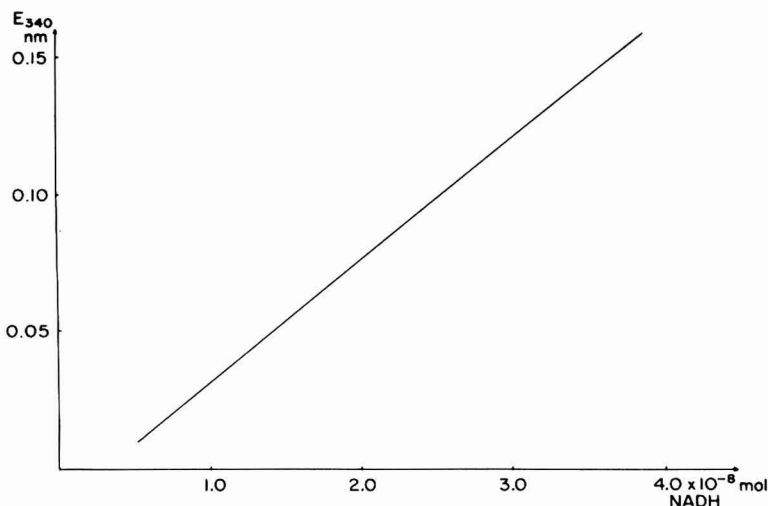
FIG. 1. Calibration curve for the sorbitol dehydrogenase activity determination.

SUMMARY

Rapid and well-reproducible methods were developed for the determination of aldose reductase and sorbitol dehydrogenase. The aldose reductase activity measurement is based on photometric o-toluidine aldose back-measurement, which is widely used in laboratories for the quantitative determination of glucose. The spectrophotometric method based on the quantitative decrease in NADH proved suitable for the measurement of sorbitol dehydrogenase activity.

Both methods could well be employed for the measurement of the aldose reductase and sorbitol dehydrogenase activities of normal and diabetic tissue homogenizates, and for the comparison of the measured values.

ACKNOWLEDGMENT

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Study of the Stability of Pyrimidine Series Cytostatics, Ftorafur and Fluorouracil Injections

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Some pyrimidine derivatives have cytostatic effects. At present, 5-fluorouracil and ftorafur (N_1-(2'-furanidil)-5-fluorouracil) are used in therapy as cytostatics from the group of antimetabolites of the primidine series. Ftorafur, which was introduced in therapy later than 5-fluorouracil, is less toxic in the therapeutical doses used and is more acceptable to the organism.

5-fluorouracil was first synthesized by Duschinsky and co-workers *(2,3)* in the laboratories of the firm Hoffmann–La Roche Inc., in 1956. A number of pyrimidine derivatives with assumed cytostatic effect have been prepared; however, only 5-fluorouracil exhibits the maximum cytostatic effect.

Many authors have studied the effect of pyrimidine derivatives on tumorous tissues *(1,4,6,8,12,13)*. The mechanism of the degradation of pyrimidine substances in an animal organism is not well understood. It has been shown experimentally that the metabolic degradation of 5-fluorouracil is analogous to that of uracil. In the first step 5-fluorouracil is converted into 5-fluorodihydrouracil and then into fluoro-β-alanine, urea, and CO_2 *(7)*.

Works dealing with changes of pyrimidine derivatives *in vitro* contribute to the clarification of the problem of stability of these cytostatics. Therefore the present work is devoted to the study of changes in fluorouracil and ftorafur injection solutions in dependence on the medium termperature, irradiation with UV light, and the medium pH. The thermal and photochemical decomposition of ftorafur and 5-fluorouracil was quantitatively monitored in alkaline and weakly acidic media, with emphasis placed upon the loss of cytostatically effective substances. We are especially interested in the rate of the degradation of 2, 4-dioxy-5-fluoropyrimidine, the substance which reacts in the place of natural metabolite uracil in the synthesis of nucleic acids in the organism.

It was found that ftorafur is considerably thermolabile and readily dissociates tetrahydrofuran on N_1 of the pyrimidine ring with formation of 5-fluorouracil. With 5-fluorouracil, the pyrimidine ring was opened be-

tween N_3 and C_4 and C_6 and N_1 with formation of urea during thermal and photochemical decomposition.

REAGENTS AND APPARATUS

Fluorouracil injections, 5%, from Roche (pH 8.81).

Ftorafur injections, 4%, from Medexport, USSR (pH 9.51).

5-Fluorouracil substance, from Roche, Basle.

Ftorafur substance, from Medexport, USSR.

Uracil substance, from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

Urea, p.a., from Lachema, Czechoslovakia.

A 0.1% aqueous solution of urea.

A 0.4% aqueous solution of 5-fluorouracil, pH 5.11.

A 0.4% aqueous solution of ftorafur, pH 5.4.

A 1% aqueous solution of 5-fluorouracil, pH 4.86.

A 1% aqueous solution of ftorafur, pH 5.15.

The organic solvents used were of p.a. purity from Lachema.

Chromatographic Plates

Silufol UV 254, 150 × 150 mm, from Kavalier, Czechoslovakia.

Lucefol and Lucefol Quick, 200 × 200 mm, from Kavalier.

Chromatographic Systems

S1:—ethyl acetate : acetone : water (30 : 25: 5).

S2:—*n*-butanol : acetic acid : water (50 : 5 : 15).

S3:—water.

S4:—cyclohexane : isopropylalcohol : 25% ammonia (25 : 65 : 10).

S5:—ethyl acetate : water : formic acid (65 : 35 : 5).

S6:—*n*-butanol : 5% ammonia (1 : 1).

S7:—*n*-butanol : 10% formic acid (9 : 1).

S8:—*n*-butanol : formic acid : water (77 : 13 : 10).

Ehrlich detection reagent. 1% --dimethylaminobenzaldehyde in 90 ml methylalcohol and 10 ml conc hydrochloric acid.

Unicam SP-700 and Varian Techtron 635 spectrophotometers.

Air-condition box Feutron 1, type 3001, German Democratic Republic.

Ultraviolet lamp Fluotest Universal, from Hanau.

Radiometer PHM 4d pH-meter.

Agla micropipette with a micrometric screw, from Burroughs Wellcome.

Microburettes MD 30 and MD 100, from Dioptra, Czechoslovakia.

Abbreviations Used

U—uracil (2,4-dioxypyrimidine).

FU—5-fluorouracil (2,4-dioxy-5-fluoropyrimidine).

FT—ftorafur (N_1-(2'-furanidil)-5-fluorouracil).
UR—urea.
p-DMAB—p-dimethylaminobenzaldehyde.

EXPERIMENTAL

I. Monitoring of the Ftorafur and 5-Fluorouracil Stability in Dependence on the Temperature

The FT and FU injection solutions were stored in the air-conditioned box at 60°C (±0.5°C) in sealed ampules of colorless glass. The injection solutions were analyzed chromatographically and spectrophotometrically in certain time intervals over 11 weeks; the decrease in the amount of the cytostatically effective substances, FT and FU, and the formation of some decomposition products were followed. Aqueous solutions 0.4% of FT and FU were stored and followed under the same conditions.

(A) Spectrophotometric Determination of Ftorafur after a Chromatographic Separation

To Silufol UV 254 plates, 0.4% FT solutions, stored at 60°C, were applied in an amount corresponding to 80–160 μg FT. Simultaneously, FT injection solutions stored at a temperature of 0–5°C and a fresh standard solution of FT were applied to the plate. The samples were applied with an Agla micropipette or with a microburette. The chromatogram was developed in an S1 system using the common ascending technique. After drying, the chromatogram was detected under a UV lamp (254 nm). The FT spots were eluted from the chromatogram with 0.1 N hydrochloric acid; the amount of the acid was selected so that the measured concentration of FT was 4 μg/ml. The eluted FT solutions were measured against the blank at an absorbance maximum at 272 nm.

(B) Determination of Urea

(a) Thin-layer chromatography. The amount of the urea formed in the FT and FU solutions was orientatively determined by thin-layer chromatography in systems S1–S6 on Silufol and Lucefol plates. Control FT and FU samples and a urea standard solution (1–5/μg) were simultaneously applied to the plates. The chromatograms were developed using a common ascending technique.

The urea was detected employing the Ehrlich reagent (after spraying the chromatogram was heated to 105°C); the urea formed intensely yellow spots on a white background.

(b) Spectrophotometry in the visible region (5,11). An amount of 0.8 ml of the FU injection solution and of 1.0 ml of the FT injection solution stored at 60°C and 1.0 ml of a 0.1% urea solution were pipetted in 10-ml volumetric flasks. To the individual solutions, 4.0 ml of a 2% solution of p-DMAB was added and the mixtures were diluted with distilled water to

the mark. The solutions of the samples and of the standard urea solution were measured on a spectrophotometer immediately after their preparation against the blank (4.0 ml of 2% p-DMAB solution in 10 ml of water) at the absorbance maximum at 414 nm. The validity of the Lambert–Beer law was verified for a concentration range of 100–400 μg urea/ml.

(C) *Chromatographic Monitoring of the Ftorafur and 5-Fluorouracil Decomposition Products*

The degree of decomposition of the FT and FU solutions stored at 60°C was determined at certain time intervals using thin-layer chromatography on silica gel and cellulose, in the systems specified in the reagents section. Control FT and FU samples were chromatographed simultaneously.

II. Monitoring of the Ftorafur Stability in Dependence on the Medium pH

The following pH values were adjusted in FT injection solutions using hydrochloric acid and sodium hydroxide under electrometric control: 1.52, 3.26, 5.87, 6.79, 7.79, 9.10, 10.01, 11.10, and 12.21. The solutions were stored in closed ground-glass stoppered bottles in the light and at laboratory temperature. The FT decomposition products were determined by thin-layer chromatography in systems specified in the reagents section. The FT solutions with various pH values were diluted to a concentration of 0.4% before the chromatographic procedure.

