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Microchemical Journal, Volume 26, Number 3, September 1981

Briefs

Cellulose Sorbents in Investigations on Self-Association of Higher Fatty Alcohols. TERESA KOWALSKA AND BEATA WALCZAK, *Institute of Chemistry, Silesian University, 9, Szkolna Street, Katowice 40-006, Poland.*

An attempt is made to physicochemically discuss the possibility of using cellulose sorbents for qualitative and semiquantitative evaluation of the ability of higher fatty alcohols to self-associate.

Microchem. J. **26**, 299–306 (1981).

A New Extraction Method for the Spectrophotometric Determination of Phosphates. V. MACHÁČEK AND M. MALÁT, Research Institute of Crop Production, 161 06 Prague 6, and Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.

The procedure is based on the extraction of the ion associates of complex anions of 12-molybdatophosphoric acid, its "molybdenum blue," and anion of 11-molybdatovanadatophosphoric acid with a cation of cationogenic tenside.

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Extraction of Pertechnetate, Molybdate, and Molybdophosphate Ions by Some Oxygen-Containing Solvents in Acid Solution: A New Procedure of Production of Technetium-99m for Medical Use. N. Z. MISAK, H. A. EL-ASRAG, M. EL-KOLALY, AND E. HALLABA, *Nuclear Chemistry Department, Atomic Energy Establishment, Cairo, Egypt.*

The effects of aqueous phase molybdophosphate concentration on the distribution coefficients of ^{99}Mo, and of organic phase molybdophosphate concentration on extraction of ^{99m}Tc, and the effect of acid concentration on molybdenum stripping from Mo-saturated organic layer were studied in the case of methyl isobutyl ketone resulting in a new procedure for the production of ^{99m}Tc for medical use.

Microchem. J. **26**, 316–328 (1981).

Microdetermination of Acetone in Aqueous Solution. S. A. RAHIM AND W. A. BASHIR, *Department of Chemistry, College of Science, Mosul University, Mosul, Iraq.*

Diazotized anthranilic acid was used as the reagent for the spectrophotometric microdetermination of acetone in aqueous solution. The determination range was 10–130 μg/ml. A possible mechanism was suggested.

Microchem. J. **26**, 329–333 (1981).

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Microchem. J. **26**, 334–339 (1981).

Atomic Absorption Spectrometry of Cadmium after Solvent Extraction with Zinc Dibenzyldithiocarbamate. MARCO TADDIA, *"G. Ciamician" Chemical Institute of the University, Via F. Selmi 2, 40126 Bologna, Italy.*

The AAS determination of cadmium extracted as dibenzyldithiocarbamate in methylisobutyl ketone was investigated. The sensitivity of the method was found to be 6 ppM.

Microchem. J. **26**, 340–343 (1981).

Recovery and Fluorometric Measurement of a Polynuclear Aromatic Hydrocarbon from Smoked Cigarettes. ABDULRAHMAN S. ATTIYAT, *Department of Chemistry, Yarmouk University, Irbid, Jordan,* AND GARY D. CHRISTIAN AND JAMES B. CALLIS, *Department of Chemistry, University of Washington, Seattle, Washington 98185*

The results demonstrate that cigarettes can be spiked with relatively large amounts of a polynuclear aromatic hydrocarbon and sufficient quantities are retained in the cigarette smoke following pyrolysis (smoking) to be useful for following the fate of the compound in biological systems.

Microchem. J. **26**, 344–353 (1981).

Studies on the Kinetics of the Ligand-Substitution Reaction of the Zinc(II)-4-(4′-Methyl-2′-Thiazolylazo)-2-Methylresorcinol with 1,2-Diaminocyclohexane-N,N,N′,N′-Tetraacetic Acid. J. J. ARIAS, F. JIMENEZ, AND F. GARCIA MONTELONGO, *Department of Analytical Chemistry, University of La Laguna, Tenerife, Canary Islands, Spain.*

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Microchem. J. **26**, 354–359 (1981).

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Spectrophotometric Determination of Osmium (VIII) with Promazine Hydrochloride. HELENA PUZANOWSKA-TARASIEWICZ, ANATOL KOJŁO, AND LUDMIŁA ZAWADZKA, *Department of Chemistry, Warsaw University Division, Bialystok, Poland.*

The reagent forms an orange oxidation product in acidic media. A study of the conditions was made.

Microchem. J. **26**, 360–364 (1981).

A Comparison of Three Methods for Cholinesterase Analysis. H. M. STAHR,* R. A. MOORE,* AND W. H. HSU,† **Veterinary Diagnostic Laboratory, and †Veterinary Physiology and Pharmacology, Iowa State University, Ames, Iowa 50011.*

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Microchem. J. **26**, 365–374 (1981).

Some Analytical Applications of Aromatic Sulfonyl Haloamines. Determination of Thiocyanate and Cyanide Ions in Metal Complexes and Salts and Thiosemicarbazide in Metal Complexes with Bromamine-T. K. S. RANGAPPA, DANDINASIVARA S. MAHADEVAPPA, AND B. T. GOWDA, *Department of Post-graduate Studies and Research in Chemistry, University of Mysore, Manasaganotri, Mysore 570006, India* AND NETKAL M. MADE GOWDA, *Division of Environmental Toxicology, Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, Texas 77550.*

The oxidation involves eight and two electron changes, respectively, with NCS^- and CN^- ions and a 12-electron stoichiometry per thiosemicarbazide molecule.

Microchem. J. **26**, 375–386 (1981).

Degradation of Uric Acid and 3-Ribosyluric Acid during Treatment with Charcoal. ROBERT C. SMITH, *Department of Animal and Dairy Sciences, Agricultural Experiment Station, Auburn University, Auburn, Alabama 36849.*

The two compounds were found to be quantitatively adsorbed to charcoal, but were not recovered as such. In both cases, there was substantial conversion to the corresponding allantoin.

Microchem. J. **26**, 387–393 (1981).

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Metallo-Amino Acid Complexes. IV. Copper Complexes. DAVID B. SABINE, *185 Old Broadway, Hastings-on-Hudson, New York 10706.*

Complexes of copper (II) with tryptophan, lysine, and aspartic acid were studied and microphotographs made.

Microchem. J. **26**, 394–398 (1981).

Chromatographic Identification of Lipids from the Aqueous Eye Humor. Z. KAŹMIERCZAK AND Z. PANDZIOCH, *Institute of Ophthalmology, Silesian Medical Academy, Katowice, Poland.*

An effort was undertaken to establish the TLC analytical conditions enabling separation and identification of lipids from the aqueous eye humor.

Microchem. J. **26**, 399–401 (1981).

The Determination of Sulfide in the Aqueous Environment. BOBBY L. WILSON, RUDOLPH R. SCHWARZER, CALLISTA O. CHUKWUENYE, AND JABER CYROUS, *Department of Chemistry, Texas Southern University, Houston, Texas 77004.*

Direct measurements were made by use of a sulfide ion-selective electrode in conjunction with a double-junction reference electrode and an Orion Model 407 A/F specific ion meter. The rate of oxidation of sulfide exposed to air was studied.

Microchem. J. **26**, 402–410 (1981).

Critical Study of the Interference of Silicic Derivatives on Fluoride Determination. J. NEVE,* M. HANOCQ,* AND C. VAN KERCHOVE.† University of Brussels, Institute of Pharmacy, Campus Plaine 205/1, B 1050 Brussels, Belgium and †Drug Testing Service, Rue Stevin, 137, B 1040 Brussels, Belgium.

Several physical properties of various silicic derivatives were examined in order to elucidate an interference with fluoride during codistillation of hexafluorosilicic acid with superheated steam.

Microchem. J. **26**, 411–417 (1981).

Stoichiometry, Ringbom Optimal Range, and Other Parameters for the Copper(I)–Bathocuproine Complex. F. SÁNCHEZ-RASERO, *Seccion de Quimica Analtica, Estación Experimental del Zaidín, Granada, Spain.*

The stoichiometry of the copper(I)–bathocuproine complex was studied and found to be in a 1:2 molar ratio.

Microchem. J. **26**, 418–425 (1981).

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Microdetermination and pK_a Measurement of Some Aliphatic Amines Using the Copper-Ion-Selective Electrode. SAAD S. M. HASSAN,* FAYEZ TADROS,* AND WALTER SELIG,† *Department of *Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt A.R.E., and †Lawrence Livermore National Laboratory, University of California, Livermore, California 94550.*

Aliphatic amines were determined by potentiometric titration with standard cupric sulfate solution in aqueous or partially aqueous (methanolic) medium. Electromotive forces were monitored with copper-ion-selective electrode or a graphite rod impregnated with silver sulfide/cupric sulfide, and a double-junction reference electrode.

Microchem. J. **26**, 426–435 (1981).

A Simple, Rapid, and Accurate Fluorometric Analysis of Epinephrine in Local Anesthetic Solutions. ALFRED E. CIARLONE, BILL W. FRY, AND RICHARD L. PARKER, *Department of Oral Biology–Pharmacology, Schools of Dentistry and Medicine, Medical College of Georgia, Augusta, Georgia 30912.*

A method is presented for the fluorometric analysis of epinephrine contained in local anesthetic solutions.

Microchem. J. **26**, 436–439 (1981).

MICROCHEMICAL JOURNAL 26, 299–306 (1981)

Cellulose Sorbents in Investigations on Self-Association of Higher Fatty Alcohols

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INTRODUCTION

In our previous papers (*2–5*) we first described the possibility of applying low active cellulose sorbents (i.e., chromatographic papers) to the qualitative comparison of selected sections of certain homologous series with respect to their ability to self-associate. In these papers (*2, 3, 5*) we discussed higher fatty alcohols and acids with an even number of carbon atoms from C_{12} to C_{18} in a molecule. A comparison of the chromatographic spot areas obtained on chromatographic paper with decalin as a low polar mobile phase proved that the step-by-step increase of those areas corresponded with prolongation of the aliphatic chains and had to do with the growing steric hindrance, which lowered the probability of intermolecular interactions. In another paper (*4*) we presented a correlation between the ability of the $HO-(CH_2)_n-OH$ glycols to intramolecularly self-associate and the spot areas obtained on chromatographic paper. In that case we noticed clear correspondence between the distance from one hydroxyl group to the other in a glycol molecule and the area of the corresponding spot. On the basis of the obtained results we came to the conclusion that the observed and qualitatively described differences in chromatographic behavior of the investigated substances could also be interpreted in physicochemical and quantitative terms. The physical meaning of various observed phenomena would undoubtedly depend upon the type of self-association interactions (either inter- or intramolecular), and—consequently—upon the mechanism of interactions between the sorbent and the molecules of the chromatographed substance.

The aim of this paper is to discuss the physicochemical meaning of differences observed with the chromatographic spot areas of higher fatty alcohols developed on cellulose sorbents.

EXPERIMENTAL

We investigated the following higher fatty alcohols: lauryl (C_{12}), myristyl (C_{14}), and cetyl (C_{16}); the sample purities in each case were

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higher than 99.5%. Carbon tetrachloride solutions were prepared with each alcohol, their concentrations as follows: 0.01, 0.025, 0.04, 0.05, and 0.1 *M*. The chromatographic paper Whatman 2 (Whatman, England) was applied as a sorbent. The introductory step depended upon drying it for 0.5 hr at 110°C; then 20-μl aliquots of each prepared sample were applied. Chromatograms were developed in decalin 16 cm high, then dried at room temperature for 24 hr and finally visualized with a 10% solution of $K_2Cr_2O_7$ in 50% H_2SO_4. The visualized chromatographic spots were immediately copied and their areas planimetrically determined. The determination error never surpassed ±5%. The obtained results are given in Table 1 and Fig. 1.

The results presented in Table 1 and Fig. 1 remain in agreement with our previous observations and conclusions (*2*, *3*, *5*) dealing with the mutual dependence between the number of carbon atoms in a molecule of investigated substance and its chromatographic spot area. On the basis of ir spectroscopic results and the model theoretical considerations we proposed a mechanism of adsorption of higher fatty alcohols on chromatographic paper (*2*), qualitatively explaining the above-mentioned dependence. In our argumentation we employed the shielding effect of the alcohol functional group with the increasing aliphatic chain, which should make self-association of the molecules more and more difficult and consequently shift it toward the lower associative multimers, i.e., such multimers which involve the least number of monomer units in their structure.

Nevertheless, the expanded experimental material given in this paper and dealing with five different concentrations of the investigated solutions enabled some further observations. Thus, in spite of quite significant error of chromatographic determinations it could be stated that (at least in the

TABLE 1
THE CHROMATOGRAPHIC SPOT AREAS ($\bar{S}$)[a] OF LAURYL, MYRISTYL, AND CETYL ALCOHOL ON THE CHROMATOGRAPHIC PAPER WHATMAN 2 (MOBILE PHASE: DECALIN) FOR VARIOUS CONCENTRATIONS OF THE APPLIED SOLUTIONS[b]

Substance	Chromatographic spot area $\bar{S}$ (mm²)				
	0.01 *M*[c]	0.025 *M*	0.04 *M*	0.05 *M*	0.1 *M*
Lauryl alcohol	75	135	200	220	285
Myristyl alcohol	110	205	325	350	430
Cetyl alcohol	200	375	490	540	805

[a] Mean values from 10 individual measurements.
[b] Constant aliquots of the CCl_4 solutions: 20 μl.
[c] Concentration of applied sample.

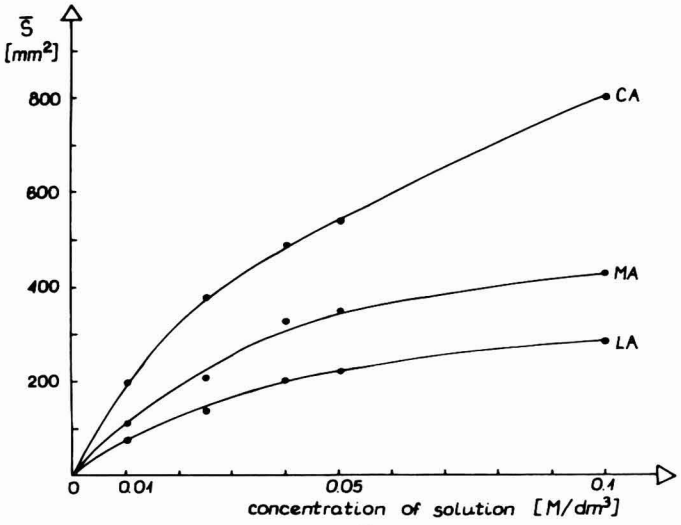


FIG. 1. The chromatographic spot areas ($\bar{S}$) of lauryl, myristyl, and cetyl alcohol vs concentrations of the applied sample solutions. Sorbent: Whatman 2; mobile phase: decalin; sample aliquots: 20 μl.

range of the applied, rather low sample concentrations) the ratios of the chromatographic spot areas for two different alcohols remained practically constant, no matter what solution concentration was taken into consideration, and—consequently—what number of moles was involved in the process of chromatography. The ratios are shown in Table 2.

The results given in Table 2 should experimentally support our considerations, presented in the following section of this paper.

In Table 3 we present ratios of the chromatographic spot areas calculated against the spot area obtained with the sample having the highest concentration.

As shown from the data given in Table 3, the $\bar{S}'/\bar{S}''$ ratio values are similar with each discussed alcohol, when the same concentrations of the

TABLE 2
RATIOS OF THE CHROMATOGRAPHIC SPOT AREAS WITH THE INVESTIGATED HIGHER FATTY ALCOHOLS

The discussed system of two alcohols	$\bar{S}'/\bar{S}''$				
	0.01 M[a]	0.025 M	0.04 M	0.05 M	0.1 M
Myristyl/lauryl	1.47	1.52	1.62	1.59	1.51
Cetyl/myristyl	1.82	1.83	1.51	1.54	1.87
Cetyl/lauryl	2.67	2.78	2.45	2.45	2.82

[a] Concentration of applied sample.

TABLE 3
RATIOS OF CHROMATOGRAPHIC SPOT AREAS CALCULATED AGAINST THE SPOT AREA OBTAINED WITH THE SAMPLE HAVING THE HIGHEST CONCENTRATION

Substance	$\bar{S}'/\bar{S}''$				
	0.01 *M*	0.025 *M*	0.04 *M*	0.05 *M*	0.1 *M*
Lauryl alcohol	3.80	2.11	1.42	1.30	1.00
Myristyl alcohol	3.91	2.10	1.32	1.23	1.00
Cetyl alcohol	4.02	2.15	1.64	1.49	1.00

Note. $\bar{S}'$ = spot area of the 0.1 *M* sample and $\bar{S}''$ = spot area of the samples for concentrations shown.

applied samples are considered. Nevertheless, when comparing the results obtained with the lowest sample concentrations (0.01 *M*), as well as those with cetyl alcohol, one can point out a not very significant, but gradual tendency toward increase of the discussed number values. This fact seems to be indisputably connected with the diminishing ability of the investigated alcohols to self-associate.