III. Monitoring of the Ftorafur and 5-Fluorouracil Stability in Dependence on Irradiation with UV Light

The FT and FU injection solutions were stored in an air-conditioned box at 20°C (±0.5°C) in sealed ampules made of colorless glass and were irraditated with UV light. 1% aqueous FT and FU solutions were stored under identical conditions. At certain time intervals these solutions were analyzed chromatographically (the formation of the decomposition products) and spectrophotometrically (the decrease in the amount of the cytostatically effective substances). The control FT and FU samples were stored in the dark at 20°C.

(A) *Spectrophotometric Determination of Ftorafur after Chromatographic Separation*

For the procedure see Section IA.

(B) *Spectrophotometric Determination of 5-Fluorouracil*

The irradiated FU solutions were diluted with 0.1 N hydrochloric acid to an FU concentration of 10 μg/ml. The absorbances of the test FU solutions, the control FU solution, and a fresh FU standard solution were measured against 0.1 N hydrochloric acid at the absorbance maximum at 267 nm.

(C) Chromatographic Monitoring of the Ftorafur and 5-Fluorouracil Decomposition Products

The procedure was analogous to that described in Section IC.

RESULTS AND DISCUSSION

The rate of the loss in the cytostatically effective substances in ftorafur and 5-fluorouracil injections was followed in dependence on the medium temperature, irradiation with UV light, and on the medium pH over a period of 14 days. The formation of the degradation products of these cytostatics was monitored simultaneously. By the effect of an elevated temperature, one, or more frequently several, substances of very different structures are formed from the medicament with the defined constitution and pharmacological effect. Naturally, the deep change in the substance structure is closely connected with changes in the biological and pharmacological effect of the substance, which are usually accompanied by an increase in the toxicity. Only exceptionally does the effect of UV light lead to a decrease in the toxicity, e.g., to a suppression of carcinogenic properties of nitrosoamines.[9] However, the pharmacological effect and toxicity of substances formed by photochemical decomposition of pharmaceuticals have not yet been systematically studied.

The degree and the rate of the thermal and photochemical decomposition of FT and FU was followed by means of thin-layer chromatography and the decrease in the content of the cytostatically effective substances was monitored spectrophotometrically. An increased temperature and UV radiation led to the colorless FT and FU injection solutions turning yellow and later yellow-brown; the FU solutions were colored more intensely than those of FT. The pH values of the test solutions did not change substantially during thermal decomposition and UV irradiation (Table 1).

On the basis of the experimental results it has been found that the first step of the thermal and photochemical decomposition of Ftorafur (I) involves dissociation of tetrahydrofuran from N_1 of the pyrimidine ring, with formation of 5-fluorouracil (II).
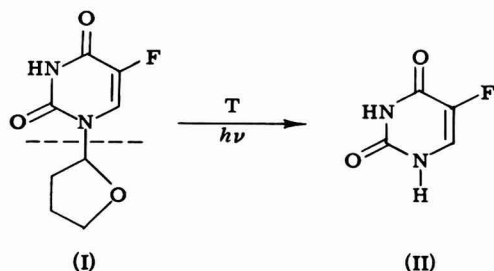

(I)   (II)

TABLE 1
Changes in the pH of FT and FU Injection Solutions and 0.4%
Solutions of FT and FU Stored at 60°C for 9 Weeks

	pH					
	Init.	1 Week	2 Weeks	5 Weeks	7 Weeks	9 Weeks
FT inj.	9.59	9.43	9.40	9.31	9.18	9.10
FU inj.	8.76	8.62	8.54	8.53	8.33	8.45
FT 0.4%	5.40	5.54	5.79	5.27	—	5.20
FU 0.4%	5.11	—	5.10	4.84	—	4.80

TABLE 2
Decrease in the FT Content in the FT Injection Solution and in a
1% FT Solution on Irradiation with UV Light

	FT content (%)					
	Before irrad.	2 Weeks	7 Weeks	9 Weeks	11 Weeks	14 Weeks
FT inj. (pH 9.51)	100	96.77	90.86	88.21	82.60	76.05
FT 1% (pH 5.15)	100	—	46.88	37.00	28.88	20.08

The rate of the decomposition of FT with formation of FU depends on the medium pH; the photochemical decomposition of FT with formation of FU is easier in weakly acidic and neutral solutions (Table 2, Fig. 1, 5). A similar dependence has also been found in the thermal decomposition of FT solutions.

As FU, which is the degradation product of FT, exhibits the maximum absorbance in the same wavelength region as FT (Table 3), the loss in the FT could not be determined spectrophotometrically directly, but after chromatographic separation. The optimum separation was attained in system S1 on Silufol. Aqueous solutions of the samples had to be applied to the plates, as FT and its degradation products could not be extracted into organic solvents immiscible with water because of their strongly polar character.

The decrease in the FT content during thermal decomposition of FT solutions can be seen in Table 4 and Fig. 2; the data on the decrease in the FT and FU contents during photochemical decomposition are given in

Table 2 and Fig. 3, and Table 5 and Fig. 4, respectively. As follows from the results given in Table 2, FT is decomposed by UV radiation in weakly acid media almost four times faster than in alkaline media.

In the second stage of the thermal and photochemical decomposition of FT solutions, the pyrimidine ring was opened between N_3 and C_4 and between C_6 and N_1, with formation of urea.

$$\text{FT} \quad \xrightarrow[h\nu]{T} \quad H_2N-CO-NH_2$$
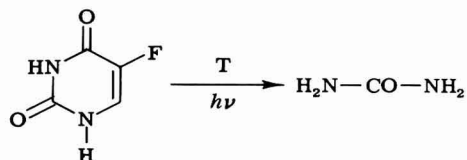
The amount of urea formed in FT and FU solutions under the given experimental conditions was orientatively determined at certain time intervals using thin-layer chromatography in the S1–S6 systems (Table 6).

The amount of urea formed during thermal decomposition of FT was determined employing the reaction of the R-NH-CO-NH$_2$ type com-
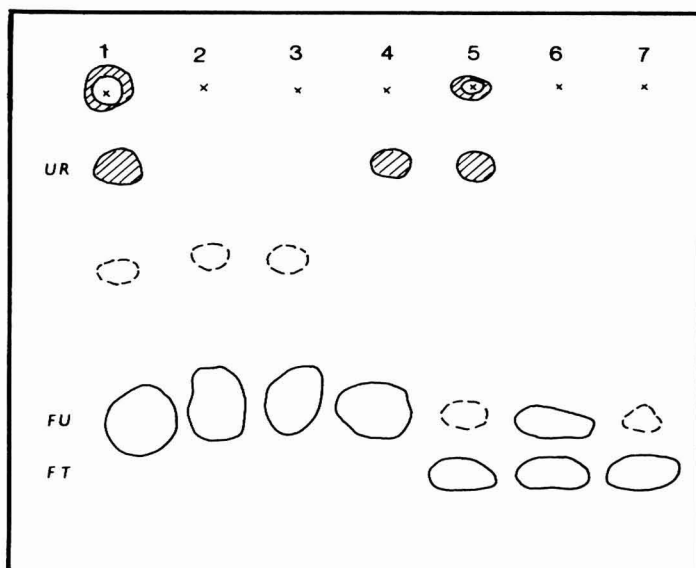
FIG. 1. Chromatographic separation of the components in FT and FU solutions irradiated for 4 weeks with UV light; Silufol UV 254, system S1, detection UV 254 nm (open figures), Ehrlich reagent (shaded figures), spots of low intensity (dashed circles). 1 FU inj. irradiated, pH 8.81 (80 μg); 2 FU inj. not irradiated (80 μg); 3 FU irradiated, solution pH 4.86 (80 μg); 4 FU standard solution (80 μg) and urea (UR) standard solution (5 μg); 5 FT inj. irradiated, pH 9.51 (80 μg); 6 FT irradiated, solution pH 5.15 (80 μg); 7 FT inj. not irradiated (80 μg).
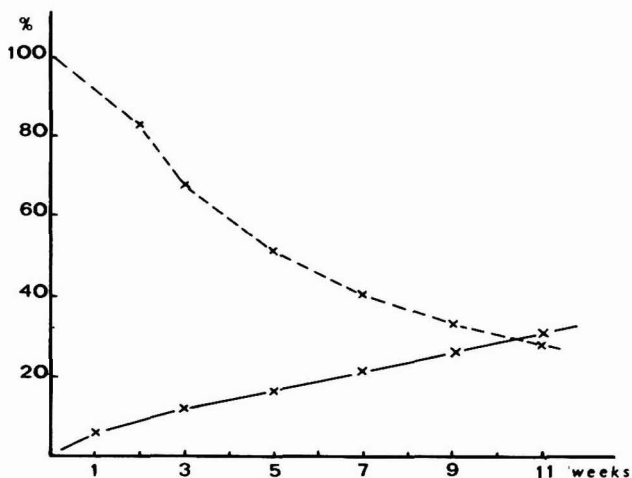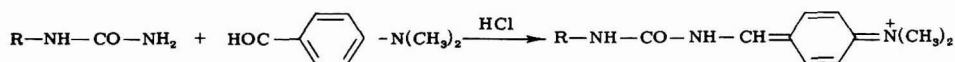
FIG. 2. Decrease in the FT content (——) and the formation of urea (——) in % in the FT injection solution stored at 60°C.

pounds with p-DMAB, in which intensely colored salts of Schiff bases are formed; these salts were then determined spectrophotometrically

$$R-NH-CO-NH_2 \ + \ HOC-\langle \rangle -N(CH_3)_2 \xrightarrow{HCl} R-NH-CO-NH-CH=\langle \rangle =\overset{+}{N}(CH_3)_2$$

The results of the determination of urea formed in the FT injection solution during thermal decomposition are given in Table 4 and Fig. 2.
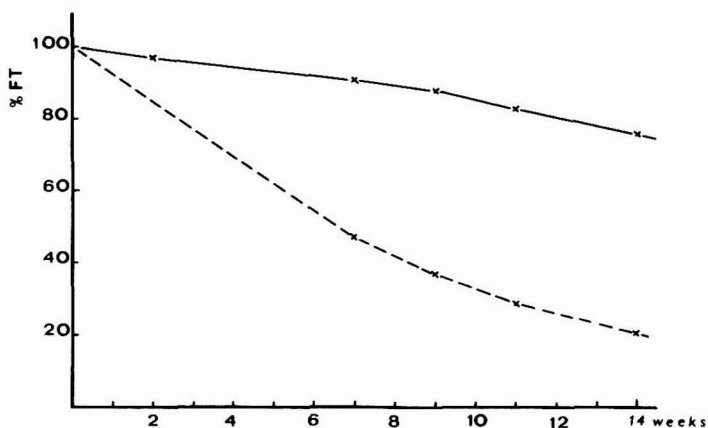
FIG. 3. Decrease in the FT content in the injection solution of pH 9.51 (——) and in an FT solution of pH 5.15 (——) on irradiation with UV light.