DISCUSSION

Let us assume now that our considerations dealing with self-association of fatty alcohols concern strongly diluted solutions, in which only monomers and linear dimers occur. Dissociation of a linear dimer can be given with the following equation:

$$\text{dimer} \rightleftharpoons \text{monomer} + \text{monomer}.$$

The equilibrium constant of this reaction equals

$$K = \frac{[\text{monomer}]^2}{[\text{dimer}]} = \frac{c\alpha^2}{1 - \alpha}, \tag{1}$$

where c is the total concentration of the discussed alcohol; α is the dissociation degree of the linear dimer. Equation (1) can also be written as

$$c\alpha^2 + K\alpha - K = 0. \tag{2}$$

Solution of Eq. (2) with regard to the dissociation degree α appears in the form of two roots:

$$\alpha_1 = \frac{-K - (K^2 + 4Kc)^{1/2}}{2c} \quad \text{and} \quad \alpha_2 = \frac{-K + (K^2 + 4Kc)^{1/2}}{2c},$$

with the first one lacking physical sense ($\alpha_1 < 0$).

Now if we compare two alcohols that are able to form dimers in solutions of equal concentration (c), the ratio of their dissociation degrees will be

$$\frac{\alpha'}{\alpha''} = \frac{K' - (K'^2 + 4K'c)^{1/2}}{K'' - (K''^2 + 4K''c)^{1/2}}. \qquad (3)$$

Then let us find the approximate solution of the above given equation; according to the widely employed approximation that if $a > b > 0$, $(a^2 + b^2)^{1/2} = 7/8 \cdot a + 1/2 \cdot b$. Thus we obtain the dependence

$$\frac{\alpha'}{\alpha''} \cong \frac{K' - [7/8 \cdot 2(K'c)^{1/2} + 1/2 \cdot K']}{K'' - [7/8 \cdot 2(K''c)^{1/2} + 1/2 \cdot K'']} \cong \frac{7(K'c)^{1/2} - 2K'}{7(K''c)^{1/2} - 2K''}. \qquad (4)$$

This solution makes it clear that in the case of serious differences between values of the equilibrium constants (K' and K'') and concentrations (c), the ratio of dissociation degrees tends toward one of the following, simplified dependences:

$$\frac{\alpha'}{\alpha''} \cong \left(\frac{K'}{K''}\right)^{1/2} \quad \text{or} \quad \frac{\alpha'}{\alpha''} \cong \frac{K'}{K''}, \qquad (5)$$

i.e., $\alpha'/\alpha'' \approx$ const when T = const, independently of the c number value.

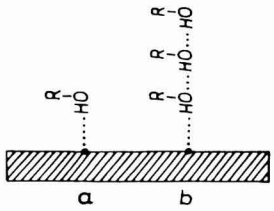
Here one should mention the entire lack in the literature of the K number values with higher fatty alcohols, which makes verification of Eq. (5) virtually impossible.

If we accept the correctness of the above-presented assumptions and conclusions, it appears only understandable that with the solutions of higher concentration, i.e., such solutions in which the reactions

$$\text{trimer} \rightleftharpoons \text{monomer} + \text{dimer};$$
$$\text{tetramer} \rightleftharpoons \text{monomer} + \text{trimer, etc.},$$

occur, ratios of the corresponding dissociation degrees (β'/β'', γ'/γ'', etc.) should also be stable at a given temperature regardless of concentration (at least in a certain range) of samples.

Now let us again consider the situation in which we obtain spots of higher fatty alcohols on a chromatographic paper. As we said in our previous works (*2*, *5*), the mechanism of formation of such spots is the following: The given active center of a sorbent layer is occupied either with (a) a monomeric alcohol molecule, or with (b) a linear multimer, in accordance with the scheme given below.



SCHEME 1.

Thus, the area of a chromatographic spot can be expressed with the equation

$$\bar{S} = p \cdot \left(A + \frac{B}{2} + \frac{C}{3} + \ldots \right), \tag{6}$$

where $\bar{S}$ is the chromatographic spot area; p is the proportionality coefficient characterizing the density of active centers upon a surface of a given sorbent; A, B, C, . . . are the number of molecules appearing as monomers, dimers, trimers, etc.

The ratio of two chromatographic spot areas with two different alcohols applied on a sorbent surface in equal volumes and from equally concentrated solutions can be given by the equation

$$\frac{\bar{S}'}{\bar{S}''} = \frac{A' + B'/2 + C'/3 + \ldots}{A'' + B''/2 + C''/3 + \ldots}, \tag{7}$$

where $A' + B' + C' + \ldots = A'' + B'' + C'' + \ldots$, and $A' \neq A''$, $B' \neq B''$, $C' \neq C''$, etc. Practically it is a function of the dissociation degrees (α, β, γ, etc.) of the corresponding multimers with two given substances.

The $\bar{S}'/\bar{S}''$ number values for three selected alcohols and five different concentrations are presented in Table 2. Their stability with each pair of substances independent of the concentration of solution used, which also means independent of the proportions of various multimers in both samples, seems to be caused by the significant difference in number values between the dissociation constants K and sample concentrations, as was explained by means of Eq. (5). Already with the difference of two number orders between the K and c values stability of the discussed $\bar{S}'/\bar{S}''$ ratios should depend almost exclusively upon K.

The situation changes when we apply upon the chromatographic sorbent solutions having different concentrations, i.e., significantly differing with respect to their association equilibria. If we assume that monomers play a rather negligible role in contributing to the chromatographic spot area, then its surface can also be described in the following way,

$$\bar{S} \cong p \cdot \frac{b}{n}, \tag{8}$$

where b is the number of all molecules of a given alcohol present upon a sorbent; n is the number of monomer units building a statistically mean linear dimer.

The ratio of chromatographic spot areas with two samples of equal volume from the solutions differing with respect to concentrations is

$$\frac{\bar{S}'}{\bar{S}''} \cong \frac{b' \cdot n''}{b'' \cdot n'}, \tag{9}$$

where $b' \neq b''$ and $n' \neq n''$. Thus its number value depends both upon the quantities of the applied samples and upon the different states of associative equilibria. If we investigate solutions having known concentrations, we are able to determine number values of b'/b''. With molar concentrations of samples and when equal volumes of both solutions are applied, the b'/b'' ratio equals

$$b'/b'' = c'/c'',$$

where c' and c'' are molar concentrations of the discussed substances.

In a given case the ratio of chromatographic spot areas equals

$$\frac{\bar{S}'}{\bar{S}''} \cong A \cdot \frac{n''}{n'}, \qquad \text{where } A = \frac{c'}{c''}, \tag{10}$$

which means that it describes the ratio of the n values in both solutions. In other words it characterizes statistically mean associative multimers in both samples. A remark should be made that our considerations neglect the influence of a sorbent and a mobile phase on self-association of the investigated compounds, and—as was explained earlier (*2*, *3*, *5*)—due to the low activity of a sorbent and low polarity of a mobile phase we simply treat them as insignificant.

The $\bar{S}'/\bar{S}''$ number values given in Table 3 illustrate the above-described case. These results concern consecutive alcohols with the changing molar concentrations of their solutions. Therefore an attempt was made to evaluate the corresponding n''/n' ratio values. The obtained results are gathered in Table 4.

As the data in Table 4 show, with growing alcohol concentration of a given sample one observes a tendency of the n'' values to increase. This statement remains in a qualitative agreement with common knowledge concerning self-association, but in our particular case (data from Table 4) the information gained is of at least semiquantitative validity.

TABLE 4

THE A AND n''/n' NUMBER VALUES[a] OF THE CCl_4 SOLUTIONS WITH THE FOLLOWING FATTY ALCOHOLS: LAURYL, MYRISTYL, AND CETYL[b]

	0.01 *M*		0.025 *M*		0.04 *M*		0.05 *M*		0.1 *M*	
Substance	*A*	n''/n'	*A*	n''/n'	*A*	n''/n'	*A*	n''/n'	A	n''/n'
Lauryl alcohol	10	0.38	4	0.53	2.5	0.57	2	0.65	1	1
Myristyl alcohol	10	0.39	4	0.53	2.5	0.53	2	0.62	1	1
Cetyl alcohol	10	0.40	4	0.54	2.5	0.66	2	0.74	1	1

[a] n' corresponds to the 0.1 *M* solution. n'' corresponds to the solutions for concentrations shown.

[b] Determination performed by means of paper chromatography (sorbent: Whatman 2; mobile phase: decalin).

There is another statement to be made dealing with cetyl alcohol. Namely, it seems correct that with cetyl alcohol the consecutive n'' values differ less when changing from one sample concentration to another compared with values for lower alcohols (i.e., the corresponding n''/n' values are relatively higher within the investigated concentration range). This is most probably caused by the greatest aliphatic chain length of cetyl alcohol, which hinders mutual interactions between molecules and shifts associative equilibria toward multimers with the least number of joined molecules.

Thus we have attempted to present a physicochemical interpretation of the phenomenon, based upon the observed suitability of the cellulose sorbents, in research aiming to qualitatively and, if possible, semiquantitatively evaluate higher fatty alcohols with respect to their ability to self-associate (which was experimentally proved in our earlier papers (*2*, *3*, *5*)).

It seems that the obtained results will enable further, more intensive studies to be made on self-association of the selected groups of compounds, taking advantage of the cellulose sorbents.

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A New Extraction Method for the Spectrophotometric Determination of Phosphates

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INTRODUCTION

Heteropolymolybdates and their reduction products, called molybdenum blues, constitute a special group of inorganic complex anions. They are still widely used in spectrophotometry owing to the fact that some of the heteroatoms are among elements which are otherwise difficult to determine, such as phosphorus, silicon, and the like. Under various reaction conditions, heteropolymolybdate anions are produced, their composition being most frequently expressed as $[X^{n+}(Mo_{12}O_{42})]^{(8-n)-}$, where X is the respective heteroatom. Their reduction produces "molybdenum blues," also called "blue oxides," whose composition is not precisely defined as yet; in the group of inorganic photometric reactions they constitute the most intensely colored products. Besides phosphorus and silicon, which have been mentioned already, the heteroatoms of complex polymolybdate anions also include arsenic, vanadium, tantalum, hafnium, titanium, germanium, zirconium, tellurium, and thorium. In aqueous medium, the heteropolymolybdates of these elements are colorless to light yellow (λ_{max} = 300 to 430 nm); they are reduced to molybdenum blue by stannous chloride, ascorbic acid, and the like. Depending on reaction conditions, the λ_{max} of the produced blue color is about 700 nm.

Yellow and blue heteropolyanions can be extracted with some oxygenic solvents (*7*).

This report shows that it is also possible to extract anions of complex 12-molybdatophosphoric acid, its molybdenum blue, and 11-molybdatovanadatophosphoric acid as well as some other heteropolymolybdates on the basis of their ion association with carbethoxypentadecyltrimethylammonium cation in chloroform stage. Extractions of complex thiocyanate (*6*) and halogenide (*2*) anions have already been studied on the same principle.

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MATERIAL AND METHODS

Reagents

Standard P solution: Dissolve 0.4397 g KH_2PO_4 in redistilled water and add water to make 1000 ml; add a drop of chloroform to the solution. Such a solution will contain 100 μg P·ml^{-1} and should be diluted 1:10 or 1:100 as required.

Mixed reagent A (Procedure b): Mix 125 ml of 2.5 *M* H_2SO_4, 37.5 ml of 4% ammonium molybdate, 75 ml of 0.1 *M* ascorbic acid, and 12.5 ml of 0.25% antimonylopotassium tartrate; this reagent must always be freshly prepared.

Mixed reagent B (Procedure c): Mix 2.14×10^{-2} *M* ammonium vanadate and 4.04×10^{-2} *M* ammonium molybdate in a 1:1 ratio.

Carbethoxypentadecyltrimethylammonium bromide (CPTB) solution 5×10^{-3} *M*: Dissolve 0.5281 g of pure substance in chloroform and get a volume of 250 ml; a 1×10^{-4} *M* solution is prepared by corresponding dilution.

The solutions of all the tested ions present had a purity p.a. Na_2SO_4 anhyd.

Apparatus

The spectrophotometric measurements were performed on the SPECTROMOM 204 spectrophotometer (MOM Budapest, Hungary) and on the SP 8-100 registration spectrophotometer (PYE-UNICAM, Cambridge, U.K.). Cuvettes with inner thickness of 1.00 cm were used in both cases.

PROCEDURES

(*a*) *Extraction of 12-molybdatophosphoric acid anion.* Add 5 ml of 5×10^{-2} *M* sodium molybdate and 5 ml of 2.5 *M* nitric acid, hydrochloric, or perchloric acid to a solution containing 2 to 10 μg P. Add redistilled water to get a volume of 25 ml. Let stand for 3–5 min. Then pour the entire solution into a 100-ml fractionating flask and extract it with two 5-ml portions of a 1×10^{-4} *M* chloroform solution of CPTB. Dry the joint extracts with anhydrous sodium sulfate and subject them to photometry at 320 nm against pure chloroform.

(*b*) *Molybdenum blue extraction.* Add 5 ml of mixed reagent A to a solution containing up to 8 μg P and make a volume of 25 ml. Leave to stand for 15 min and extract with two 5 ml portions of 5×10^{-3} *M* solution of CPTB in chloroform. Dry the joint extracts with anhydrous sodium sulfate and perform the photometry at 690 nm against blank sample.

(*c*) *Extraction of 11-molybdatovanadatophosphoric acid anion.* Add (stepwise) 3.5 ml of 1.25 *M* sulfuric acid or 5 ml of 2.5 *M* perchloric acid and 2.5 ml of mixed reagent B to a solution containing 5 to 15 μg P and add

water to get a volume of 25 ml. Extract the solution after 30 min in the same way as in method (b), using 5×10^{-3} *M* chloroform solution of CPTB. The photometry of the organic stage should be performed at 335 nm against a blank sample.

The same procedure can also be followed when determining 5 to 70 μg P, if the photometric measurement is done at 430 nm.

RESULTS AND DISCUSSION

Absorption Spectra

The measurement of the spectra of the ion associates of the complex anions of 12-molybdatophosphoric acid, the assumed anion of its molybdenum blue, or anion of 11-molybdatovanadatophosphoric acid together with the cation of carbethoxypentadecyltrimethylammonium in the chloroform stage gives evidence of their existence and at the same time it provides the basic information of the course of the respective absorption curves, as indicated in Fig. 1. At the studied values of λ_{max} the optimum conditions of the investigated extractions and the respective determinations were studied.

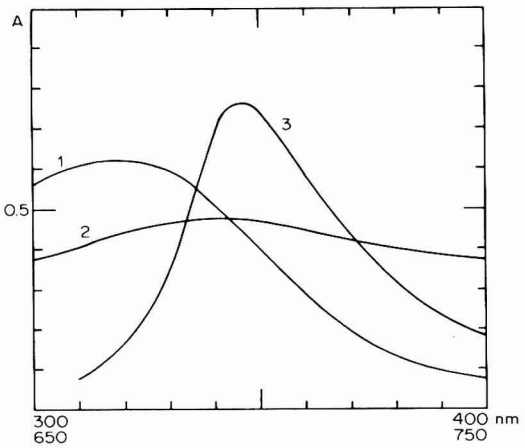

FIG. 1. Absorption curves of the ion associates of complex heteropolyanions of phosphorus and CPTB in chloroform medium. Curve: 1—anion of 12-molybdatophosphoric acid ($c_P = 9.0 \times 10^{-6}$ *M*, $c_{Mo} = 1 \times 10^{-2}$ *M*, $c_{CPTB} = 1 \times 10^{-4}$ *M*, $c_{HNO_3} = 0.5$ *M*); 2—molybdenum blue of 12-molybdatophosphoric acid, ($c_P = 6.45 \times 10^{-6}$ *M*, $c_{Mo} = 1 \times 10^{-3}$ *M*, $c_{CPTB} = 5 \times 10^{-3}$ *M*, $c_{H_2SO_4} = 0.29$ *M*); 3—anion of 11-molybdatovanadatophosphoric acid ($c_P = 1 \times 10^{-4}$ *M*, $c_{Mo} = 4.04 \times 10^{-3}$ *M*, $c_V = 2.12 \times 10^{-3}$ *M*, $c_{CPTB} = 5 \times 10^{-3}$ *M*, $c_{H_2SO_4} = 0.1$ *M*). 1 = 1.00 cm, $V_{H_2O} = 25.0$ ml, $V_0 = 10.0$ ml. Curves 1 and 3 are measured within 300–400 nm, curve 2 within 650–750 nm. All measurements were made against a blank sample.)

Optimum Extraction Conditions, Beer's Law

All the ion associates mentioned are extracted from the medium of mineral acids, and this is the reason why the best acidity of the most-suitable acids was studied; these acids are the following: nitric, sulfuric, hydrochloric, perchloric. The extraction of the complex 12-molybdatophosphoric anion takes place in 0.3–0.7 *M* HNO_3, 0.3–0.4 *M* H_2SO_4, and 0.3–0.6 *M* HCl or $HClO_4$, but 0.5 *M* HNO_3 or $HClO_4$ are by far the best. In the case of the 11-molybdatovanadatophosphoric anion, the extraction can be performed in 0.7–1.3 *M* HCl, 0.45–1.0 *M* HNO_3, 0.2–1.1 *M* $HClO_4$, or 0.07–0.6 *M* H_2SO_4; for practical reasons, either 0.8 *M* H_2SO_4 or 0.5 *M* $HClO_4$ is the best. The extraction of molybdenum blue takes the best course in the medium of 0.29–0.6 *M* H_2SO_4, if ascorbic acid is used for reduction in the presence of antimonylopotassium tartrate (*8*).

The optimum concentration of molybdenum and/or vanadate and the concentration of CPTB in chloroform were also studied for each complex anion. For the anion of 12-molybdatophosphate the values $c_{Mo} = 1 \times 10^{-2}$ *M* and $c_{CPTB} = 1 \times 10^{-4}$ *M* were found, for the 11-molybdatovanadatophosphoric anion $c_{Mo} = 2 \times 10^{-3}$ *M* and $c_{CPTB} = 5 \times 10^{-3}$ *M*, and for molybdenum blue $c_{Mo} = 5 \times 10^{-3}$ *M* and $c_{CPTB} = 5 \times 10^{-3}$ *M*.

It was also found that the extraction could take place after 5 or 30 min standing in the aqueous stage, as indicated in instructions under (a) and (c); in the case of molybdenum blue the standing time is 15 min. The absorbance of the extracts of all three complex anions remained unchanged throughout a 60-min period.

For all the newly studied methods, the applicability of Beer's law was tested and the results of these measurements are surveyed in Table 1. The method of molar ratios was used for both complex anions for the study of the P:Mo ratio, which was found to correspond to the same value as that for the aqueous stage, i.e., 1:12 or 1:11. In the case of method (a) it was found that 84% of the phosphate contained in the aqueous solution was extracted.

Phosphate can also be extracted with chloroform solution of dimethyllaurylbenzylammonium bromide (Ajatin) or tetradecyldimethylbenzylammonium bromide (Zephiramin).

Effect of Foreign Ions

The study of the foreign ions present in the reactions was mainly aimed at the determination of those which might influence determination in soils and plants. Soil analysis is not disturbed by the ions liberated from the sample by the so-called neutral extraction agents (e.g., 1×10^{-2} *M* $CaCl_2$, 1×10^{-2} *M* K_2SO_4), i.e., Na^+, Mg^{2+}, NH_4^+ and NO_3^-; similarly, there is no disturbance effect of the Na, K, Ca, and Mg contained in a plant

TABLE 1
METHODS OF PHOSPHATE DETERMINATION

Procedure		Validity of Beer's law[a]	λ (nm)	Regress. coeff. of calibr. curve eq. a	b	ϵ'	S^b ($\mu g \cdot cm^{-2}$)
Mo-P[c]	0.5 *M* HNO_3	0.1–1.0	320	0.077	0.558	70.543	0.00055
	0.5 *M* $HClO_4$	0.1–1.0		0.082	0.499	65.698	0.00062
Mo-Blue	0.29 *M* H_2SO_4	0.1–0.8	690	0.0061	0.821	64.857	0.00048
Mo-V-P[d]	0.18 *M* H_2SO_4	0.5–1.5	335	0.061	0.520	40.310	0.00066
	0.5 *M* $HClO_4$	0.5–1.5		0.059	0.410	31.007	0.00884

[a] Concentration extent of P in $\mu g \cdot ml^{-1}$ org.
[b] Sensitivity according to Sandell for A = 0.005.
[c] 12-Molybdatophosphoric acid.
[d] 11-Molybdatovanadatophosphoric acid.

sample after mineralization, and the same is true of the Se (about 60 mg) added to the sulfuric acid used for mineralization. If a 12-molybdatophosphate anion is extracted from a $HClO_4$ medium, there is no disturbing effect from arsenic that is present; if the anion of 11-molybdatovanadatophosphoric acid is extracted, arsenic does not disturb the reaction up to a content of 100 μg. Of the currently occurring elements, the effect of Fe (III) was tested as an example in which the determination can be made up to a P:Fe ratio of 1:100 without masking or reduction of Fe.

Determination of Phosphorus in Soils, Plants, and Percolation Waters

The described extraction spectrophotometric determinations have been practically tested in the analysis of the samples of brown soils at a pH 5.2 containing available P (27 ppm) and K (225 ppm), Mg (60 ppm), Na (32 ppm), and inorganic N (16.6 ppm), or in plant material represented by wheat grain, which contained in its dry matter (percentual data) P (0.47), and N (2.35), K (0.56), Na (0.04), Ca (0.05), and Mg (0.19).

In soil samples, methods (a) and (b) were tested after the liberation of phosphate by the anex procedure; in method (b) soil extraction with 1×10^{-2} *M* $CaCl_2$ had also been made in advance. In the first case, 1 g of exactly weighed sample (granularity 0.25 mm) together with 25 ml of distilled water is shaken for 2 hr and then 1 g (conversion to D.M.) of anex OSTION AT[1] is added to the specimen; shaking for 80 min follows. Then

[1] Strongly basic anex; Dowex 1 X-8 or Amberlite IRA-400 can also be used.

TABLE 2

DETERMINATION OF P (AS PO_4^{3-}) IN SOIL, PLANT MATERIAL, AND PERCOLATION WATER

Kind	Sample Adjustment[a]	Vol. (ml)	Method (type, acidity)	Found P[b] (μg)	n[c]	Range	SD	Variation coefficient (%)
Soil	Anex	5	a, 0.5 *M* HCl	3.09 (3.25)[d]	5	0.45	0.193	6.26
		5	a, 0.5 *M* HCl	3.89 (3.90)[d]	5	0.33	0.142	3.65
		5	a, 0.5 *M* HCl	2.88 (2.90)[d]	5	0.35	0.150	5.22
		5	a, 0.5 *M* $HClO_4$	3.23 (3.25)[d]	5	0.30	0.129	3.99
		5	a, 0.5 *M* $HClO_4$	3.90 (3.90)[d]	5	0.15	0.064	1.65
		5	a, 0.5 *M* $HClO_4$	2.95 (2.90)[d]	5	0.15	0.064	2.19
		5	a, 0.5 *M* HNO_3	3.17 (3.25)[d]	5	0.55	0.236	7.46
		5	a, 0.5 *M* HNO_3	3.93 (3.90)[d]	5	0.25	0.107	2.73
		5	a, 0.5 *M* HNO_3	2.96 (2.90)[d]	5	0.25	0.107	3.63
		5	b, 0.29 *M* H_2SO_4	3.40 (3.30)[d]	5	0.15	0.064	1.88
		5	b, 0.29 *M* H_2SO_4	1.73 (1.70)[d]	5	0.20	0.086	4.97
		5	b, 0.29 *M* H_2SO_4	5.25 (5.20)[d]	5	0.20	0.086	1.64

	$CaCl_2$	5	b, 0.29 *M* H_2SO_4	3.11 (3.09)[d]	5	0.08	0.034	1.11
		5	b, 0.29 *M* H_2SO_4	1.49 (1.45)[d]	5	0.12	0.052	3.46
		5	b, 0.29 *M* H_2SO_4	2.17 (2.17)[d]	5	0.08	0.034	1.58
Wheat grain	Mineralization	3	c, 0.18 *M* H_2SO_4	38.76 (39.0)[e]	5	2.3	0.99	2.55
		3	c, 0.18 *M* H_2SO_4	34.02 (33.0)[e]	5	3.3	1.42	4.17
		3	c, 0.18 *M* H_2SO_4	28.08 (28.4)[e]	5	3.0	1.29	4.59
Percolation water		20	b, 0.29 *M* H_2SO_4	1.72	5	0.14	0.072	3.27
		20	b, 0.29 *M* H_2SO_4	1.94	5	0.21	0.063	2.06
		20	b, 0.29 *M* H_2SO_4	2.06	5	0.17	0.059	3.40

[a] In soil—release of PO_4^{3-} from anex or extraction with $CaCl_2$.
[b] Results of control method given in parentheses.
[c] Number of determinations.
[d] Method of Mo-blue adapted according to Ref. (*4*).
[e] Method of 11-molybdatovanadatophosphoric acid.

the mixture is poured through a PE net (0.4-mm mesh) and the captured anex is washed with water. Phosphorus, or phosphate, is liberated from the anex by double extraction with 0.1 *M* hydrochloric acid for about 20 min (with shaking from time to time) (*5*). From the extract prepared in this way, 5 ml is usually pipetted for the determination itself, for which method (a) or (b) is used.

If the 1×10^{-2} *M* $CaCl_2$ extraction is made, the procedure is as follows: Add 100 ml extraction solution to 20.0 g of soil, granularity 2 mm, and shake the mixture for 5 min. Filter the mixture and pipet 5 to 15 ml from the filtrate for method (a) or 5 to 20 ml for procedure (b).

Method (b) can also be used for the determination of the kinetics of the phosphorus released from the soil according to Cook (1).

In the case of plant material, weigh exactly 1 g of specimen, add about 60 mg of metallic selenium, 25 ml of concd. sulfuric acid, and mineralize by heating this mixture. When the mineralization is finished, add sulfuric acid to get a volume of 15 ± 0.5 ml and dilute in measuring flask to a volume of 250 ml. Filter the solution of the specimen. Pipet 3 to 15 ml of this solution for determination by procedure (c).

Methods (a) and (b) were also used in the determination of phosphate in percolation waters from lysimetric experiments; 20 ml of specimen is taken for the study.

The results of the determination of phosphates in the samples of soil, grain, and percolation water are shown in Table 2.

CONCLUSIONS

The methods described in this paper constitute three new possibilities for the extraction photometric determination of phosphates. They are based on the extraction of the ion associates of complex polymolybdate anions of phosphorus with a cation of cationogenic tenside.

The new spectrophotometric determination methods are expedient, simple, and less laborious, when compared with the hitherto described extraction procedures using basic dyes, and are more sensitive than the extraction of these complex phosphorus anions with oxygenic solvents. The determination procedures were practically applied to the measurement of the content of phosphorus in soils, plants, and waters obtained in lysimetric experiments; good results were obtained in all these cases. The described determination procedures will be applied to automatic series spectrophotometry (*3*).

The same extraction principle was studied in all the other heteropolymolybdate anions containing silicon, arsenic, vanadium, germanium, titanium, and the like (*3*).

SUMMARY

The paper describes new procedures of extraction spectrophotometric determination of phosphates, based on the extraction of the ion associates of complex anions of 12-molybdatophosphoric acid, its "molybdenum blue," and anion of 11-molybdatovanadatophosphoric acid with a cation of cationogenic tenside. The methods were applied to the determination of phosphorus in the samples of soil, plants, and water from lysimetric studies.

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Extraction of Pertechnetate, Molybdate, and Molybdophosphate Ions by Some Oxygen-Containing Solvents in Acid Solution: A New Procedure of Production of Technetium-99m for Medical Use

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INTRODUCTION

Studies of the extraction behavior of pertechnetate, molybdate, and molybdophosphate ions are important for the production of ^{99m}Tc from neutron-irradiated molybdenum compounds and for analytical and separation purposes. ^{99m}Tc for medical use is prepared by its extraction in ketones from alkaline ^{99}Mo solutions followed by its recovery from the organic solvent (*2, 8, 9, 16*). Such extraction, but with the use of a neutral ^{99}Mo solution to avoid aldol condensation impurities, has been suggested by this laboratory (*7*).

Another procedure for production of ^{99m}Tc for physicochemical and biological research is to extract molybdenum selectively from acid solution by HDEHP with the direct milking of ^{99m}Tc from the organic extract (*1*). Since heteropolyacids can be selectively extracted by many oxygen-containing organic solvents (*13*), extraction of molybdophosphate, molybdate (always present with molybdophosphate), and pertechnetate ions by some of these solvents in acid solution was investigated to evaluate the possibility of production of ^{99m}Tc for medical use by direct milking. Possible separations deduced from this study are also considered.

The effects of aqueous phase molybdophosphate concentration on the distribution coefficients of ^{99}Mo, of organic phase molybdophosphate concentration on extraction of ^{99m}Tc, and of acid concentration on molybdenum stripping from a Mo-saturated organic layer were studied for methyl isobutyl ketone (MIBK), with a new procedure recommended for production of ^{99m}Tc for medical use.

EXPERIMENTAL

Materials. ^{99}Mo was obtained by neutron irradiation of MoO_3 and used as a tracer for Mo in the molybdate and molybdophosphate ions. A neu-

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tral (pH = 7.2) molybdate solution was prepared by dissolution of MoO_3 in 5 *N* NaOH with the addition of H_2O_2 to ensure a hexavalent state for Mo, followed by heating. Molybdophosphoric acid was prepared by addition of the requisite amounts of NaH_2PO_4 and HCl to Na_2MoO_4, following the equation (*13*): $12\ Na_2MoO_4 + NaH_2PO_4 + 25\ HCl \rightarrow H_3[P(Mo_3O_{10})_4] + 25\ NaCl + 12\ H_2O$. The resulting solution was stored in a polyethylene bottle protected from light. The molybdate and molybdophosphate aqueous solutions used in the distribution measurements contained, unless otherwise stated, 17 mg MoO_3/ml. ^{99m}Tc was separated from 5 *N* NaOH by methyl ethyl ketone (MEK) extraction and a sodium pertechnetate ($NaTcO_4$) solution in 0.9% NaCl was used. Its purity was checked by following its decay and by γ-spectroscopy.

Apparatus. A γ-ionization chamber, Model 238 (Isotope assay Calibrator), D. A. Pitman Ltd., England, was used for relatively high radioactivity determination. For relatively small radioactivities, a scintillation counter, ECKO type N 664 A, fitted with NaI(Tl) well-type crystal (1 × 1 in.) type N 553 A and connected to an automatic scaler, type ECKO N 530 F, was used. A single channel analyzer, digital autoscaler MS Str. 1104/1, was used in the γ-spectroscopic study.

Distribution ratio study. Five milliliters of the aqueous phase of the desired composition was shaken at room temperature with 5 ml of MEK, MIBK, DIPK (diisopropyl ketone), or DEE (diethyl ether) for 10 min, which is sufficient to achieve equilibrium. The phases were separated and 1 ml of each phase counted. ^{99m}Tc is counted directly while ^{99}Mo is counted after cutting the γ-rays of ^{99m}Tc by a lead absorber of 6 mm thickness. The distribution coefficient (K_d) is calculated by the equation:

$$K_d = \frac{\text{Specific activity of organic phase}}{\text{Specific activity of aqueous phase}} \qquad (1)$$

In case of stripping of molybdenum from MIBK by aqueous acidic solutions, the reaction is very slow and the K_d values correspond to a shaking time of 10 min.

RESULTS AND DISCUSSION

Extraction Behavior and Mechanisms

The extraction of molybdate, molybdophosphate, and pertechnetate ions by MEK, MIBK, DIPK, and DEE in HCl acidity range of 0.012–4.8 *M* is shown in Figs. 1, 2, 3, and 4, respectively. In case of molybdophosphate, this acidity is the acidity in excess of that needed for the formation of molybdophosphate according to Eq. (1).