TABLE 3

MAXIMA AND MINIMA ON THE ABSORPTION CURVES OF FT AND FU IN WATER,
0.1 N HYDROCHLORIC ACID, AND 0.1 N SODIUM HYDROXIDE
(20 μg FT/ml, 10 μg FT/ml, 10 μg FU/ml, Unicam SP 700).

		Medium		
		H_2O	0.1 N HCl	0.1 N NaOH
FT (nm)	max	271	272	271
	min	235	235	247
FU (nm)	max	266	267	271
	min	232	232	247

The study of the stability of FT solutions in dependence on the medium pH showed that the opening of the pyrimidine ring with formation of urea is easier in alkaline media (Figs. 5,6). The FT stability could not be followed down to very acidic solutions, as FT crystals separated soon after the pH adjustment. In alkaline solutions, pH 11.10 and 12.21, the FT solutions turned yellow perceptibly.

It was assumed that during the thermal and photochemical decomposition of FT and FU the pyrimidine ring may be dehalogenated on C_5 with formation of uracil, analogously to the 5-chloro- and 5-iodo- derivatives of pyrimidine.[10] Thin-layer chromatography in several separating systems was used for the uracil detection (Table 5). However, uracil was not detected in the studied solutions of FT and FU; thus it may be assumed
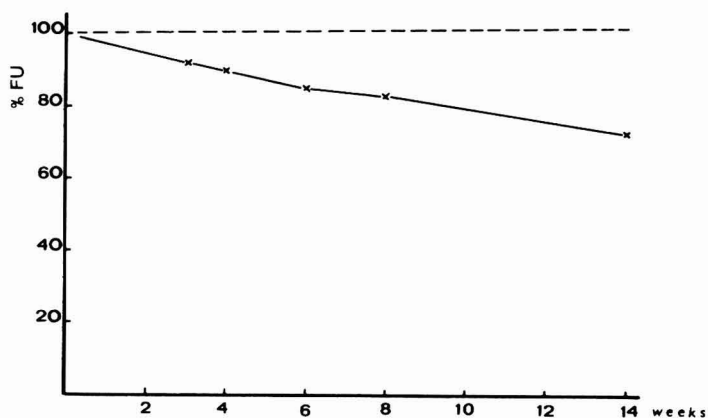

FIG. 4. Decrease in the FU content in the FU injection solution of pH 8.81 (———) and in an FU solution of pH 4.86 (——) on irradiation with UV light.

TABLE 4

SPECTROPHOTOMETRIC DETERMINATION OF THE LOSS IN FT AND THE UREA
FORMED IN THE FT INJECTION SOLUTION STORED AT 60°C FOR 11 WEEKS

	Before decomp.	1 Day	3 Days	1 Week	2 Weeks	3 Weeks	5 Weeks	7 Weeks	9 Weeks	11 Weeks
				FT injection solution						
FT	99.92	98.08	97.11	93.10	83.34	68.30	52.05	40.06	33.80	28.12
Urea	—	—	—	6.04	8.32	12.0	15.8	21.07	26.6	30.05
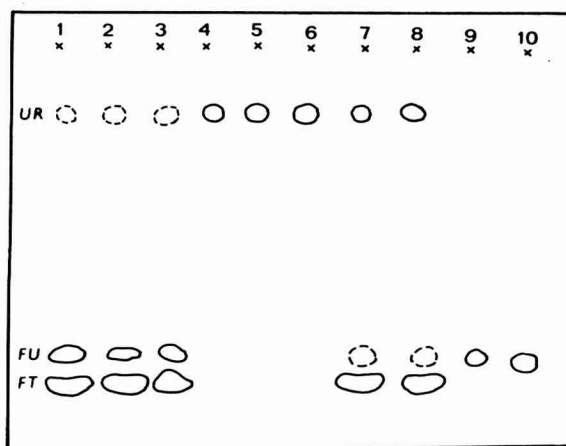
FIG. 5. Chromatographic study of FT injection solutions in dependence on the medium pH after 3-month storage at laboratory temperature (Lucefol Quick, system S1). **1** FT inj. solution, pH 7.79 (160 μg); **2** FT inj. solution, pH 9.10 (160 μg); **3** FT inj. solution, pH 10.0 (160 μg); **4** urea standard solution (1 μg); **5** urea standard solution (1.5 μg); **6** urea standard solution (2 μg); **7** FT inj. solution, pH 11.1 (160 μg); **8** FT inj. solution, pH 12.21 (160 μg); **9** FU standard solution (2 μg); **10** FU standard solution (5 μg); (dashed circles, spots of low intensity).
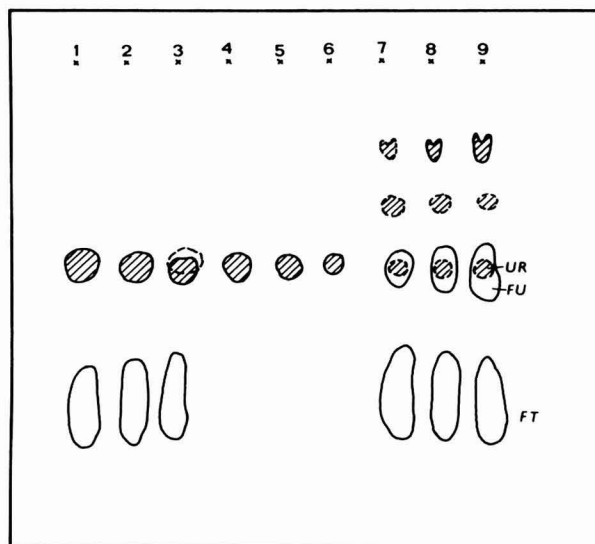
FIG. 6. Chromatographic study of FT injection solutions in dependence on the medium pH after 7-month storage at laboratory temperature (Lucefol Quick, system S8, detection UV 254 nm (open figures), Ehrlich reagent (shaded figures), spots of low intensity (dashed circles)). **1** FT inj. solution, pH 12.21 (160 μg); **2** FT inj. solution, pH 11.10 (160 μg); **3** FT inj. solution, pH 10.0 (160 μg); **4** urea standard solution (2 μg); **5** urea standard solution (1.5 μg); **6** urea standard solution (1 μg); **7** FT inj. solution, pH 9.10 (160 μg); **8** FT inj. solution, pH 7.79 (160 μg); **9** FT inj. solution, pH 6.79 (160 μg).

TABLE 5

DECREASE IN THE FU CONTENT IN THE FU INJECTION SOLUTION AND IN A
1% FU SOLUTION ON IRRADIATION WITH UV LIGHT

	FU content, %							
	Before irrad.	3 Weeks	4 Weeks	5 Weeks	6 Weeks	8 Weeks	12 Weeks	14 Weeks
FU inj. (pH 8.81)	100	91.78	89.1	87.2	86.25	82.8	—	73.55
FU 1% (pH 4.86)	100	—	100	—	—	102.5	103	103.5

that the 5-fluoro pyrimidine derivatives are not dehalogenated under the given experimental conditions, in contrast to the 5-chloro- and 5-iodo-derivatives.

Differences between the thermal and the photochemical decomposition of the studied cytostatics were encountered only in higher decomposition stages. During the photochemical decomposition of FU and FT, a substance with R_F 0.22 was formed (Fig. 7), which produced instantaneously an intensely yellow coloration with the Ehrlich reagent; not even traces of this substance were formed during the thermal decomposition. In contrast during the thermal decomposition of the cytostatics; a substance with R_F to the photochemical decomposition, two substances were formed 0.76 produced a blue coloration with the Ehrlich reagent, while the other substance (R_F 0.86) turned red (Fig. 7). These degradation products were not more closely identified; we assume that these probably are condensation or oxidation products of the primary degradation products, which need not be considered from the point of view of the substance stability during the time of its therapeutical applicability.

CONCLUSION

The stability of the pyrimidine series cytostatics—ftorafur and fluorouracil injections—was followed in dependence on the irradiation with UV light and on the medium pH and temperature. The degree and the rate of the thermal and photochemical decomposition of FT and FU and their stability as a function of the medium pH were studied using thin-layer chromatography (Table 6) and the decrease in the content of the cytostatically effective substances was followed spectrophotometrically (Tables 2, 4, and 5).

In the first stage of the thermal and photochemical decomposition of FT, tetrahydrofuran is dissociated from N_1 of the pyrimidine ring with formation of 5-fluorouracil which is also cytostatically effective. It was
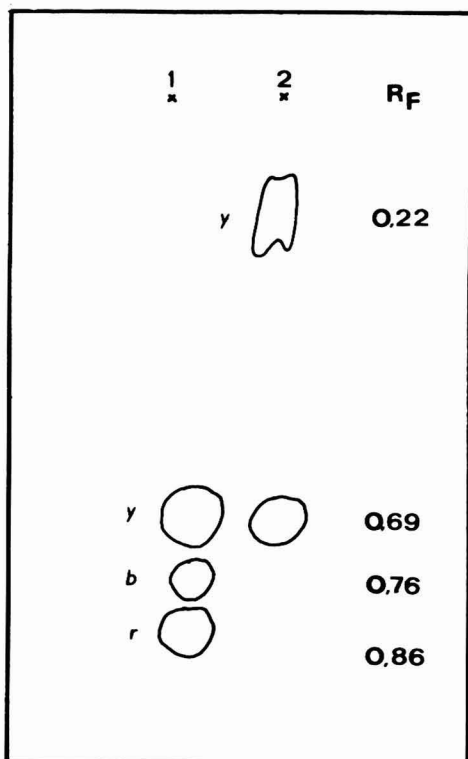
FIG. 7. Chromatographic study of FU injection solutions irradiated with UV light and of FU injection solutions stored at 60°C (Lucefol Quick, system S8, detection with the Ehrlich reagent). 1 FU inj. solution (100 μg) after 14-week thermal decomposition; 2 FU inj. solution (100 μg) after 14-week irradiation with UV light; y, yellow; r, red; b, blue.

found that the decomposition was strongly pH-dependent; the tetrahydrofuran dissociated more easily in weakly acidic and neutral solutions (Table 2, Figs. 1,5).