Extraction of molybdate. The change of K_d is very irregular in case of

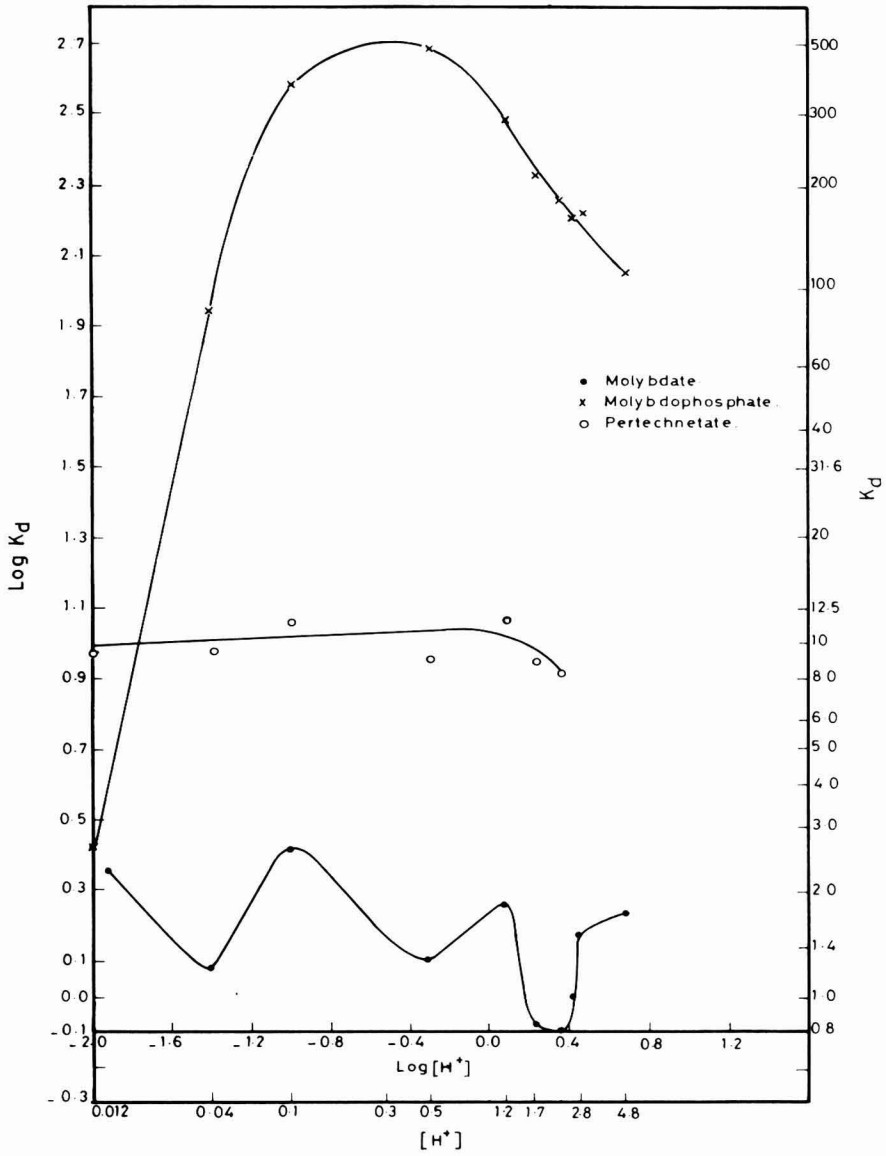

FIG. 1. Effect of HCl concentration on the extraction of MoO_4^{2-}, $PMo_{12}O_{40}^{3-}$, and TcO_4^- by MEK.

MEK (Fig. 1) whereas in other solvents (Figs. 2–4) a significant increase of K_d is observed only at acidities $> \sim 2\,M$. The order of extractability is generally the following: MEK > MIBK > DIPK > DEE. For the ketones (Figs. 1–3), increase of extractability is consistent with an increase of the basicity (solvating power) and dielectric constant of the solvent. In addi-

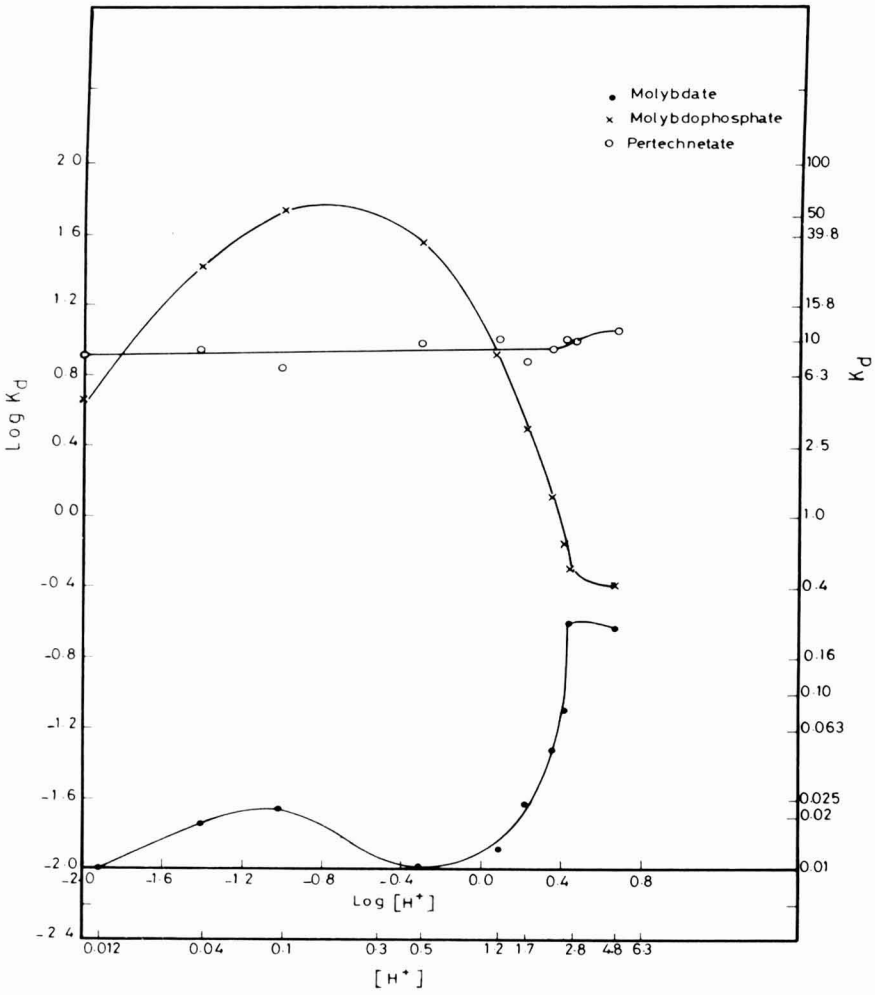

FIG. 2. Effect of HCl concentration on the extraction of MoO_4^{2-}, $PMo_{12}O_{40}^{3-}$, and TcO_4^- by MIBK.

tion, this order is consistent with a decrease in K_d with increased branching of the alkyl group (*12*). DEE has a very low dielectric constant but a higher solvating power than the ketones. The low extractability (Fig. 4) in this case may therefore indicate that the extracted molybdate species is at least partially dissociated. This together with the fact that K_d is little affected by acidity over a relatively large acidity range (Figs. 1–4) may indicate that the extracted species is Na_2MoO_4 and not species of the type HMO_4 suggested before for metals that form oxyanions in their higher oxidation state (*12*). The relatively large increase of K_d at the higher acidities (Figs. 2–4) may probably be related to the formation at these

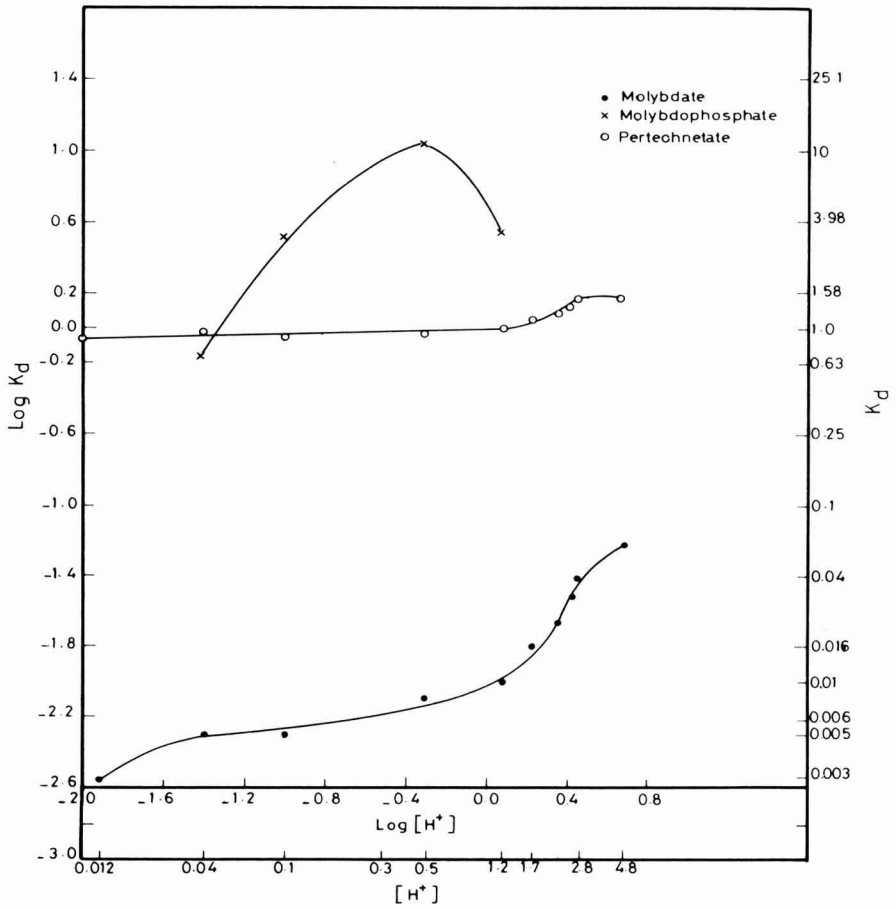

FIG. 3. Effect of HCl concentration on the extraction of MoO_4^{2-}, $PMo_{12}O_{40}^{3-}$, and TcO_4^- by DIPK.

acidities of more extractable isopolymolybdate anions (*5*) and to a dehydration effect of high acidity which drives the extracted species to the organic phase where they can be solvated by the organic molecules (*14*).

Extraction of molybdophosphate. Here, K_d increases in all cases (Figs. 1–4) with acidity to about 0.4 *M* (~0.2 *M* for MIBK, Fig. 2), after which it decreases continuously. The acidity here is in excess of the value necessary for the formation of the yellow molybdophosphoric acid.

Ptushkina and Lebedeva (*15*) suggested that the molybdophosphate anions form coordinate bonds with the solvent while Simon and Boltz (*17*) suggested an "onium-salt" mechanism in which the solvent coordinates with a hydrated hydronium ion and enters into an ion pair formation with the phosphomolybdate anion to form the extracted species. The former authors gave infrared evidence for their suggestion. The increase of K_d

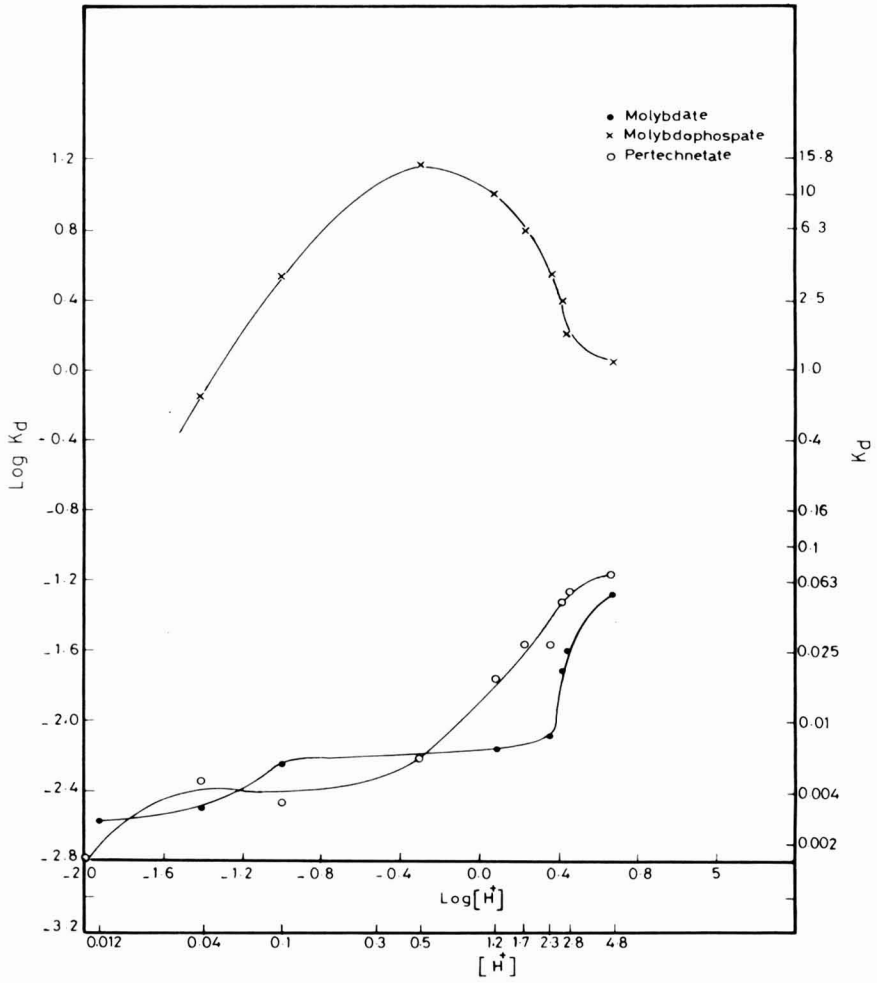

FIG. 4. Effect of HCl concentration on the extraction of MoO_4^{2-}, $PMo_{12}O_{40}^{3-}$, and TcO_4^- by DEE.

with acidity in this case may be due to the suppression of the dissociation of the molybdophosphoric acid with a corresponding weakening in the hydration of its anion (*10*). However, the large effect of acidity on K_d in the initial relatively low acidity range (Figs. 1–4) seems to indicate that the "onium-salt" mechanism is the predominant one.

The decrease of K_d after a certain acidity may be due to the decomposition of the molybdophosphoric acid (*17*), at the higher acidities. Such a decrease may also be due to the gross changes at these acidities in the phase volumes on equilibration, leading to the dissolution of a large

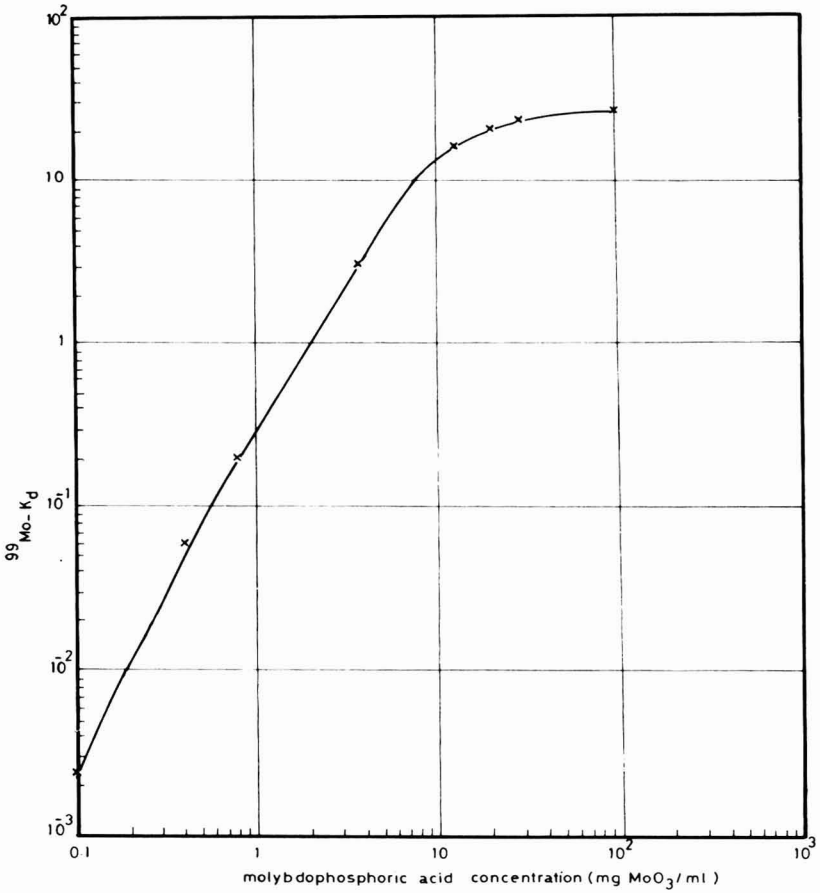

FIG. 5. Effect of molybdophosphoric acid concentration on ^{99}Mo-K_d in MIBK at total 0.7 *M* HCl.

amount of the solvent in the aqueous phase (*12*). This factor is probably important in the more acid-soluble DEE and MEK.

As in the case of molybdate, the order of extractability is: MEK > MIBK > DIPK ≈ DEE. As already mentioned, this order for the ketones agrees with considerations of basicity, dielectric constant, and steric hindrance. Again, the large effect of dielectric constant revealed by the low extracting power of DEE is consistent with the assumption of dissociation of molybdophosphoric acid in the organic phase. Such dissociation has been proved in acetone and ether (*4*), isopentanol (*18*), and 20% dioxane-water (*19*).

The extraction of molybdophosphate can be represented by the equation (*6*):

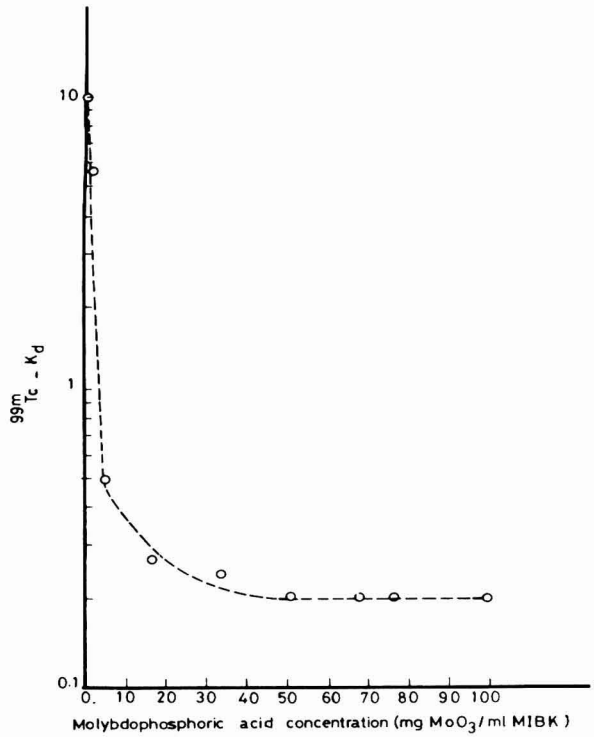

FIG. 6. Effect of molybdophosphoric acid concentration in MIBK on ^{99m}Tc-K_d at 0.7 *M* HCl.

$$[\mathrm{MPA}^{n-}]_{aq} + n[\mathrm{H}^+] + m[\mathrm{S}] \rightleftharpoons [\mathrm{H}_n\mathrm{MPA} - \mathrm{S}_m]_{org} \qquad (2)$$

where MPA is the molybdophosphate anion and S is the organic solvent. For this reaction, we have

$$K = [\mathrm{H}_n\mathrm{MPA} - \mathrm{S}_m]_{org}/[\mathrm{MPA}^{n-}]\,[\mathrm{H}^+]^n\,[\mathrm{S}]^m. \qquad (3)$$

Since

$$K_d = \frac{[\mathrm{H}_n\mathrm{MPA} - \mathrm{S}_m]_{org}}{[\mathrm{MPA}^n]_{aq}}, \qquad (4)$$

it follows that

$$\log K_d = \log K + n \log [\mathrm{H}^+] + m \log [\mathrm{S}].$$

Therefore, the number of H^+ ions involved in the extraction will be equal to the slope of the log *D* - log $[H^+]$ straight line obtained at a constant [S]. The very approximate values obtained from the initial positive slopes in Figs. 1–4 are equal to 2.5, 1.3, 1.6, and 1.7 for MEK, MIBK, DIPK, and

DEE, respectively. These values may be compared with a value of 2.6 for DEE (*17*) and 3 for 1-butanol in kerosene (*13*). However, as pointed out by Simon and Boltz (*17*), such a procedure may not give the correct number in systems where the solvent plays a definite coordinating role in forming the extractable species.

Extraction of pertechnetate. In all ketones (Figs. 1–3), K_d of the pertechnetate changes very little with change of acidity while for DEE (Fig. 4), K_d changes little with acidity up to about 0.3 *M* while a significant increase occurs thereafter.

Boyd and Larson (*3*) have shown that ^{99m}Tc is extracted in the form of pertechnetic acid from acid solution by oxygen-containing solvents and that the extraction increases with the increase of the dielectric constant of the organic phase, suggesting that the extracted acid is appreciably dissociated in that phase. Therefore, one would have expected that the extraction of pertechnetate by the oxygen-containing solvents might increase with the acidity of the aqueous solution. The relative independence of K_d on the HCl concentration observed here with the ketones (Figs. 1–3) may be due to an increase of reduction of heptavalent technetium with an increase of HCl concentration to lower valence states that are less extractable (*8*), which counteracts the increase of K_d with increase of acidity. The increase of K_d at relatively large acidities in case of DEE (Fig. 4) may be due to a dehydration effect of the acid that was perceptible here due to the initially very low K_d values obtained.

The extractability of pertechnetate follows the order: MEK > MIBK >> DIPK >> DEE. This is the order of increasing basicity and dielectric constant and decreasing steric hindrance of the ketones. As in the case of molybdate and molybdophosphate, the dielectric constant is a predominant factor since the extraction by DEE, having the highest basicity, is least due to its very low dielectric constant. This is in accord with the work of Boyd and Larson (*3*) who have found that quantitative extraction of heptavlant technetium was obtained when the active solvent (containing a basic oxygen or nitrogen atom) also possessed an appreciable dielectric constant.

Separation of Ions in Different Solvents

The highest separation (Figs. 1–4) between the molybdophosphate and molybdate ions is achieved at an acidity of about 0.4 *M* in all the solvents. The separation factors (K_d molybdophosphate/K_d molybdate) at this acidity are 60, 4000, 1500, and 2000 in MEK, MIBK, DIPK, and DEE, respectively.

The highest separation factor between the pertechnetate and molybdate (K_d pertechnetate/K_d molybdate) is obtained at an acidity of about 0.25 *M* in MEK where the separation factor is about 12, of 0.01–0.4 *M* in MIBK

where the separation factor is about 500–1000, and of about 0.01 *M* in DIPK where the separation factor is about 300, while separation factors close to unity (almost no separation) are received in DEE. The high separation factors obtained in MIBK together with the fact that over 90% of ^{99m}Tc and less than 2% of molybdate are extracted in one extraction by MIBK at a wide HCl acidity range of 0.01–0.4 *M* indicate that this solvent is a very promising one for a selective extraction and production of ^{99m}Tc.

The highest separation factors between molybdophosphate and pertechnetate (K_d molybdophosphate/K_d pertechnetate) are obtained at an acidity of about 0.4 *M* in MEK, DIPK, and DEE and of about 0.1 *M* in MIBK. The separation factors at these acidities are about 50, 5, 10, and 1500 in MEK, MIBK, DIPK, and DEE, respectively.

It is therefore seen that in all these solvents, Mo in the form of molybdophosphate can be selectively extracted relative to ^{99m}Tc. This can form the basis for the ultimate goal of a selective extraction of ^{99}Mo from which ^{99m}Tc can be directly milked into the desired aqueous solution. The separation factor is not the only important factor for such a procedure. The solvent of choice must have a sufficiently high MPA (molybdophosphate)-K_d so as to ensure an almost complete extraction of Mo in one extraction and almost no Mo release in the milking of ^{99m}Tc from the extract, a relatively low ^{99m}Tc-K_d so as to achieve an efficient Tc milking from the organic Mo extract, a negligible extraction of molybdate-Mo, so as to obtain a Mo-free Tc solution, a negligible dissolution in the aqueous acidic solution, and a sufficiently low volatility. Although the MPA/Tc separation factor in DEE is highest, this solvent is excluded due to its high solubility in acid. MEK, although showing a higher selectivity for MPA than MIBK, still shows a rather appreciable extraction of Mo-molybdate. In addition, MIBK is much less soluble than MEK in the acid solution and has a lower volatility. Although DIPK shows a higher MPA/Tc separation factor than MIBK, its selectivity for MPA is much lower than that of MIBK. It seems therefore that MIBK is the solvent which provides a compromise between all the factors and it is this solvent that was used in the present work for the development of a new procedure for the production of ^{99m}Tc.

New Procedure for Production of ^{99m}Tc

The variation of ^{99}Mo-K_d with variation of molybdophosphoric acid concentration in the aqueous phase at a total initial HCl acidity of 0.7 *M* is given in Fig. 5. It is seen that the increase of K_d tends to a limiting value of about 25 at a concentration of about 50 mg MoO_3/ml or higher.

The ^{99m}Tc-K_d for technetium extraction from aqueous solution (0.7 *M* HCl) by MIBK loaded with different amounts of molybdophosphoric acid

(Fig. 6) is shown to decrease drastically with increase of this acid concentration until a limiting value of about 0.1 is achieved at a concentration of about 50 mg MoO_3/ml or higher. This shows the ease of release of technetium in these conditions.

Finally, stripping of ^{99}Mo from an organic layer loaded with 17 mg MoO_3/ml in the form of molybdophosphate by different concentrations of HCl is given in Fig. 7. The K_d values given in this figure, as previously mentioned, are not equilibrium values and correspond to a shaking time of 10 min. This figure shows that at an acidity of about 0.7–1.7 M, Mo is strongly retained in MIBK.

The recommended procedure therefore involves the selective extraction of molybdophosphate (50 mg MoO_3/ml) from 10 ml aqueous solution (containing the respective amounts of Na_2MoO_4 and NaH_2PO_4) at an HCl acidity of 0.05–0.5 *M* (Fig. 2, total acidity added 0.7–1.1 *M*) by 10 ml MIBK. The extraction is carried out in a lead-shielded solvent extractor similar to that of Mani and Narasimhan (*11*). The charged MIBK is then

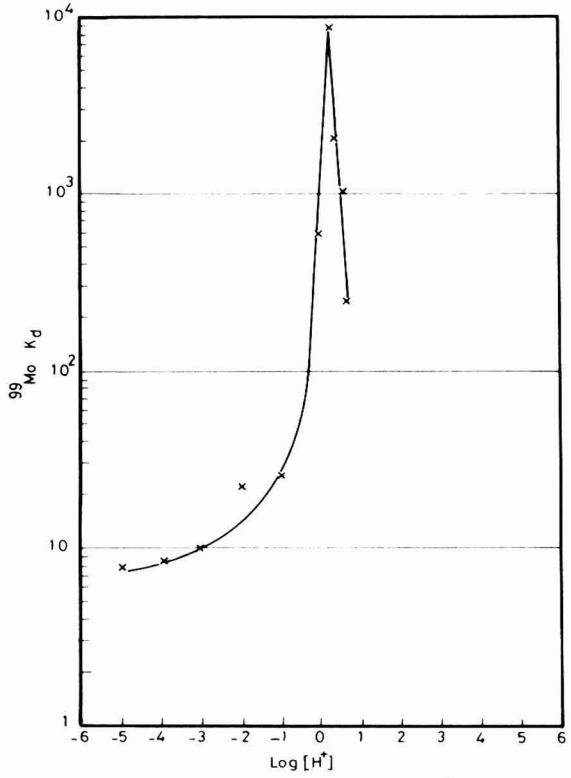

FIG. 7. Effect of HCl concentration of the aqueous layer on Mo-K_d for molybdophosphoric acid (17 mg MoO_3/ml) charged in MIBK, shaking time 10 min.

washed with 10 ml 0.7 *M* HCl saturated with MIBK to remove any impurities of molybdate ions.

It is now ready for milking every 24 hr with an equal volume of 0.7 *M* HCl for 10 min.

The aqueous layer containing the radioactive ^{99m}Tc is separated, passed through a small alumina column to remove any contaminant, then adjusted to neutral pH and sterilized for medical use.

If the aqueous molybdophosphate solution contains 1.5 g MoO_3 (irradiated for 48 hr at 10^{13} n/cm^2/sec) we could obtain a generator yielding 100 mCi ^{99m}Tc on milking.

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MICROCHEMICAL JOURNAL 26, 329–333 (1981)

Microdetermination of Acetone in Aqueous Solution

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INTRODUCTION

Acetone is becoming increasingly common as a water pollutant, especially in industry (*3*). A review for the spectrophotometric determination of acetone in aqueous solution revealed that the methods, among other things, lack speed and sensitivity (*1*, *5*). Consequently in an attempt to overcome the drawbacks of the literature methods, an investigation was made to develop an analytical procedure for acetone microdetermination in aqueous solution. The method described in this paper involved the reaction of acetone with diazotized anthranilic acid as a reagent. This approach proves to be simple, rapid, and far more sensitive than the literature methods (*1*, *5*).

MATERIALS AND METHODS

All reagents used were of analytical or microanalytical reagent grade. Doubly distilled water was employed throughout the work.

Acetone solution. A standard (100 ppm) solution of acetone in water was prepared.

Anthranilic acid solution. This solution was prepared by dissolving 1.0 g of anthranilic acid in 100 ml of hydrochloric acid (0.25 *N*) solution.

Sodium nitrite solution. Freshly prepared 8% solution.

Diazotized anthranilic acid solution. A volume of 100 ml of 1% anthranilic acid and 10 ml 8% sodium nitrite solution were cooled separately to about 5°C; the solutions were mixed and the mixture was shaken occasionally for 15 min. The resulting solution was stable for 20 hours when stored in a refrigerator (at about 5°C).

Potassium hydroxide solution. 50% solution was prepared.

Starch solution. It was prepared by dissolving 0.05 g of starch in distilled water and the volume was made to the mark in a 100-ml volumetric flask.

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TABLE 1
THE EFFECT OF POTASSIUM HYDROXIDE CONCENTRATION ON THE ABSORBANCE

Volume of potassium hydroxide solution (ml)	Absorbance
0.5	0.380
1.0	0.610
1.1	0.630
1.2	0.660
1.25	0.620
1.3	0.590
1.4	0.550

Apparatus. All absorbance measurements were made in 1.0-cm glass cells with a Unicam SP 800 recording spectrophotometer and Unicam SP 600 spectrophotometer.

Procedure. To a series of 20-ml calibrated flasks, each one containing from 0.1 to 1.3 ml of aqueous sample solution (1 ml contains 100 μg acetone) were added, 10 ml of doubly distilled water, 2 ml of 1% diazotized reagent solution, 1.2 ml of 50% potassium hydroxide solution, and 1 ml of 0.05% starch solution. The volume was made to the mark with distilled water and the reaction mixture was allowed to stand for 30 min. The absorbances were measured against a reagent blank at 450 nm within 10 min. A straight-line calibration curve passing through the origin was obtained, indicating that Beer's law was followed over the concentration range 10–130 μg of acetone in a final volume of 20 ml, i.e., 0.5–6.5 ppm. The molar absorptivity in the region of least photometric error was found to be 9.28×10^3 liter·mol^{-1}·cm^{-1}.

RESULTS AND DISCUSSION

In the preliminary experiments, the determination of acetone in aqueous solution was attempted under the experimental conditions described for the determination of acetone in acetic acid with diazotized anthranilic acid

TABLE 2
THE EFFECT OF STARCH CONCENTRATION ON THE ABSORBANCE

Volume of starch solution (ml)	Absorbance
0.5	0.670
0.7	0.72
1.0	0.780
2.0	0.780

TABLE 3
COLOR DEVELOPMENT AND STABILITY IN PRESENCE OF STARCH

Time (min)	Absorbance	
	In absence of starch	In presence of starch
0	0.080	0.060
10	0.460	0.500
20	0.580	0.650
30	0.660	0.770
35	0.630	0.770
40	0.590	0.770
45	0.530	0.740
50	0.450	0.700

(*2*). However, the color intensity of the product thus obtained was not measurable, and optimum reaction conditions were therefore sought.

The effect of hydrochloric acid concentration on the color intensity was first studied. Concentrations from 0.05 to 0.3 *N* HCl were used; 0.25 *N* was selected because of maximum absorption. Next, anthranilic acid concentration was examined and 1% solution was selected as the optimal concentration. Consequently, the effect of sodium nitrite was tested and 8% was chosen as the maximal concentration.