In the second stage of the thermal and photochemical decomposition of FT, the pyrimidine ring opened between N_3 and C_4 and between C_6 and N_1, with formation of urea. The pyrimidine ring opened more easily in alkaline media (Figs. 5,6). The results of the determination of the urea formed during the thermal decomposition of FT injection solutions are summarized in Table 4.

Differences between the thermal and the photochemical decomposition of the cytostatics were observed only in further phases of the decomposition (Fig. 7).

It has been found that FT is considerably thermolabile; after 11 weeks of storage at 60°C the decrease in the FT content in the injection solution amounted to almost 72% (Table 4). The degree of the photochemical

TABLE 6
CHROMATOGRAPHIC SEPARATION OF URACIL (U), 5-FLUOROURACIL (FU),
FTORAFUR (FT), AND UREA (UR) IN SYSTEMS S1–S6^a

	S1	S2	S3	S4	S5$_{Luc}$	S6$_{Luc\ Q}$
U	0.45	0.73	0.35	0.42	0.40	0.41
FU	0.57	0.75	0.28	0.62	0.55	0.26
FT	0.79	0.39	0.44	0.75	0.70	0.20
UR	0.20	0.76	0.29	0.63	0.55	0.33

^{a} Luc–Lucefol, Luc Q–Lucefol Quick; in other cases Silufol UV 254 was employed; detection: UV 254 nm, Ehrlich reagent.

decomposition of FT depended considerably on the medium pH; at pH 9.51 the decrease in the FT content in the injection solutions was 24% after 14-week irradiation, while at pH 5.15 it was 79% (Table 2).

Further it has been found that, in contrast to the 5-chloro- and 5-iodo-derivatives of pyrimidine, the studied cytostatics were not dehalogenated in position 5 on the pyrimidine ring during the thermal and photochemical decomposition. The decomposition products formed during prolonged thermal and photochemical decomposition of the cytostatics were studied only chromatographically and were not more closely identified; we assume that they are condensation or oxidation products of the primary degradation products, which need not be considered from the point of view of the stability of the pharmaceuticals during the period of their medical applicability.

SUMMARY

The stability of the cytostatics, ftorafur and fluorouracil injections, was studied in dependence on irradiation with UV light and on the medium temperature and pH. In the first stage of the thermal and photochemical decomposition of ftorafur, tetrahydrofuran dissociated from position N_1 in the pyrimidine ring and 5-fluorouracil was formed. In the second stage of the thermal and photochemical decomposition of ftorafur, the pyrimidine ring opened between N_3 and C_4 and between C_6 and N_1, with formation of urea. Ftorafur, fluorouracil, and urea were determined using thin-layer chromatography and spectrophotometry in the UV and visible region.

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Serotonin in Blood Platelets: Studies on a Simple Assay[1]

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INTRODUCTION

Serotonin (5-HT), a metabolite of the amino acid tryptophan (TRY), is a biogenic amine found widely distributed in nature. In man it is located primarily in enterochromaffin cells of the intestine, serotonergic neurons of the brain, and platelets of the blood (2,3,6,16,20,26). The main sites of 5-HT biosynthesis are the brain and the intestine. 5-HT in platelets is actively transported from sites of intestinal synthesis and stored in subcellular organelles called dense bodies (1,4,21). This amine is a known vasoconstrictor (17,22,26) and a suspected neurotransmitter. As a putative neurotransmitter, 5-HT has been implicated in many emotional and behavioral disorders (10,11,14,18,24). Peripheral platelet 5-HT levels may be an index of central, neuronal 5-HT levels, and may therefore be of diagnostic and prognostic value in psychiatric medicine.

Although most assays for 5-HT depend on either natural fluorescence of the molecule or on fluorescence of a suitable derivative, the methods involve elaborate and tedious extraction procedures (7,9,15,23,28,30,32), and generally have both low sensitivity and poor recovery. Several simplified procedures for measuring platelet 5-HT have been reported (5,8,25,31). The present assay is based on the natural fluorescence of 5-HT at 335 nm when excited at 301 nm in near-neutral solutions. The assay is applied to the measurement of 5-HT levels in blood platelets; platelets are readily available as a biological specimen from humans. The precision of the assay, the effects of lowered pH values on the sensitivity of the assay, recovery studies using platelet samples, and interferences in the assay by 5-HT-related compounds are evaluated.

MATERIALS AND METHODS

Reagents and Apparatus

An aqueous stock solution of 5-HT, 100 μg/ml, was prepared from serotonin creatinine sulfate (available from Sigma Chemical Company, St.

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Louis, Mo.). This solution is stable for several months when stored at 5°C. Aqueous working standards, ranging from 0.001 to 1.0 $\mu g/ml$, were made up daily. In addition, protein-containing control samples were prepared by diluting stock 5-HT with aqueous 1% EDTA plasma (non-lipemic, nonjaundiced). The concentration of 5-HT in these controls was similar to that in platelet samples. Approximately 400 aliquots were taken and stored at $-20°C$ where the 5-HT is stable for several months. After an acceptable range of values for 5-HT concentrations in the control samples was established ($\bar{x}$ = 0.179 $\mu g/ml$, SD = 0.009 $\mu g/ml$, n = 20), the control sample was used as a daily index of the precision of the assay.

Other chemicals were ACS grade.

A Perkin—Elmer Model 203 fluorescence spectrophotometer, equipped with a medium pressure mercury lamp, was used for fluorescent measurements. Determinations of pH were performed using a Radiometer pH Meter, Model 26. EDTA vacutainers (7.0 ml) were used for the collection of blood samples.

Procedures

Blood specimens were collected in EDTA vacutainers and spun at 150g for 45 min to get platelet-rich plasma (PRP). PRP was transferred with siliconized Pasteur pipettes (pretreated with Siliclad by Clay Adams, No. 1950, available from Becton, Dickinson and Company, Parsippany, N.J. 07054) to a plastic tube. (Platelet counts of whole blood and PRP were done using 1% ammonium oxalate and standard Neubauer haemocytometers.) A measured volume of PRP, in duplicate, was transferred to plastic tubes and spun at 800g for 20 min. The plasma was removed. The platelet pellets were washed twice with 0.85% saline and frozen ($-20°C$) for subsequent assay of 5-HT and protein *(12,19)*. Platelet pellets were reconstituted with a measured volume of water; to facilitate lysis of the platelets and release of 5-HT, the sample was mixed vigorously using a Vortex Jr. mixer, frozen for a short time, and thawed. Measured volumes (1.0 ml) of platelet lysates, control samples, standards and blank (water) were taken in triplicate. Aqueous EDTA plasma (2 ml of 1%) was added to each tube. To all tubes, 0.6 ml of 10% $ZnSO_4$ and 0.3 ml of 1 N NaOH was added for deproteinization. The tubes were mixed and spun at 800g for 20 min. Supernatants were drawn off. The flurorescence of all supernatants was measured directly by setting the spectrophotofluorometer at 0 and 100 units of intensity, using the blank and standard of maximum concentration, at λ_{max} 301 and 335 nm. Quinine sulfate (0.04 $\mu g/ml$ in 0.1 N H_2SO_4) was used to standardize the instrument at λ_{max} 365 and 448 nm. The concentrations of 5-HT in the samples were determined from a standard curve (using a least-square analysis) and expressed in $\mu g/mg$ platelet protein, $\mu g/billion$ platelets, and $\mu g/ml$ whole blood.

Within-run and between-run precisions of the assay were determined by calculating the coefficient of variation (C.V.) from a series of control values determined during one run, and over several runs.

The effect of decreased pH on the sensitivity of the assay was determined by comparing the fluorescence of aqueous 5-HT standards (λ_{max} 301 and 335 nm) to the fluorescence of standards made 3 N in HCl (λ_{max} 301 and 335 plus λ_{max} 301 and 535 nm).

In the recovery studies, water and varying concentrations of aqueous 5-HT standards were used to reconstitute platelet pellets prepared from the same PRP. The amount of 5-HT added in each case was compared to the amount found by assay.

Interference of other indoles in the serotonin assay was assessed by adding TRY, 5-hydroxytryptophan (5-HTP), 5-hydroxyindole acetic acid (5-HIAA), and N, N-dimethyltryptamine (DMT) to aqueous standard solutions of 5-HT. The fluorescence of these "contaminated" standards was compared to that of "pure" standards.

RESULTS AND DISCUSSION

Platelet pellets were usually prepared from 1.0 ml of PRP; they were reconstituted with 5.0 ml water. The concentration of 5-HT in these platelet lysates ranged from 0.05 to 0.3 μg/ml. The assay used is capable of measuring levels of 5-HT at least as low as 1.0 ng/ml. Therefore, if necessary, much smaller samples of PRP could be used in preparing platelet pellets for 5-HT analysis using the present assay.

Precision of the Assay

Within-run precision of the assay was determined by measuring 5-HT in 21 control samples. The mean 5-HT concentration was 0.179 μg/ml with a standard deviation of 0.008 μg/ml. The C.V. was 4.5%. Of a similar set of experiments, this is the highest within-run C.V.; the lowest was 2.1%. Similar calculations were done on the concentrations of control samples determined from 24 separate assays. The mean 5-HT concentration was 0.174 μg/ml with a standard deviation of 0.013 μg/ml Therefore the C.V. for between-run analyses is 7.5%. Within-run and between-run variation in the 5-HT assay are therefore small enough to consider the results reproducible on a routine basis.