The effect of potassium hydroxide (50%) solution was investigated for maximal color intensity. Table 1 shows that 1.2 ml of 50% potassium hydroxide solution gave maximum absorption for the colored system. Under these conditions the order of reagents addition has to be the sample solution, diazotized reagent, and potassium hydroxide solution.

Under the above optimized conditions, the colored species formed was not stable. Accordingly, it was decided to use some surfactant (*4*) to attempt retarding the decomposition of the colored species. Starch was found to be the most effective protective colloid.

Effect of starch. The effect of starch on the sensitivity of the colored system was examined and a volume of 1 ml of 0.05% starch solution,

TABLE 4
DETERMINATION OF ACETONE IN AQUEOUS SOLUTION

Acetone taken (μg)	Error* %
10	+2.0
60	+1.2
130	−1.2

* Errors obtained are from the standard calibration curve.

TABLE 5
INTERFERING EFFECT OF SOME ASSOCIATED ORGANIC COMPOUNDS

Organic compound added	Permissible limit in the presence of 50 μg acetone/20 ml
Formaldehyde	650
Ethanol	260
Methanol	250
Ethyl acetate	300

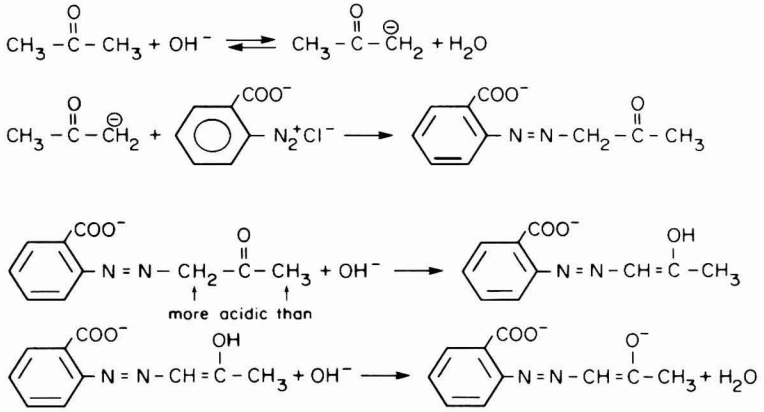
when introduced after all other reagents, was selected as shown in Table 2.

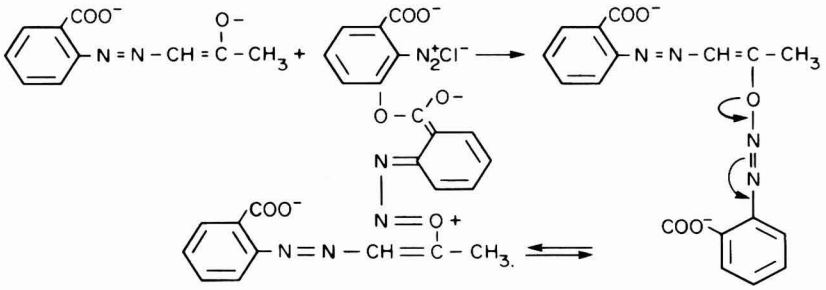
Next, the effect of starch on the stability of the colored species was examined. The color was developed as usual and the absorbance readings were made at various time intervals and compared with a reference containing all the reagents except starch. Measurements were performed against a reagent blank. Table 3 shows that the color reached equilibrium after 30 min and remained stable for another 10 min.

Under these optimized conditions, the colored species showed maximum absorption centered at 450 nm. This new method is suitable for determining acetone in aqueous solution in the range of approximately 10–30 μg/20 ml with errors for three different amounts of acetone that range between approximately −1.5% and +2% (Table 4). The coefficients of variation were 2.8, 1.1, and 0.8 for 10, 60, and 130 μg of acetone, respectively (five replicates). The molar absorptivity was 9.28×10^3 liter · $mol^{-1} \cdot cm^{-1}$.

The possible interfering effect of some organic compounds commonly associated with acetone in aqueous solution was tested and their tolerable amounts are shown in Table 5. When compared with the most recent (modified) method (*1*), it was found to be far more sensitive and simple.

Regarding the reaction mechanism, it is suggested to proceed as follows:





SUMMARY

Diazotized anthranilic acid has been found to be a possible reagent for the spectrophotometric microdetermination of acetone in aqueous solution. The determination range is 10–130 μg/ml. The coefficient of variation does not usually exceed 1.2% for 60–130 μg of acetone but increases to 2.8% at the 10-μg level. The average relative error for five determinations ranges from −1.5 to 2%. The molar absorptivity is 9.28×10^3 liter·mol^{-1}·cm^{-1} in the presence of starch as a surfactant. Possible reaction path mechanism has been suggested for the colored body formation.

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Iodometric Microdetermination of Carboxyl Function by Indirect and Direct Procedures

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INTRODUCTION

Determination of a strong acid by titration with a standard alkali solution is easy and simple. But this is not so with weakly dissociated carboxylic acids, especially at the micro level. On titration with alkali the inflection on the pH-neutralization curve is much less pronounced for weak acids than that for strong acids and progressively shortens as more and more dilute solutions are involved. The color change of a neutralization indicator is not easily discernible if the inflection range is less than 2 pH units (*7*). This difficulty is further accentuated by the fact that the phenolphthalein color at the endpoint fades away rapidly when 0.01 *N* alkali is used as the titrant (*2*). A sample containing 0.05 meq of a weak acid upon titration with 0.01 *N* alkali using phenolphthalein indicator can give an error as large as ±2% (*8*). The alkalimetric determination is interfered with by carbon dioxide.

For samples that give poor titration curves in aqueous solution a nonaqueous medium can be used. However, the endpoint is then usually located potentiometrically, especially when microsamples are involved. Further, solutions of certain titrants such as sodium triphenylmethyl and lithium ammonium hydride are too unstable to be used as 0.01 *N* standards (*2*). The potentiometric titration of a weak acid with a strong base entails costly equipment and is time consuming. The formation of a buffer too can influence the titration results (*4*).

Acids can also be determined iodometrically by treating the sample with an excess of neutral iodide and iodate solution followed by the titration of the liberated iodine. The method gives excellent results even with dilute solutions of strong acids but failed when applied to weak acids. Kolthoff (*3*) showed that tartaric, citric, succinic, acetic, and benzoic acid reacted slowly and hence could not be determined iodometrically. The

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reaction mixture could not be heated to accelerate the reaction rate due to volatility of iodine. Addition of magnesium or calcium salt to the reaction mixture has been recommended when certain organic hydroxy acids could be determined at the macro level by allowing 30 min for the reaction. Another modification due to Kolthoff consisted of adding a measured excess of thiosulfate to the acid sample containing an excess of iodide and iodate solution and subsequently titrating the unused thiosulfate with iodine. The time required for the completion of the reaction is 15–30 min for 0.1 *N* acid and could be much larger with 0.01 *N* and more dilute solutions. Moreover, there is a possibility of the thiosulfate reacting with the acid (*5*) during the long reaction period. This procedure has been adopted by several workers for estimating the acid content in various products but usually the reaction period is 2–24 hr (*6*). Some authors recommend heating of the reaction mixture (*9*) but then the extent of the reaction between the acid and the thiosulfate is much larger.

In the proposed indirect method (procedure 1) the sample solution, in the presence of sufficient potassium iodide, is heated with a known excess of potassium iodate for 3–5 min. Later, the residual iodate is determined iodometrically. Because this procedure is based on the measurement of the iodate consumed rather than on the titration of the liberated iodine, the reaction mixture can be heated to enhance the reaction rate.

With organic acids of more complex nature there is a tendency for the unreacted iodate to enter into secondary reactions so that the stoichiometry of the main reaction is vitiated. For such acids a direct iodometric method (procedure 2) has been used. The reason for the failure of the direct iodometric determination of organic acids is their slow reaction rate when treated with iodide and iodate reagents. Attempts were made to speed up the reaction by increasing the volume of iodide and iodate solution. But then the overall volume of the reaction mixture increased, which had an adverse effect on the reaction velocity so that the quantitative liberation of iodine could not be achieved. Solid potassium iodate and iodide have, therefore, been added instead of their aqueous solutions. In the case of acids having poor solubility in water, an alcohol–water mixture has been used as a solvent. The effect of change in the amount of iodide and iodate and in the reaction period has also been investigated.

MATERIALS AND METHOD

Reagents. Potassium iodate, 0.0166 *M* (0.1 *N*), was prepared; this was used to standardize the thiosulfate solution. The 0.01 *N* solutions were then prepared by diluting with conductivity water.

Potassium iodide solution, 10% (m/v), aqueous.

Sulfuric acid, 4 *N*.

Aqueous starch 1% (m/v), aqueous.

Solutions (0.05 *N*) of acids studied were prepared and standardized by alkalimetry. These were then diluted as required to prepare the test solutions. Acids poorly soluble in water were dissolved in a 1:1 water–ethanol mixture.

Procedure 1. Pipet 5–10 ml of the sample solution containing 0.03–0.1 meq of the acid into a 100-ml conical flask. Add 10 ml of 10% potassium iodide and a known excess of 0.01 *N* potassium iodate. Immerse the flask in a boiling water bath for 3–5 min. Cool the contents of the flask under tap immediately and precisely neutralize the iodine with thiosulfate using starch indicator. Acidify the mixture by adding 2–3 ml of 4 *N* sulfuric acid and titrate the liberated iodine with 0.005–0.01 *N* thiosulfate. Run a blank also. The difference between the blank and the experimental titer gives the amount of acid in terms of thiosulfate solution used. The quantity of iodate added should be such that the blank titer is approximately double the experimental titer; this ensures that a 100% excess of iodate has been added. The reactions involved are:

$$IO_3^- + 6H^+ + 5I^- = 3H_2O + 3I_2,$$
$$3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6,$$
$$6COOH \equiv 6H^+ \equiv IO_3^- \equiv 6Na_2S_2O_3.$$

1 ml of 0.01 *N* thiosulfate ≡ 0.45 mg of COOH group.

Procedure 2. Pipet 5–10 ml of a solution containing 0.025–0.1 meq of acid into a 100-ml iodine flask. Add 0.5–2.0 g solid potassium iodate and 0.5–2 g of potassium iodide. Swirl the flask for about a minute to dissolve the iodate. Keep for 3–5 min, shaking the flask frequently during this period. Titrate the liberated iodine with 0.005–0.01 *N* thiosulfate using starch indicator.

RESULTS AND DISCUSSION

The proposed procedures have been applied to determine certain organic acids of varied nature. The results in Tables 1 and 2 show that the average deviation is in the range of 0.1–0.3%. The methods, being iodometric, give sharp endpoints with 0.01 *N* or even more dilute solutions of thiosulfate. Ashworth (*1*) has commented that the iodometric determination can be used as an alternative, often more accurate, to the titration of acids with alkali reagents. For acids that are sparingly soluble in water such as *p*-nitrobenzoic acid and 1:5 dinitrobenzoic acid, a 1:1 water–ethyl alcohol mixture has been used as a solvent. The resultant solution is slightly yellow in color and alkalimetry below a 0.1-meq level involves a considerable positive error. The iodometric finish in such cases has a distinct advantage. In some instances, such as in the determination of carboxylic acid in lac, the lac resin is saponified, rendering titration with alkali unsuitable. Here, the iodometric method provides a better alternative.

TABLE 1
MICRODETERMINATION OF CERTAIN ORGANIC ACIDS BY PROCEDURE 1

	Alkalimetry				Iodometry		
Acids	Amount	Amount[a]	% deviation	% standard deviation	Amount found (mg)	% deviation	% standard deviation
Formic	4.600	4.634	0.75	0.42	4.596	0.08	0.20
	2.300	2.331	1.35	0.52	2.308	0.33	0.26
Acetic	6.000	6.035	0.58	0.38	6.000	0.00	0.00
	1.500	1.520	1.33	0.61	1.497	0.20	0.20
Propionic	7.400	7.456	0.76	0.52	7.406	0.08	0.20
	3.700	3.749	1.33	0.61	3.695	0.13	0.20
Butyric	8.810	8.876	0.75	0.52	8.803	0.08	0.20
	2.202	2.228	1.18	0.58	2.208	0.27	0.26
Isobutyric	8.810	8.868	0.66	0.52	8.810	0.00	0.00
	2.202	2.232	1.36	0.61	2.198	0.18	0.20
Valeric	10.213	10.298	0.83	0.41	10.221	0.08	0.20
	2.553	2.591	1.49	0.58	2.546	0.27	0.26
Oxalic	6.304	6.367	1.00	0.48	6.304	0.00	0.00
	1.576	1.600	1.52	0.52	1.580	0.25	0.26
Succinic	5.905	5.935	0.51	0.45	5.910	0.08	0.20
	1.476	1.496	1.36	0.61	1.474	0.16	0.20

Note. In case of titration with alkali the phenolphthalein color fades rapidly at the endpoint. The first appearance of pink color was noted.

[a] Calculated from six determinations.

For determining simple acids such as formic, acetic, propionic, etc., the indirect method can be used. Because the reaction is carried out at 100°C, the time required for its completion is about 3–5 min even with 0.025 meq of the sample. The amount of iodate added is much smaller than that required in the direct procedure. But with acids of complex nature, a positive error is observed if the heating period exceeds 5 min and an undue, large excess of iodate is added. In such cases the direct method is to be preferred. This procedure also requires 3–5 min for the completion of the reaction at 30–32°C. However, a very large excess of iodide and iodate in the solid form has to be added.

Both procedures proposed do not involve any costly equipment or any special reagent. The methods are simple, rapid, and accurate and are quite suitable for the quantitative analysis of dilute solutions of the weak acids studied.

TABLE 2
MICRODETERMINATION OF CERTAIN ORGANIC ACIDS BY PROCEDURE 2

	Alkalimetry				Iodometry				
	Amount taken (mg)	Amount[a] found (mg)	% deviation	% st. deviation	KI added (g)	KIO_3 added (mg)	Amount[a] found (mg)	% deviation	% st. deviation
α-Chloropropionic	10.853	10.925	0.66	0.52	0.25	0.25	10.844	0.08	0.20
	2.714	2.755	1.50	0.69	0.25	0.25	2.705	0.33	0.26
α-Hydroxyisobutyric	10.410	10.505	0.91	0.49	0.5	0.25	10.367	0.04	0.20
	2.602	2.637	1.35	0.61	0.5	0.25	2.610	0.31	0.26
Itaconic	6.500	6.538	0.58	0.38	2	2	6.500	0.00	0.00
	1.625	1.655	1.85	0.74	2	2	1.623	0.12	0.20
p-Nitrobenzoic	16.712	16.837	0.75	0.42	0.5	0.5	16.740	0.17	0.20
	4.178	4.248	1.68	0.82	0.5	0.5	4.164	0.33	0.26
1,5-Dinitrobenzoic	21.212	21.353	0.66	0.42	0.25	0.25	21.194	0.08	0.20
	5.303	5.400	1.83	0.74	0.25	0.25	5.320	0.32	0.26
Benzilic	22.900	23.072	0.75	0.42	0.5	0.5	22.862	0.17	0.26
	5.725	5.840	2.00	0.72	0.5	0.5	5.744	0.33	0.26
Galacturonic	19.400	19.562	0.83	0.52	0.5	0.25	19.384	0.08	0.20
	4.850	4.930	1.65	0.82	0.5	0.25	4.858	0.16	0.20
Acetylsalicyclic[b]	18.015	18.285	1.50	0.45	—	—	18.00	0.08	0.20
	4.504	4.594	2.00	0.63	—	—	4.519	0.33	0.26

Note. In case of titration with alkali the phenolphthalein color fades rapidly at the endpoint. The first appearance of pink color was noted.

[a] Calculated from six determinations.

[b] 10.0 ml, 0.01 *N* KIO_3 and 10 ml, 5% KI solutions were added.

SUMMARY

Two iodometric procedures have been described for the microdetermination of certain organic acids which can be adopted for evaluating 0.03–0.1 meq of these acids. These methods consist of treating the acid sample with an excess of neutral potassium iodide and iodate. In the indirect method the iodate used up is measured whereas the direct procedure is based on the titration of the liberated iodine. The latter procedure has been applied to determine acids in a water–alcohol medium also. The effect of various factors influencing the stoichiometry of the reactions involved has also been studied.