Effects of pH

Aqueous solutions of 5-HT as the creatinine sulphate complex are slightly acid (pH = 6.0 $\pm$ 0.1). The pH of the supernatant, after protein precipitation in the present assay, is slightly higher (pH = 6.5 $\pm$ 0.1). In both solutions the fluorescence of 5-HT (λ_{max} 301 and 335 nm) is directly proportional to concentration in the range studied (0.001 to 1.0 μg/

ml). The fluorescent intensity of 5-HT solutions does not vary apprec-
iably between pH values of 2 and 10; in very acidic media (pH less
than 1.0) fluorescence at 335 nm decreases, and a new peak of emis-
sion appears at 535 nm *(27)*. Comparisons of fluorescent intensities of
aqueous 5-HT standards and 5-HT solutions made 3 *N* in HCl are
illustrated in Fig. 1. Figure 1A depicts calibration curves for 5-HT in
HCl at concentrations ranging from 0.15 to 0.75 μg/ml using 3 *N* HCl
as a blank, measured at λ_{max} 301/335 and 301/535 nm. Figure 1B is a
calibration curve for aqueous 5-HT ranging from 0.001 to 0.005 μg/ml,
using water as a blank, measured at λ_{max} 301 and 335 nm. (Aqueous
solutions of 5-HT do not fluoresce at 535 nm.) All fluorescent measure-
ments in Fig. 1 were taken at the same sensitivity settings. These calibra-
tion curves illustrate that the fluorescence of 5-HT in HCl (3*N*) is en-
hanced when measured at 535 nm, compared to measurement at 335 nm.
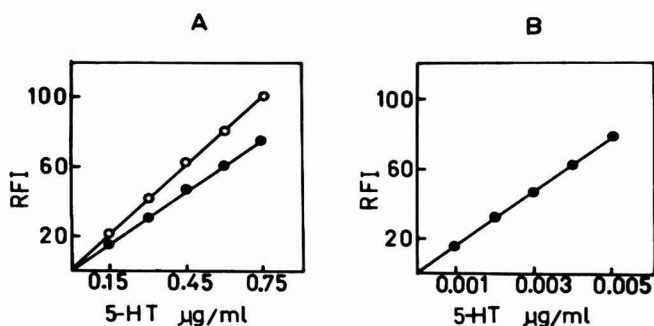
FIG. 1. Effect of pH on serotonin fluorescence. (A) Calibration curves of 5-HT in 3 *N* HCl
(pH < 1.0), concentrations ranging from 0.15 to 0.75 μg/ml; $\bigcirc$, excitation λ = 301 nm,
analyzer λ = 535 nm; $\bullet$, excitation λ = 301 nm, analyzer λ = 335 nm. (B) Calibration curve
of aqueous 5-HT (pH ~ 6), concentrations ranging from 0.001 to 0.005 μg/ml; excitation λ =
301 nm, analyzer λ = 335 nm. RFI: relative fluorescent intensity. Each point on the calibra-
tion curve represents an average of two readings. Sensitivity settings on the spectrophoto-
fluorometer were the same for A and B.

However, in order to obtain a comparable level of fluorescent intensity,
the concentration of 5-HT in the acidic medium must be 120-fold (assayed
at 535 nm) or 150-fold (assayed at 335 nm) the concentration of 5-HT in
water. Thus an assay where fluorescent measurements on 5-HT are taken
in a slightly acid environment is substantially more sensitive than one
where measurements are taken in a highly acidic medium (3 *N* HCl). (The
possibility of contaminants in the HCl, which could quench 5-HT fluores-
cence, was not investigated.) Therefore, where increased senstivity is an
advantage, the assay at near-neutral pH should be used.

Recovery Studies

Aqueous standards, and platelet pellets reconstituted with either water or aqueous standards, were analyzed using the routine 5-HT assay. The fluorescent intensities of the standards of the samples are illustrated in Fig. 2. The endogenous level of 5-HT in the platelet lysate was 0.08 $\mu g/ml$. Recovery of 5-HT added to the platelets averaged 98% (96–100%), indicating almost complete recovery. In extraction procedures as much as 50% of the 5-HT is lost. Therefore, the present assay is potentially more sensitive because the analyte is almost completely recovered.

Fluorescence of Related Indoles in the 5-HT Assay

Neither TRY nor DMT, at concentrations as high as 10 times the 5-HT concentrations, exhibited interference (positive or negative) in the assay. A positive interference for equivalent amounts of 5-HTP averaged 10%, indicating the similar though less intense fluorescent pattern of 5-HTP. Since 5-HT is not synthesized in platelets, the interference of 5-HTP in the 5-HT assay is of no practical significance when the sample being analyzed is platelets. Aqueous 5-HIAA exhibits λ_{max} of 301 and 335 nm; it was found to introduce a positive error of 60% in 5-HT samples of the same molar concentration. Although 5-HIAA is a metabolite of 5-HT and may thus be considered a potential interference in the present assay for 5-HT
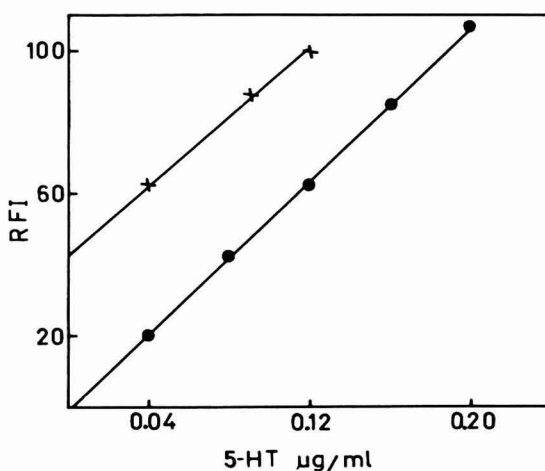
FIG. 2. Recovery of serotonin added to platelet pellets. ●, Aqueous standards were assayed to produce a calibration curve. Each point is an average of readings on three samples. ×, Platelet pellets were prepared from 1.0-ml samples of the same PRP. Pellets were then reconstituted with water and varying concentrations of aqueous 5-HT standards. Fluorescent intensities of the platelet samples were then determined in the 5-HT assay. Each point on the curve is an average of readings on four samples. RFI: Relative fluorescent intensity.

in platelets, its concentration is normally low, averaging about 3% of the 5-HT concentration (29). If abnormal metabolism of 5-HT to 5-HIAA were suspected, the indoles could be separated using ion-exchange chromatography as outlined by Guilbault (13), and then measured using the natural fluorescence of the separate fractions. Such a separation was successful in this laboratory, and 5-HIAA was indeed found to be present in only very small quantities in the platelet lysates.

SUMMARY

A simple, sensitive assay for aqueous solutions of 5-HT, especially platelet lysates, has been outlined. The precision of the assay is acceptable for routine use (C.V. less than 10%). The fluorescent intensity of 5-HT is reduced drastically when the pH of the medium falls below 1.0, whether the emission intensity is measured at 535 or 335 nm; thus the present assay of 5-HT in near-neutral solution is much more sensitive than the common assays which use a highly acidic environment for measurement of the final fluorescence. Interferences from the indoles TRY and DMT are negligible, but interferences from 5-HTP and 5-HIAA are 10 and 60%, respectively; therefore the assay is not specific for 5-HT. However, ion-exchange chromatography can readily be combined with the present assay to allow specific analysis of 5-HT and other indoles which have similar fluroescent patterns.

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Separation and Gravimetric Determination of Barium Using Sulfadimethoxin Salicylaldimine

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INTRODUCTION

Perusal of literature reveals that condensation products of sulfadiazines with salicylaldehyde and substituted salicylaldehyde are not only good bacteriostatic agents *(12, 13)*, but are also good complexing agents *(5–8)*. Sulfadimethoxin salicylaldimine (SUDMSA) has the system $-0-C=C-C=N$, similar to many precipitating agents used for a number of transition metal ions. The reagents having such a system have been investigated by a number of workers *(4)*. The present work deals with chelates formation of SUDMSA with Ba(II).

EXPERIMENTAL

Chemicals

All chemicals employed were of Analar grade.

Reagents

The aqueous stock solution of barium chloride was prepared by dissolving 1 g of $BaCl_2$ in a liter of double-distilled water. The contents of barium were determined volumetrically *(15)* and a stock solution of the 0.001 *M* concentration was prepared by appropriate dilution.

SUDMSA was synthesized by the procedure described earlier *(9)*.

A 1% solution of the masking reagents was prepared by dissolving the requisite amount in double-distilled water.

Procedure

Barium chloride solution (5 ml, 0.01 *M*) was diluted to 100 ml and warmed. To the hot solution, a solution of the reagent (a slight excess was used) was added with stirring. The pH of the resulting solution was adjusted to 6.00. The white precipitate of the complex formed settled down on digestion for 30 min on water bath leaving a clear supernatant liquid. The precipitates were filtered through a sintered glass crucible of the porosity G-4 and were washed thoroughly with hot water and finally with 20% alcohol–water solution. The barium complex thus obtained was dried at 115–120°C. and weighed as $(C_{19}H_{17}O_5N_4S)_2Ba$.

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Separation of Barium from Other Metal Ions

With a view to separate barium from the other metal ions, a 14.56 ml (0.01 M) solution of barium chloride was mixed with a known amount of desired foreign metal ions (40 mg) and diluted to 100 ml. A 5-ml portion of 1% masking reagent solution was added and the contents were warmed. To the hot solution, a solution of the reagent (a slight excess was used) was added and the pH was adjusted to 6.0. The complex so formed was allowed to stand on water bath for 30 min. Thus, barium could be separated and precipitated quantitatively in the presence of Ag(I), Mn(II), Cu(II), Ni(II), Co(II), Cd(II), Zn(II), Hg(II), Pd(II), Ca(II), Sr(II), Cr(III), Fe(III), Mg(II), As(III), Sb(III), Sn(IV), Tl(I), and ZrO(II)(Tables 1 & 2).

TABLE 1
DETERMINATION OF BARIUM

S. No	0.01 M BaCl$_2$ (ml)	Barium taken (mg)	Weight of complex (mg)	Barium found (mg)	Error (mg)
1.	10.00	13.70	96.30	13.73	0.03
2.	20.00	27.50	192.60	27.46	−0.04
3.	30.00	41.20	289.00	41.21	0.01

RESULTS AND DISCUSSION

Analysis

A known amount of barium complex was decomposed with a mixture of perchloric acid, sulfuric acid, and nitric acid, and barium contents were determined volumetrically (15).

Nitrogen was estimated by a modified Kjeldahl procedure (1) and sulfur by Messenger's method (11).

	N (%)	S (%)	Ba (%)
Calculated	11.68	6.67	14.02
Found	11.78	6.72	13.98

IR Spectral Data

Sulfadimethoxin salicylaldimine gives two bands at 3580 cm^{-1} and 2600 cm^{-1}, which may be assigned to intramolecular hydrogen bonding and chelated OH involving N (2, 14). These two bands are absent in barium chelate.

A strong band at 1640 cm^{-1} assignable to C=N stretch (3) of SUDMSA is observed in the chelate at 1660 cm^{-1}. The observed shift obtained in C=N stretch after chelation suggests that azomethine nitrogen is coordinated to the metal ions.
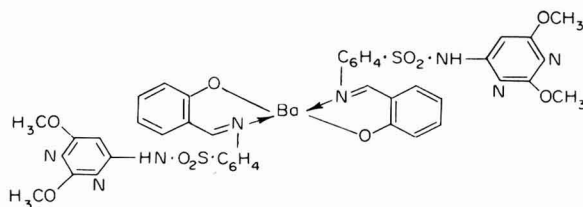
TABLE 2

Ba(II) taken (mg)	Foreign ion added (40 mg)	Masking reagent used	Ba(II) estimated (mg)	Error (mg)
1	2	3	4	5
10.00	Ca(II)	KCN	9.98	−0.02
10.00	Sr(II)	—[a]	9.98	−0.02
10.00	Mg(II)	—	9.98	−0.02
10.00	Sn(IV)	—	9.96	−0.04
10.00	Hg(II)	—	9.99	−0.01
10.00	Tl(I)	—	9.98	−0.02
10.00	ZrO(II)	—	9.98	−0.02
10.00	Cr(III)	—	9.97	−0.03
10.00	Ni(II)	KCN	10.02	+0.02
10.00	Cu(II)	KCN	9.97	−0.03
10.00	Co(II)	KCN	9.99	−0.01
10.00	Mn(II)	KCN	9.98	−0.02
10.00	Zn(II)	NH_4F	9.98	−0.02
10.00	Cd(II)	NH_4F	9.96	−0.04
10.00	Pd(II)	KCN	9.98	−0.02
10.00	Ag(I)	KCN	9.98	−0.02
10.00	Fe(III)	Tartrate	9.98	−0.02
10.00	As(III)	Tartrate	10.02	+0.02
10.00	Sb(III)	Tartrate	9.98	−0.02

[a] Masking reagent not required.

The band at 1275 cm⁻¹ in SUDMSA may be assigned to phenolic C–O stretching in SUDMSA. Similarly the band at 1315 cm⁻¹ in chelate may be assigned to phenolic C–O stretching in chelate and also possibly to combination frequency of M–O bonds and M–N linkages.

These observations lead to the following conclusions: (i) In Ba-chelates the azomethine nitrogen has taken part in the coordinate bond formation. As a result of this, the bond order of carbon to the nitrogen link is increased. (ii) Disappearance of hydrogen-bonded –OH in the chelate and high frequency shift of the phenolic C–O stretch are suggestive of M–O bond formation.

On the basis of the above data the structure of barium chelate may be represented as:

SUMMARY

Sulfadimethoxin salicylaldimine (SUDMSA) has been found to be a wide spectrum precipitant of a number of metal ions. The chelates are granular, stable, and quantitatively formed. SUDMSA has been utilized for the gravimetric determination of barium in the presence of Ca(II), Sr(II), Fe(III), As(III), Sb(III), Cr(III), Ag(I), Cu(II), Ni(II), Co(II), Mn(II), Cd(II), Zn(II), Hg(II), Pd(II), Mg(II), Tl(I), ZrO(II), Sn(IV). The St. of the chelate was confirmed by elemental analysis and IR data.

ACKNOWLEDGMENTS

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Microdetermination of α-Amino Acids by Colorimetry and Atomic Absorption Spectrophotometry

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INTRODUCTION

In previous work *(6, 7)* two procedures were described for the titrimetric microdetermination of α-amino acids based upon chelation with Cu(II) ions. In an attempt to increase the sensitivity, two further reliable methods were developed for such a determination involving also the use of Cu(II) as a pilot ion for the coversion of α-amino acid to its corresponding Cu(II) chelate product. The colorimetric method is based on allowing the soluble copper amino-acid complex to react with CN^- whereby the chelated Cu(II) is first converted to cyanocuprate(I) complex. The latter simply functions as a reductant to the phosphomolybdic acid, added in presence of HCl, giving rise to the familiar molybdenum blue complex whose absorbance is measured as usual *(17)*. In the second method, the absorbance of the copper chelate is recorded directly on aspiration and atomization in the atomic absorption spectrophotometer.

EXPERIMENTAL METHODS

Reagents and materials. The reagents and amino acids used were of analytical grade and the water was always bidistilled. Washed cupric phosphate suspension and sodium metaborate buffer solution of pH 9.1, both prepared as described by Schroeder *et al, (13)*. Copper sulfate, standard solution 1000 ppm in copper, prepared by dissolving 0.392965 g of $CuSO_4 \cdot 5H_2O$ in 100 ml of water. Potassium cyanide and phosphomolybdic acid (aqueous 1% solutions), and hydrochloric acid (1 *M*). Amino acids, test solutions, made by dissolving 50 mg in 100 ml of water.

Apparatus. Pye Unicam SP 1300 Colorimeter, series 2, with 1-cm cell, and Pye Unicam SP 90 Atomic Absorption Spectrophotometer.

Procedure

(i) Colorimetric procedure. Transfer a portion of solution containing 0.5-3.0 mg of the amino acid into a 10-ml polythene centrifuge tube, add 2 ml of the Cu(II) phosphate suspension, dilute to 8 ml with water, and cen-

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trifuge for ca. 5 min at 7000 rpm. Pipette an aliquot (2–5 ml) of the supernatant solution into a 25-ml flask, add 1 ml of each of potassium cyanide, phosphomolybdic acid, and hydrochloric acid in the sequential order quoted and make up to the mark with water. Leave for 2 min and then measure the absorbance of the molybdenum blue formed on the Pye Unicam colorimeter at 725 nm using 1-cm cell and the red filter. The copper content of the amino acid complex is deduced from a calibration curve previously constructed for copper sulphate solutions containing 20–300 μg Cu/25 ml) following exactly the same procedure described by Tobia *et al, (17)*. Similarly, another calibration curve was constructed for alanine, as a test amino acid, by measuring the absorbance due to the copper contents (20–300 μg/25 ml) of the corresponding copper alanine complexes obtained through applying the same procedure described above for actual amino acid determination.

Calculate the α-amino group content based on a copper–amino acid complex of ratio 1:2.

63.54 mg of copper ≡ 32.05 mg of NH_2.

(ii) Atomic absorption procedure. Transfer 1–4 ml of the amino acid solution (0.5–2.0 mg) into a 10-ml polythene tube and proceed as exactly described for the colorimetric procedure till the end of the centrifugation step. In case of amino acid contents between 0.5 and 1.0 mg, transfer the total supernatant solution quantitatively into a 25-ml flask, while with higher amounts (1.5–2.0 mg), transfer the solution preferably into a 50-ml flask and in both cases make the volume up to the mark with water. Then, pour an aliquot (ca. 2 ml) into the cuvette of the atomic absorption spectrophotometer and read the absorbance using a copper hollow cathode lamp at the resonance line 324.8 nm under the following instrumental conditions conventionally recommended for the copper determination.

Fuel: air–acetylene mixture, acetylene pressure 0.7 kg/cm², compressed air 30 psi, air flow rate 5 ml/min, burner height 1 cm, maximum lamp current 5 mA, operating current 4 mA, slit width 0.08 mm, scale expansion 2, gain control 4, and an optimum copper concentration level of 2.0–20.0 ppm.

Calculate the copper content in the chelate product by correlating the read absorbance to the corresponding Cu amount with the aid of a calibration curve predrawn using various solutions of copper sulfate containing 2–20 ppm Cu and their respective absorbance as read directly on the instrument. Also, a second calibration curve was drawn for alanine following the actual atomic absorption procedure for amino acid determination.

Calculate the α-amino group content using the same relation given under the colorimetric procedure.

RESULTS AND DISCUSSION

The complexometric procedure, previously described (6) for the determination of α-amino acids which is based upon isolation of the Cu-complex, its oxidative destruction with nitric acid, and titration of the resulting cupric nitrate with EDTA seems not to be applicable with the required accuracy to amounts less than 2.5 mg of the amino acid.

For this reason, the search was directed towards possible instrumental methods known to enable the accurate determination of amounts much lower than 2.5 mg of the amino acid. The two approaches studied in the present work to satisfy such a requirement are the application of colorimetric and atomic absorption spectrophotometric methods which are discussed below separately.

(i) The colorimetric method. Although many organic substances (16), e.g., nitro and nitroso-compounds, quinones, and ninhydrin were utilized for the direct colorimetric determination of α-amino acids, yet the latter is still considered to be the most widely popular reagent owing to its high sensitivity. However, it was reported (11) that consistent and precise analysis with ninhydrin is difficult to be achieved because of the instability of the colored product due to side oxidative reactions and the nonstoichiometry stated for some amino acids. Recently (18, 19), the spirolactone resulting from condensation of ninhydrin with phenylacetaldehyde has been proposed for the sensitive fluorometric assay of amines and amino acids. On the other hand, indirect colorimetric methods are also available, involving mainly coversion to the corresponding copper–amino acid complex followed by treatment with organic reagents for colorimetric copper determination, e.g., diethyldithiocarbamate (20), biscyclohexanone oxalyldihydrazone (cuprizone) (2, 10) or the respective oxalyldihydrazide (10), 2, 9-dimethyl-1, 10-phenanthroline (neocuproin) (12), 1-fluoro-2, 4-dinitrobenzene (14), and tetraethylenepentamine (4). In most of these indirect methods, careful experimental conditions are necessary since the majority of these reagents are sensitive towards light, pH and temperature alterations, besides being difficult to obtain or to prepare. Thus, our interest was motivated towards the development of a simple and reliable colorimetric procedure involving the use of readily available and common reagents. However, this was achieved by allowing the Cu(II) ions consumed in the formation of the amino acid complex to react directly with cyanide and phosphomolybdic acid, whereby molybdenum blue is spontaneously formed presumably due to reduction of molybdenum (VI) by the cyanocuprate(I) complex (17). The sensitivity of molybdenum blue is attested by its higher molar absorbancy (5) of 25×10^4 as compared (2), for example with 16×10^3 for cuprizone, 12.7×10^3 for diethyldithiocarbamate or 7.95×10^3 for neocuproin (15).