ACKNOWLEDGMENTS

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Atomic Absorption Spectrometry of Cadmium after Solvent Extraction with Zinc Dibenzyldithiocarbamate

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INTRODUCTION

The efficiency of solvent extraction techniques as a means of sensitivity enhancement in atomic absorption spectrometry (AAS) is well documented (*1*). This increase in sensitivity is caused by two factors: a preconcentration achieved by a favorable phase–volume ratio and the effect of organic solvent on the atomic absorption signal. A better nebulization efficiency and aspiration rate seem to govern the mechanism producing this enhancement (*2*, *6*). Solvent extraction–atomic absorption procedures have been applied to the determination of cadmium. Extractions with dithizone in a variety of organic solvents (*7*) and ammonium pyrrolidindithiocarbamate (APDC)–diethylammonium diethyldithiocarbamate (DDDC) mixtures in methyl isobutyl ketone (MIBK) (*4*), have been extensively investigated. The present work concerns the extraction of cadmium with zinc dibenzyldithiocarbamate (ZnDBC) in MIBK and subsequent atomic absorption determination using an air–acetylene flame. It is well known that dibenzyldithiocarbamic acid forms extractable chelates with some metal ions (*5*). However, only for copper has the AAS determination as dibenzyldithiocarbamate been reported (*3*). A critical study of the solvent extraction–AAS procedure was made and the utility of various solvents for the CuDBC extraction was examined (*2*).

EXPERIMENTAL

Apparatus. Atomic absorption measurements were made on a Perkin–Elmer Mod. 372A atomic absorption spectrophotometer equipped with a cadmium hollow-cathode lamp (228.8 nm). Premixed air–acetylene flame was used with a standard single-slot burner. Conventional working conditions as given in the Perkin–Elmer instructions manual were followed.

Reagents. All reagents were analytical grade. A cadium stock solution (1000 ppm) was prepared by dissolving the appropriate amount of cad-

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mium sulfate in water. ZnDBC solution (0.05% w/v) was prepared by dissolving ZnDBC (Merck) in MIBK by shaking for 20 min. The solution was stored in the dark. Acetate buffer solution (pH 5.0) was prepared by mixing 0.1 *M* acetic acid and 0.2 *M* sodium acetate in suitable ratio.

General extraction procedure. An aliquot of cadmium solution was transferred into a 50-ml flask. Two milliliters of the acetate buffer was added to adjust the pH of the final solution to 5 ± 0.5. The solution was diluted to the mark, then was transferred into a 100-ml separatory funnel. Ten milliliters of the ZnDBC–MIBK solution was added, the mixture was shaken for 2 min, then allowed to sit for 10 min. After physically separating the two layers, the organic extract was centrifuged for 5 min at 5000 rpm. The absorbance of cadmium in the extract was measured against a reference consisting of the solvent saturated with water.

RESULTS AND DISCUSSION

Effect of shaking time. The effect of shaking time on the extraction of the CdDBC complex from aqueous solution at pH 5.0 into MIBK was studied. No significant change in the absorbance of the organic phase was observed at shaking time from 2 to 6 min. Therefore a shaking time of 2 min was adopted in further experiments.

Effect of acidity. To find the optimum conditions for extraction of cadmium the percent extraction vs. pH was determined. The general extraction procedure was followed and the aqueous layer was analyzed for cadmium content by AAS. As shown in Fig. 1, MIBK gave complete extraction of cadmium dibenzyldithiocarbamate over the pH range 4.0–7.5. For practical reasons pH 5 was used in further extractions.

Stability. Since it is necessary that the extracted metal complex remain stable in the solvent as long as it takes to carry out a number of extractions and AAS measurements, stability tests were performed. The absorbance of the organic phase was checked at different times after

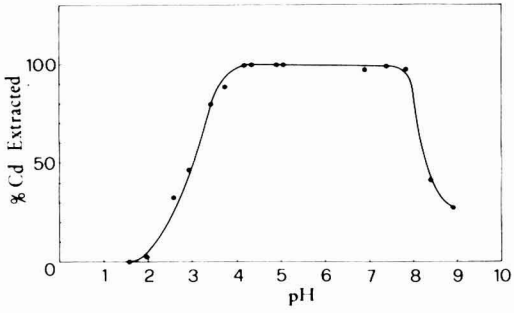

FIG. 1. Effect of pH on extraction of cadmium dibenzyldithiocarbamate with MIBK.

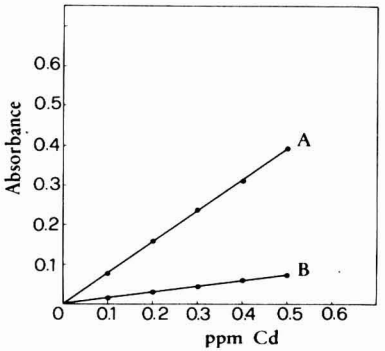

FIG. 2. Effect of solvent on atomic absorption of cadmium. A—MIBK. B—H_2SO_4, 0.05 *N*.

extraction. No significant difference in the absorbance occurred up to 12 hr.

Sensitivity. A linear calibration curve in the range 0.1–0.5 ppm Cd was obtained (Fig. 2). The sensitivity (1% absorption) was found to be 6 ppM. The enhancement expressed by the ratio of the cadmium absorbance in MIBK and water was found to be 5.23.

Precision. There were ten replicate extractions and AAS determinations of cadmium at the 40-ppM level in the original aqueous phase. The precision expressed as coefficient of variation was 1.5%.

Interferences. The effect of a number of different cations and anions on the AAS determination of CdDBC in MIBK was examined. A 50-fold excess of Cu(II), Pb(II), Fe(III), Zn(II), Co(II), Mn(II), Ni(II), Cr(III), V(V), and Ca(II) was added to aqueous solutions containing 40 ppM Cd. Each ion was tested individually. In all cases no significant interference appeared. The same result was obtained by adding phosphate, sulfate, nitrate, chloride, jodide, bromide, and citrate at the 0.01 *M* concentration. Higher concentrations of cations able to form dibenzyldithiocarbamate may eventually interfere because of their consumption of chelating agent.

Conclusion. The cadmium extraction with ZnDBC in MIBK is suitable for a subsequent AAS determination. The procedure is simple and fast. Sensitivity and precision are good enough to permit the determination at the trace level.

SUMMARY

The AAS determination of cadmium extracted as dibenzyldithiocarbamate in methyl isobutyl ketone was investigated. Cadmium was quantitatively removed from the aqueous phase at pH 5 ± 0.5 with a single extraction by shaking for 2 min. The sensitivity of the method was found to be 6 ppm (1% absorption). The precision expressed as coefficient of variation was 1.5% at the 40-ppM level. Interference from foreign cations and anions was examined.

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Recovery and Fluorometric Measurement of a Polynuclear Aromatic Hydrocarbon from Smoked Cigarettes

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INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are well-known chemical carcinogens. It is evident now that they exist in the atmosphere (*9*). Since the discovery that these compounds exist in the environment as pollutants, their study has attracted many researchers (*23*).

Many methods have been developed to separate, identify, and quantitate PAHs. High-performance liquid chromatography (HPLC) with fluorescence means of detection was used to determine them in automobile exhaust and oils (*15*), water pollution samples (*7*), and atmospheric particulate matter (*5*). Variable wavelength uv detection (*8*) and simultaneous monitoring of absorbance and fluorescence (*13*) have also been used to determine polynuclear aromatic hydrocarbons and their metabolic products eluted form an HPLC. PAH identification from different sources was demonstrated by low-temperature fluorescence spectrophotometry (Shpol'skii effect) as a method of HPLC detection (*4*).

Solvent extraction, thin-layer chromatography, and spectrofluorimetry were used to determine PAHs in marine sediments (*12*). Benzo(*a*)pyrene has been determined in air using thin-layer chromatography with a fluorescence spectrophotometric thin-layer plate scanning device (*22*). Benzo(*a*)pyrene and its synthesized derivatives were determined by gas chromatography–mass spectrometry (GC–MS) after trimethysilinization of the derivatives (*21*).

Gas chromatography has been used to determine PAH compounds in particulate matter (*2*, *3*, *11*, *16*, *21*) and in lake sediments (*6*).

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Snook *et al.* used GC, HPLC, and GC–MS to identify high molecular weight polynuclear aromatic hydrocarbons and multi-alkylated PAH in tobacco smoke (*17–20*). A large variety of these compounds were identified and many of them were quantitated. Lee and co-workers used GC–MS and NMR spectroscopy to identify and quantitate PAHs in tobacco and marijuana smoke (*10*). About 150 compounds were quantitated. Both studies used large sample size. There were 90 to 270 cigarettes used in the first study and 1000 cigarettes used in the second. The level of each polynuclear aromatic hydrocarbon is too low to be followed in biological systems. The level of perylene, for example, was found to be 0–3 μg/100 cigarettes (*17*) and that for benzopyrene (both isomers) was 3 μg/100 cigarettes (*10*).

The effect of filters on the levels of selected PAHs was reported by Severson *et al.* (*17*). Tritium-labeled benzo(*a*)pyrene was used to study the formation of water soluble metabolic products in hamster embryo cell cultures (*1*, *14*).

In order to follow the metabolism of a certain PAH in tobacco smoke, a considerable quantity of this compound must be present. In this study the retention of one polynuclear aromatic hydrocarbon (perylene) in the smoke from a single cigarette spiked with that particular hydrocarbon is demonstrated. The ability of the cigarette filter to retain varying amounts of the hydrocarbon was also studied.

EXPERIMENTAL

Reagents

The following reagents were used: cyclohexane and methanol (distilled in glass) from Burdick and Jackson Laboratories, Muskegon, Mich.; benzene (spectro ACS) from Eastman–Kodak Company, Rochester, New York; diethyl ether (anhydrous) from Scientific Products, McGaw Park, Illinois; *n*-octane from Aldrich Chemical Company, Milwaukee, Wisconsin; perylene standard solution (100 ppm perylene in xylene) from Chem. Service, Westchester, Pennsylvania; NaOH solution, pH 13; HCl solution, pH 0.8.

Apparatus

A Perkin–Elmer 650-10S fluorescence spectrophotometer (P-E 150 xenon power supply) interfaced with an X-Y recorder was used to record fluorescence spectra.

A smoking machine was constructed as follows. Two test tubes (16 × 125 mm), each with a side arm, were connected in series. (The side arm of the first was connected to the mouth of the second via a Tygon tube and a glass tube penetrating a rubber stopper in the mouth of the second tube.)

The side arm of the second tube was connected to a water aspirator for suction. A glass tube (eyedropper tube) with a rubber adaptor (to fit the cigarette) was inserted into the mouth of the first tube through a rubber stopper. Both test tubes acted as traps to the smokes and were cooled in a dry ice/acetone bath.

Procedure

Cigarette smoke condensate (CSC). Six commercial-type cigarettes (Marlboro 100's) with filters were spiked (in the tobacco portion) with 2, 4, 6, 8, 10, and 20 μg perylene, respectively (equivalent to a total of 20, 40, 60, 80, 100, and 200 μl of the above perylene solution). Spiking was accomplished by inserting a Hamilton syringe needle lengthwise in the cigarette and delivering five equal portions at 1-cm intervals (starting at 1 cm from the top of the cigarette) for 2 to 8 μg and ten equal portions at 0.5-cm intervals for 10 or 20 μg. The cigarette was 6.5 cm long, exclusive of the filter. After allowing to dry for 3 hr, these cigarettes were smoked separately on the smoking machine (a puff every 30 sec for a duration of 5 sec by controlling the aspirator). The filters were saved, and the smoke condensates were dissolved in 25 ml of the solvent mixture of benzene:methanol:diethyl ether (2:1:2), followed by a cleanup procedure summarized in Fig. 1.

Control sample. An unspiked cigarette of the same type, smoked in the same way and treated the same as the spiked cigarette, was used as a control sample. The filter was also saved.

Filter extracts. Each filter of the smoked cigarettes (spiked and unspiked) was cut into small pieces and was soaked overnight in 20 ml of the same solvent mixture used to dissolve the smoke condensate. Each extract was transferred to a container and the residue of the filter was washed with 2 × 10 ml of the solvent mixture. The combined solvent mixture (40 ml) of each filter was transferred to a separatory funnel and the cleanup procedure shown in Fig. 1 was followed.

Analytical recovery from the CSC. Ten micrograms of perylene (100 μl perylene solution) were added to the first tube of the smoking machine. An unspiked cigarette was smoked. The CSC (from both tubes) was dissolved in 45 ml of the solvent mixture and treated as in Fig. 1.

Analytical recovery from the filters. Five micrograms of perylene (50 μl perylene solution) were added to the filters of smoked (unspiked) cigarettes and left overnight. The filters were then treated similarly to the sample filters as above.

Fluorescence analysis. Standard perylene spectra were recorded for perylene in *n*-octane using an excitation wavelength of 412 nm and slit widths of 2 nm for both excitation and emission. The spectrum was scanned between 420 and 520 nm. Sample and control spectra were ob-

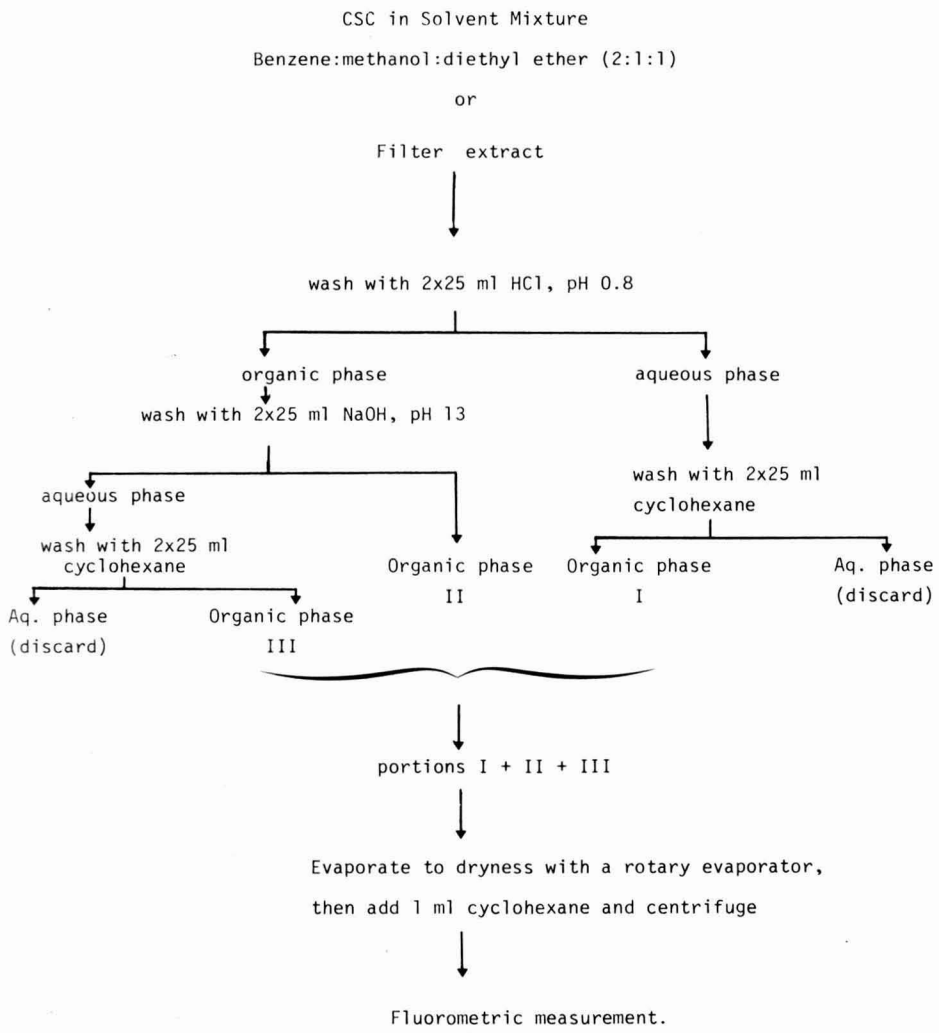

FIG. 1. Flow chart of the sample cleanup procedure prior to fluorescence analysis.

tained by diluting 10 μl of each prepared sample solution to 2 ml using *n*-octane in a fluorescence cell. The spectra were recorded using the same conditions as for the standard spectra.

Shpol'skii spectra. Liquid nitrogen temperature spectra were recorded for a standard perylene solution in *n*-octane (330 ng/ml) and for the control sample and one CSC sample (from the cigarette spiked with 10 μg perylene). Five microliters of the control and the CSC solutions were diluted each to 1 ml using *n*-octane, then the spectra were recorded.