The suitability of the method was first ascertained from the reproduci-

ble calibration curve primarily constructed for different copper sulfate concentrations (20–300 μg Cu/25 ml) following the previously *(17)* recommended procedure. However, in order to obtain a more representative and applicable calibration curve, appropriate concentrations of alanine, selected as a test amino acid, were run through the same actual procedure developed. Fortunately, the two curves coincided satisfactorily indicating that the method is equally applicable for the colorimetric determination of both inorganic and chelated copper.

Applying the colorimetric procedure finally developed for the determination of the α-amino group in a variety of 9 amino acids, satisfactory figures were obtained. Table 1 includes only representative results showing a maximum deviation of 0.64% NH_2 and an average error of $\mp 0.3\%$.

Although, the present procedure is not meant to oust the famous ninhydrin method particularly as regards the sensitivity, it still offers clear advantages, namely, simplicity, reagent availability, remarkable rapidity as exemplified by the very short time (1–2 min) required for the development of molybdenum blue, and color stability, in addition to the reasonable sensitivity enabling the accurate determination of as low as 5 ppm of α-amino acid.

TABLE 1

COLORIMETRIC DETERMINATION OF α-AMINO ACIDS BY MOLYBDENUM BLUE

Amino acid	Wt (mg)		Defference (mg)	$NH_2\%^a$		Error %
	Taken	Found		Calcd	Found	
Glycine	2.000	2.003	+0.003	21.35	21.38	+0.03
	0.500	0.486	−0.014		20.75	−0.60
L(+)-α-Alanine	2.000	1.935	−0.065	17.99	17.40	−0.59
	0.500	0.482	−0.018		17.35	−0.64
Valine	3.000	3.097	+0.097	13.68	14.12	+0.44
	0.500	0.508	+0.008		13.92	+0.24
DL-Tyrosine	3.000	3.023	+0.023	8.84	8.91	+0.07
	0.500	0.507	0.007		8.98	+0.14
DL-Serine	1.000	1.029	+0.029	15.25	15.69	+0.44
	0.500	0.489	−0.011		14.93	−0.32
Glutamic acid	1.000	1.001	+0.001	10.89	10.89	0.00
	0.500	0.504	+0.004		10.79	−0.10
L-Lysine−HCl	3.000	2.898	−0.102	8.77	8.47	−0.30
	0.500	0.517	+0.017		9.08	+0.31
Aspartic acid	2.000	1.927	−0.073	12.04	11.60	−0.44
	0.500	0.503	+0.003		12.10	+0.06
L-Threonine	1.000	0.975	−0.025	13.47	13.11	−0.34
	0.500	0.517	+0.017		13.92	+0.47

[a] Results are calculated on the basis of one amino group which is in α-position to the carboxylic group.

(ii) The atomic absorption method. The application of flame photo-
metry for the macrodetermination of α-amino acids (100–400 mg), firstly
introduced by Beauchéne *et al.* *(1)*, is based on measuring the copper
bound in the amino acid complex. Since then, despite the rapid progress
in the use of atomic absorption spectrophotometry only few methods
became available recently utilizing this attractive tool for the determina-
tion of total α-amino nitrogen, in urine *(3, 8)* and plasma *(9)* also through
chelation with copper (II), in amounts *(8, 9)* not less than 2.4 and 1.39 mg
nitrogen/25 ml solution, respectively.

As clear, the determination of such relatively high amounts of total
amino acids as present in urine or plasma does not warrant the use of the
atomic absorption spectrophotometer; such amounts can be easily deter-
mined by using any common noninstrumental method. However, since
the use of atomic absorption is known to afford a high level of sensitivity,
it was thus desirable to develop the present method which not only ver-
ified this purpose but also enabled the individual determination of a good
variety of α-amino acids.

Preliminary studies involved measurement as cupric nitrate, which is
produced after destructive oxidation of the Cu–amino acid complex with
nitric acid. The method was then simplified further by direct aspiration
and atomization of the copper chelate after slight acidification with nitric
acid, as the only chemical treatment required. Later, it was realized that
acidification is even unnecessary since essentially the same results were
obtained in presence and absence of nitric acid. This indicated that the
copper complexes of α-amino acids do not undergo any hydrolysis pro-
cesses in the absence of acid provided that the absorbance is recorded
within a reasonable time after chelation.

Similar to the case with the colorimetric method, no substantial differ-
ence was observed between the two calibration curves constructed using
copper sulphate or alanine as standards. Therefore, the calibration curve
of copper sulphate was selected for convenience.

In short, the procedure developed proved successful for the determina-
tion of the α-amino group in a series of 10 amino acids. The results (Table
2) obtained show an average error of ±0.18% and a maximum error of
0.5%.

Comparison with the aforementioned methods for urine *(8)* and plasma
(9), reveals that a much better sensitivity is obtainable using the present
procedure since this calculates, in terms of α-amino nitrogen, to a value of
38 μg/25 ml on a weight basis of 0.5 mg of L-lysine hydrochloride.

The potential aspect of sensitivity of the atomic absorption technique,
was further exploited on analyzing as low as 60 μg/25 ml of glycine (i.e.,
2.4 ppm), as a representative amino acid, employing the apparatus highest
scale expansion value of 10. The amino group content obtained is 21.48%

TABLE 2

DETERMINATION OF α-AMINO ACIDS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

Amino acid	Wt (mg)		Defference (mg)	$NH_2\%$[a]		Error %
	Taken	Found		Calcd	Found	
Glycine	1.500	1.501	+0.001	21.35	21.35	+0.00
	1.000	0.989	−0.011		21.12	−0.23
L(+)-α-Alanine	2.000	1.963	−0.037	17.99	17.65	−0.34
	0.500	0.504	+0.004		18.16	+0.17
Valine	1.000	0.995	−0.005	13.68	13.62	−0.06
	0.500	0.505	+0.005		13.87	+0.19
DL-Tyrosine	1.500	1.504	+0.004	8.84	8.87	+0.03
	0.500	0.524	+0.024		9.33	+0.49
DL-Serine	1.000	1.001	+0.001	15.25	15.26	+0.01
	0.500	0.496	−0.004		15.13	−0.12
Glutamic acid	2.000	2.014	+0.014	10.89	10.97	+0.08
	0.500	0.5189	+0.018		11.34	+0.45
L-Lysine − HCl	2.000	2.007	+0.007	8.77	8.80	+0.03
	0.500	0.470	−0.030		8.27	−0.50
Aspartic acid	2.000	2.001	+0.001	12.04	12.04	0.00
	0.500	0.492	−0.008		11.86	−0.18
L-Threonine	2.000	2.006	+0.006	13.45	13.490	+0.04
	0.500	0.506	+0.006		13.62	+0.17
L-Proline	1.500	1.468	−0.032	13.92	13.62	−0.30
	1.000	1.001	+0.001		14.09	+0.17

[a] Results are calculated on the basis of one amino group which is in α-position to the carboxylic group.

showing an error of only +0.13%. This was calculated from a calibration curve prepared specially for glycine using solutions of copper sulfate containing 0.1–2.5 ppm Cu, at a scale expansion value of 10, under otherwise the same experimental conditions already described in procedure (ii).

SUMMARY

The present work describes two simple, rapid, sensitive, and reliable methods for the determination of α-amino acids after conversion to their corresponding Cu(II) chelates. In the first method, the copper complex is treated with cyanide and phosphomolybdic acid in HCl medium followed by colorimetric measurement of the molybdenum blue produced. In the second method, the absorbance of the copper chelate is directly recorded on aspiration in the atomic absorption spectrophotometer. A variety comprising 9–10 α-amino acids were successfully determined by each of the two methods with average errors not exceeding ±0.30 and ±0.18%, respectively.

ACKNOWLEDGMENT

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BOOK REVIEWS

Chromtatography, A Laboratory Handbook of Chromatographic and Electrophoretic Methods, Third Edition. by Erich Heftmann. Van Nostrand, New York, 1975. xxx + 969 pp., $47.50.

The Third Edition follows the previous editions in the respect that the book is divided into two parts. The first, 389 pages, covers primarily the theory, techniques, and terminology of chromatographic and related procedures. Part Two, the remainder of the book, covers applications of chromatography. The book is, indeed, a comprehensive summary of chromatographic and electrophoretic methods along with their applications to a wide variety of substances. It is written by a large number of contributing authors who are specialists in their particular area. Accordingly, the interest of the authors is reflected in each of the chapters. In some sections the literature citations are not too current; the manuscript for the book appeared to have been prepared in 1973, based upon the most recent literature citation. There is no question about the utility of the book and the information presented in it. Information can be found in it on any type of chromatographic separation. However, because of the rapid changes in chromatography, the section on HPLC could be considered out of date, and insufficient examples are presented. One of the major revisions, according to the editor, has been Chapter 21, which now covers not only the alkaloids but the entire field of pharmaceuticals with the exception of certain drugs that are taken up in other chapters. Unfortunately, the rapidity of change in analysis limits the application of HPLC to only two paragraphs and the interesting statement, "In drug analysis this technique has attracted only sporadic attention." Similarly, the rapidly expanding technique of immunoelectrophoresis is only very briefly covered. A wealth of information is presented in the more established techniques of thin-layer and gas chromatography. The book is recommended; information will certainly be found on any area of chromatographic work which includes inorganic-ion chromatography, ion-exchange chromatography, gel chromatography, instrumentation methods in chromatography, as well as applications to all types of material.

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Methods in Olfactory Research. Edited by D. G. Moulton, Amos Turk, and J. W. Johnston, Jr. Academic Press, New York, 1975. xii + 497 pp., $28.50.

The NATO-sponsored Summer School on Odor Perception held at Utrecht in 1970 pointed out the need for a text to summarize as well as act as a guide to the methods used in olfactory research. Although this volume is primarily a reference work, it does contain a substantial amount of new material. The combination admirably fulfills the objectives set by the editors.

Each of the 14 chapters has an ample bibliography and in addition there are separate author and subject indices at the end of the volume.

The chapter headings give a convenient overview of the contents: Instrumental Aspects of Olfactometry, The Purity of Odorant Substances, Biological and Chemical Methodology in the Study of Insect Communication, The Application of Transmission Electron Microscopy

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on the Study of the Olfactory Epithelium of Vertebrates, Application of Scanning Electron Microscopy and Autoradiography in the Study of Olfactory Mucosa, Correlative Anatomical Methods for Studying the Intrinsic Organization of the Olfactory Bulb, Techniques for Investigating Single Unit Activity in the Vertebrate Olfactory Epithelium, Methods in Electrophysical Studies of the Olfactory System, Human Psychophysics in Olfaction, Laboratory Methods for Obtaining Olfactory Discrimination in Rodents, Determination of Odor Preferences in Rodents, The Simultaneous Measurements of Neural Response and Performance in Rats and Rabbits involved in an Odor Detection Task, Psychophysical and Electrophysiological Experiments with Binary Mixtures of Acetophenone and Eugenol, and Pharmacological Aspects of Olfaction.

This is truly a multidisciplined exposition and for the microchemist particular attention might be drawn to the work with microelectrodes and micropipettes but certainly not limited thereto.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

The Pathogenic Anaerobic Bacteria. By LOUIS DS. SMITH. CHARLES C THOMAS, Springfield, 1975. xiii + 430 pp, Second Edition. $26.75.

This text is a fine survey of the anaerobic pathogenic bacteria of humans and animals. Topics included for each group of organisms are: ecologic niche, identifying characteristics, mechanisms of pathogenesis, pathology, and methods of disease control. An excellent bibliography of about 1000 references is included. The book is well edited and attractively printed. The index of 10 pages is too lean.

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Rosalind Franklin and DNA. By ANNE SAYRE. Norton, New York, 1975. 221 pp., $8.95

Everyone who has read "The Double Helix" (and who hasn't?) will want to read this book. Had Dr. Franklin lived, she would have undoubtedly shared the Nobel Prize with Crick, Watson, and Wilkins.

Whether Dr. Franklin's personality was a "prickly" as Dr. Watson implies in "The Double Helix" or as sweet as Mrs. Sayre claims in this book is unimportant. What matters is that Rosalind was imaginative and brilliant; a careful, painstaking, and exhaustive researcher who believed in hard facts. According to Sayre, "She had no vested interest in any technique . . . she did not even describe herself as a crystallographer," but her X-ray crystallographic pictures were superb. The beautifully clear X-ray picture of hydrated DNA (the β-form) went far to establish the helical structure of the genetic molecule. It was Watson's perceptive ability with "tinker-toy" models that supplied the finishing touch.

Only Dr. Franklin's premature death prevented the full recognition of her contributions. Sayre's book presents additional and interesting information about the final determination of the DNA structure but is unnecessarily polemical. There was glory enough for all. Crick and Watson *did* acknowledge Franklin's unpublished experimental results and ideas in their epochal paper in *Nature*. Furthermore, it is useless to speculate whether or not Crick and Watson would have reached their conclusions without Franklin's X-ray pictures or whether Franklin would have done it without Watson's models. The facts are that neither did.

As Rosalind's friend, Sayre cannot be faulted for some bias. But where does Watson say "She [Dr. Franklin] cherished *categorical* [emphasis by Sayre] objections to the models method"? Watson does say that the use of models was a "last resort," but that would not appear to indicate total dependence on that method. Also, by what telepathy can Sayre know that the evidence for a helix had "been overwhelming to Rosalind some time before" either Crick, Watson or Wilkins? Watson says "Six weeks of listening to Francis [Crick] had me realize that the crux of the matter was whether Rosy's new X-ray pictures would lend any support for a helical DNA structure."

Strangely, "The Double Helix," by James Watson, a scientist, is far more readable than "Rosalind Franklin and DNA" by Anne Sayre, a professional novelist. Dr. Watson's account is a rollicking and rather irreverent but fully enjoyable tale of two men who enjoyed both their life-styles and their work; Mrs. Sayre's book is dull and pedantic with strangely derived words, e.g., "unorthodoxly," "profitlessly," and many awkwardly constructed sentences. As an attempt to "rehabilitate" a neglected figure in science it is unnecessary; as a biography of an accomplished scientist, it is well worthwhile. As such, it is highly recommended to scientists of all disciplines.

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New Techniques in Biophysics and Cell Biology, Vol. 2. By R. H. PAIN AND B. J. SMITH. Wiley—Interscience, New York, 1975. 397 pp., $39.50.

The second volume in this series includes nine topics reviewing recent advances in the application of physicochemical techniques to problems of biological importance.

Chapter 1—Cellular Membrane Fractionation (by J. M. Graham)—includes discussions on markers (morphological, enzyme, intrinsic, and extrinsic), homogenization, and fractionation (zonal centrifugation and other techniques).

Chapter 2—Techniques in the Isolation and Fractionation of Eukaryotic Chromosomes (by C. V. Hanson)—discusses isolation, properties, and fractionation (by differential sedimentation velocity, isopycnic sedimentation, selective filtration, electro-focusing).

Chapter 3—Microcalorimetry (by I. Wadso)—includes a description of the types of calorimeters and the sources of errors in the experiments, the principles of calorimetry, as well as specific applications to determination of insulin activity and bacterial growth. The potential of the method to measure thermodynamics of enzyme—substrate binding is illustrated with the example of binding of N-acetylglucosamine oligomers to lysozyme.

Chapter 4—The Techniques of Plant Cell Culture and Somatic Cell Hybridization (by P. K. Evans and E. C. Cocking)—includes isolation of cells, protoplasts, culture of cells and protoplasts, and protoplast fusion and somatic hybridization.

Chapter 5—New Techniques in Detection of Antibodies to Viral Antigens and Tumor-Associated Antigens (by C. C. Ting and R. B. Herberman)—discusses cytotoxicity tests haemadsorption, haemadsorption inhibition and mixed haemadsorption tests, immunoelectron microscopy, radioimmune precipitation assay, radioiodine-labeled antibody techniques.

Chapter 6—Dielectric Spectroscopy as a Tool for Studying Hydration (by A. Suggett)—discusses a technique still in its infancy, but which potentially is unique in its ability to discern the sites of hydration in macromolecules in hydrophobic, hydrophilic areas as well as hydration surrounding ionic groups. This technique on an inherently faster time scale than nuclear magnetic relaxation, is complementary to the latter.

Chapter 7—Nanosecond Pulsefluorimetry (by P. Wahl)—describes fluorescence kinetics and measurement of fluorescence decay by single photoelectron counting as well as

mathematical analysis of experimental data.

Chapter 8—The Application of Carbon-13 NMR Techniques to Biological Problems (by J. Feeney)—discusses (a) the spectral parameters observed; (b) the methods of sensitivity improvements; (c) applications to proteins, nucleosides, nucleotides, phospholipids, interactions of small (usually ^{13}C-enriched) molecules with proteins. This topic is also discussed in several other recent treatises.

Chapter 9—The Mossbauer Effect and Its Applications in Biology (by R. Cammack)—describes the principles, experimental techniques, and applications to haemoglobin, cytochrome c, and some other ^{57}Fe-requiring proteins, rubredoxin and two-iron ferrodoxins.

These volumes are valuable additions to biophysical chemistry. They are written by experts on topics of intense current interest. The references are up to date (well into 1974). This reviewer particularly appreciated the fact that the methods, instrumentation, and pitfalls are critically examined. This along with the presentation of recent applications allows one to judge whether or not the method presented has a potential for solving a particular research problem.

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Microcrystal Polymer Science. By O. A. BATTISTA. McGraw—Hill, New York, 1975. x + 208 pp., $18.50.

Dr. Battista, who is now the head of Research Services Corporation, has written this interesting book, principally covering research that he carried out with co-workers at the FMC Corporation and later at Avicon, Inc. Microcrystals, as defined by the author, are discrete particles extracted from within the matrix of polymers that have dimensions ranging from 50 to 10,000 Å. The author notes that these particles can be produced in a form remarkably uniform in size (100—300 Å) and shape, and that such microcrystals may constitute the basic building blocks for the gross morphology of high-molecular-weight natural and synthetic linear polymers.

Microcrystals were originally produced from rayon cellulose by acid hydrolysis to the so-called level-off degree of polymerization. Severe mechanical agitation in water unhinged the crystals and produced thick stable colloidal suspensions. These suspensions have found numerous applications, for example, as noncaloric thickeners of food that impart remarkable shelf-life to various formulated products, such as sauces and ice cream. Microcrystalline cellulose is also a useful binder for pharmaceutical tableting, as an emulsifier for latexes, a binder for ceramics, etc. Analytical chemists have used the material in column and thin-layer chromatography.

Subsequent work has shown that a wide variety of other polymers can be similarly converted to the microcrystalline form. These polymers include collagen, polyamides, polyesters, polyolefins, and amylose among the organic polymers, and even silicates. The microcrystals have such diverse applications as wound dressings, bioassimilable adhesives and prostheses, catalyst supports, fireproof paper, coatings for glass fibers, powders for fluidized-bed coating, and additives for control of rheological properties.

The preparation, properties, and uses are described in detail for all of these different microcrystals, except for some proprietary information. Battista's style is clear and even enthusiastic; he obviously feels that this subject is important and he points out many potential applications and fields of study.

Microcrystals have been described in several prior reviews. This book can be highly recommended both to those who are not already familiar with this interesting subject and to those who wish to have an up-to-date description of the state of our current knowledge of polymeric microcrystals.

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