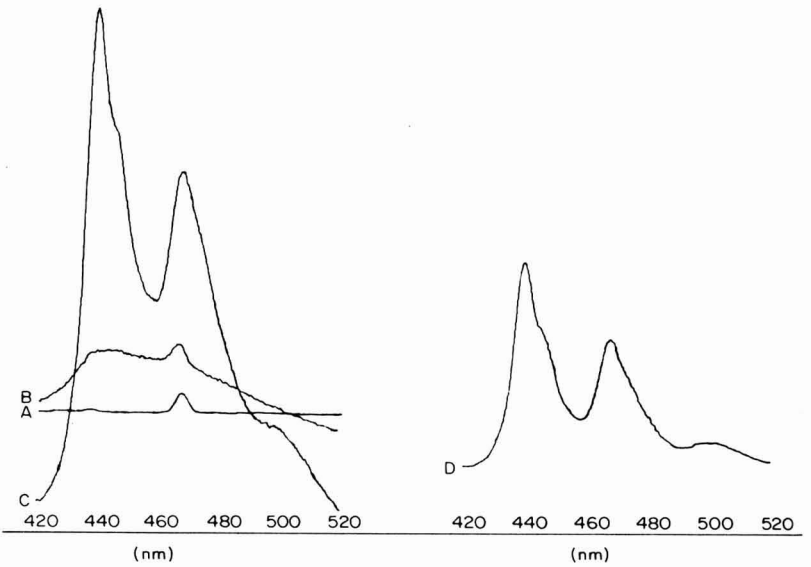

FIG. 2. Fluorescence spectra of (A) the solvent (*n*-octane), (B) the control sample, (C) the sample derived from the cigarette which was spiked with 6 μg perylene, and (D) perylene standard solution, 2.5 ng/ml. All samples in *n*-octane. The spectra are offset from one another at the start of the scan.

RESULTS

Spectra. All the spectra of the spiked samples (CSC and filters) show the characteristic peaks of perylene (at 440, 468, and 500 nm and the shoulder at 445 nm). Figure 2 shows the spectra of (A) the solvent, (B) the control sample, (C) the CSC sample spiked with 6 μg perylene, and (D) a standard perylene solution in *n*-octane (2.5 ng/ml). The solvent (*n*-octane) has a small peak at 468 nm which coincides with the perylene peak and is ascribed to the Raman peak of the solvent. The CSC background, which is represented by the spectrum of the control sample, is relatively small, but it is large if the acid–base wash is eliminated.

Shpol'skii spectra. Figure 3 shows the Shpol'skii spectra for (a) a standard perylene solution, (b) a CSC sample of the cigarette spiked with 10 μg perylene, and (c) a control sample.

It is clear that spectra (a) and (b) are identical in the position of the peaks (446, 452, 460, 474, 478, 487, 505, 513, and 522 nm). These high-resolution spectra are extremely valuable when high background, due to other fluorescent species in the sample, is a problem.

Analytical recovery. Table 1 shows the analytical recovery of perylene from CSC. The average recovery was 68.4% of the added amount. Table 2 shows the analytical recovery of the perylene from the filters of smoked

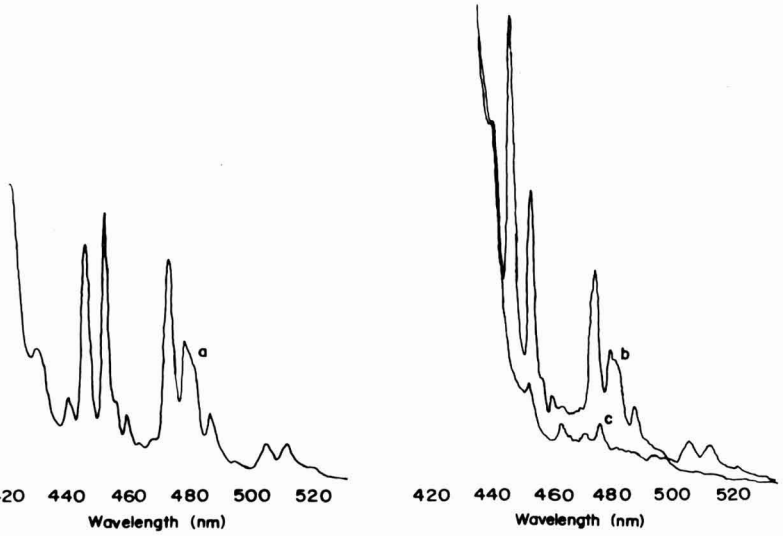

FIG. 3. Shpol'skii spectra for (a) the standard perylene solution in *n*-octane (330 ng/ml), (b) the sample derived from the cigarette which was spiked with 10 μg/perylene, and (c) the control sample derived from the unspiked cigarette.

cigarettes. An average of 81.4% of the added amount was recovered. Precisions were very good. The lower apparent recovery from the CSC sample may be due to fluorescence quenching by compounds present in the CSC.

Recovery from CSC. Curve A in Fig. 4 shows that the fraction of perylene recovered from the spiked cigarettes (CSC plus filter) ranges between 72 and 34%. The range found in the CSC is between 3 and 24% of the added amount, while 69 to 17% was retained in the filters. These values are corrected for the analytical recovery.

The percentage recovered from the CSC increases with the amount added to a certain point then tends to be constant (curve C). At the same time, the percentage retained in the filters as well as the total percentage recovered is small at the beginning, then levels off at the higher concen-

TABLE 1
ANALYTICAL RECOVERY OF PERYLENE FROM CSC

Amount added (μg)	Amount recovered (μg)	Percentage recovered
10.0	6.57	65.7
10.0	6.70	67.0
10.0	7.24	72.4
	Ave.	68.4

Note. cv = 5.2%.

TABLE 2
RECOVERY OF PERYLENE FROM THE FILTERS

Amount added (μg)	Amount recovered (μg)	Percentage recovered
5	4.00	80.0
5	4.07	81.4
5	4.14	82.8
	Ave.	81.4

Note. cv = 1.7%.

trations, almost at the same point as the CSC (curves A and B). The sample corresponding to the cigarette which was spiked with 6 μg perylene was found to have an exceptionally high percentage of perylene in the CSC, while the filter of the same cigarette retained a low percentage.

Precision. The analytical precision, including extraction and the cleanup procedure, was excellent, as shown in Tables 1 and 2. Figure 5 illustrates the degree of reproducibility in the amount of the perylene retained by the filters of the spiked cigarettes. The coefficient of variation

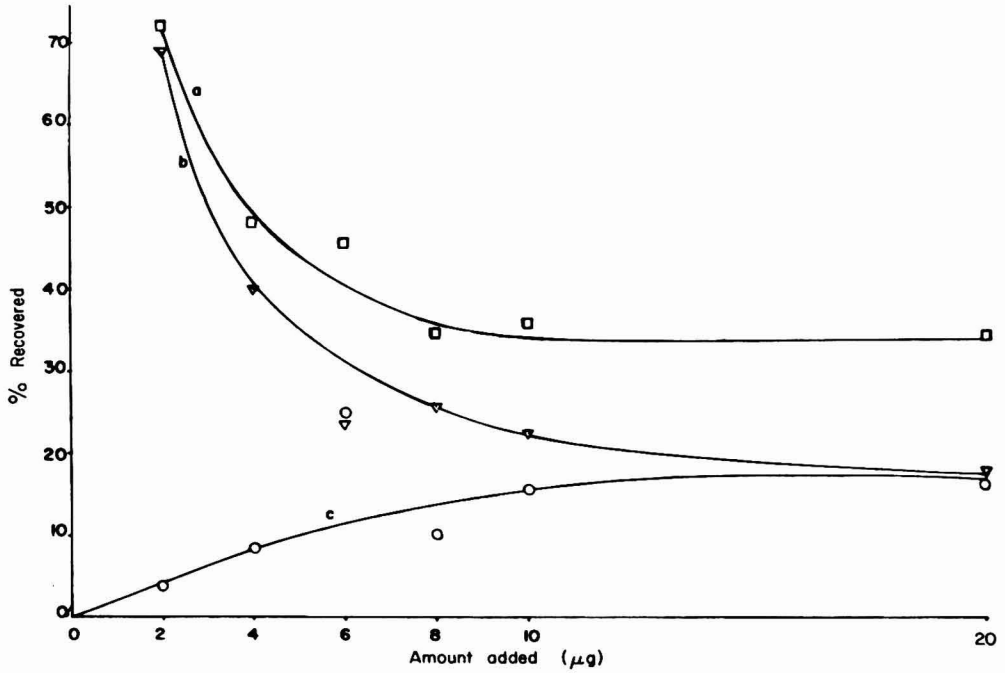

FIG. 4. The percentage of perylene recovered as a function of the amount added to the cigarettes. (a) Total recovery, (b) recovery from the filters, (c) recovery from the CSC.

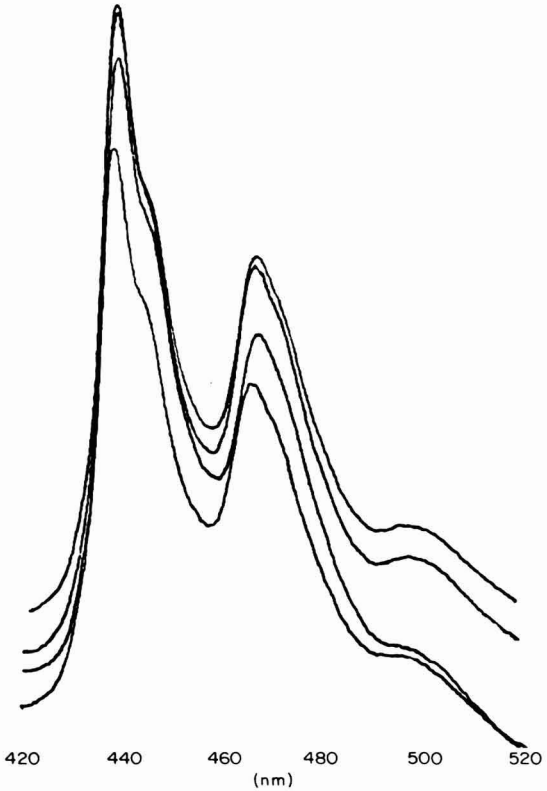

FIG. 5. Spectra of four samples derived from the filters of four cigarettes which were spiked with 20 μg perylene each. The spectra are offset from one another at the start of the scan.

was 6% (n = 4). The coefficient of variation for the amount found in the CSC was 36% (n = 3). The higher variation in the CSC is due largely to the pyrolysis and combustion processes that take place during the smoking process, as well as the escape of some smoke in the trap.

DISCUSSION

The results demonstrate that cigarettes can be spiked with relatively large amounts of a polynuclear aromatic hydrocarbon and sufficient quantities are retained in the cigarette smoke following pyrolysis (smoking) to be useful for following the fate of the compound in biological systems (e.g., in rats taught to smoke). We have not searched for pyrolysis products at this stage, but it is likely that with adequate spiking, these will be produced in sufficient quantities to detect and monitor their biological fate.

We have also demonstrated that cigarette filters retain a significant fraction of the polynuclear aromatic hydrocarbon, probably on particu-

lates, depending on the amount present. The efficiency appears to increase with decreasing amounts of PAH.

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MICROCHEMICAL JOURNAL 26, 354–359 (1981)

Studies on the Kinetics of the Ligand-Substitution Reaction of the Zinc(II)-4-(4′-Methyl-2′-Thiazolylazo)-2-Methylresorcinol with 1,2-Diaminocyclohexane-N,N,N′,N′-Tetraacetic Acid

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INTRODUCTION

As a part of our studies on a group of organic compounds derived from thiazolyl-azo-phenols and thiazolyl-azo-diamines, as new spectrophotometric reagents as well as metallochromic indicators for metal ions, we are interested in the kinetics of the substitution reaction of metal chelates with multidentate ligands, which is often involved in the complexometric titration of metal ions.

Some studies of the kinetics of ligand-substitution reactions of metal-indicator chelates with EDTA and EGTA have been reported (*1*, *2*, *6*, *7*, *9*), and Nakagawa and co-workers (*3–5*, *8*) have studied the influence of auxiliary complex-forming agents on the rate of metallochromic indicator color changes.

In the work described in this paper the rate of the substitution of Zn(II)–MTAMR complex with DCTA is studied.

EXPERIMENTAL

Apparatus. The apparatus used included a Beckman 25 recording spectrophotometer with thermostated cell compartment, and 1-cm path length silica cells; a Radiometer PHM51 pH meter with glass and saturated calomel electrodes; and a Colora Messtechnik GMBH thermoelectric circulating bath.

Reagents. Analytical reagent grade chemicals were used throughout, without further purification.

4-(4′-methyl-2′-thiazolylazo)-2-methyl-resorcinol: 10^{-3} *M* in absolute methanol.

Disodium dihydrogen 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetate: 10^{-1} *M*. Standardized with zinc oxide and eriochrome black T.

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Zinc perchlorate: 10^{-1} *M*, prepared from zinc oxide and perchloric acid, and standardized complexometrically.

Procedure for measuring the substitution reaction rate. The Zn(II)–MTAMR complex solutions (50% v/v methanol–water), with different MTAMR excess were buffered with the suitable borax–HCl buffer, and the ionic strength adjusted to 0.25 ($NaClO_4$). This solution and the DCTA solution were brought to equilibrium in the bath kept at 25 ± 0.1°C. The substitution reaction was started by adding the DCTA solution to the complex solution in the silica cell. Absorbance variation at 550 nm were automatically recorded as a function of the reaction time.

RESULTS AND DISCUSSION

As previously reported Zn(II) and MTAMR form complexes $ZnHR^+$ and ZnR in excess of metal ion, and $Zn(HR)_2$ and ZnR_2^{2-} in excess reagent (*8*), whose formation constants and spectrophotometric characteristics are given in Table I.

Under the present experimental conditions, equilibrium

$$ZnR_2 + Y \rightleftharpoons ZnY + 2R$$

(charges are omitted for sake of simplicity) is much favored to the right; the substitution reaction of the Zn(II)–MTAMR complex with DCTA thus goes to completion, and the reverse reaction can be neglected in the kinetic study.

The order of the reaction with respect to ZnR_2 concentration was obtained by plotting $\log (A_0 - A_\infty)/(A_t - A_\infty)$ vs. t, where t is the time elapsed from the beginning of the reaction, and A_0, A_t and A_∞ are the absorbances of the reaction system at $t = 0$, t, and ∞, respectively. If the reaction is first order, the plot must result in straight lines whose slopes are the conditional rate consant involving the concentrations of DCTA, MTAMR, and hydrogen ion, for every temperature. Plots in Fig. 1, show that reaction is first order with respect to the ZnR_2 concentration and that the following rate equation holds:

$$v = -\frac{d[ZnR_2]}{dt} = K_{(R,H,Y)}[ZnR_2].$$

The influence of the temperature was studied by applying the Arrhenius equation for the kinetic coefficient,

$$K_{(R,H,Y)} = K_{o(R,H,Y)} \exp(-E_a/RT).$$

the plot of $\ln K_{(R,H,Y)}$ vs. T^{-1} (°K) gave a straight line $Y = 22.295 - 7202.82X$ (cc = 0.997), whose slope gives the activation energy $E_a = 14.30$

TABLE 1
VALUES OF THE DISSOCIATION CONSTANTS OF MTAMR AND OF FORMATION CONSTANTS FOR THE Zn–MTAMR COMPLEXES

Reagent species	pK_a	Complex species	log β
H_3R^+	1.21	$ZnHR^+$	13.73
H_2R	6.49	ZnR	13.95
HR^-	11.07	$Zn(HR)_2$	19.30
R^{2-}		ZnR_2^{2-}	18.45

kcal·mol^{-1}, and the frequency factor, $K_{0(R,H,Y)} = 8.016 \times 10^7$ sec^{-1}, is evaluated from the intercept on the Y-axes.

The conditional rate constant, $K_{(R,H,Y)}$, was also determined at various concentrations of MTAMR, at constant concentrations of DCTA and hydrogen ion. Values are plotted in Fig. 2-2, and are related by the equation $Y = 0.1458 \times 10^{-4} + 6.568 \times 10^{-8}X$ (cc = 0.9928) showing clearly the linear relationship between $K_{(R,H,Y)}$ and 1/[HR$^-$], where HR$^-$ is the predominant species of MTAMR in the pH range 8.40 to 9.40, and from whose slope $K_{(H,Y)R} = 6.568 \times 10^{-8}$ liter·mol^{-1}·sec^{-1} can be evaluated. Thus, the rate law can be expressed as

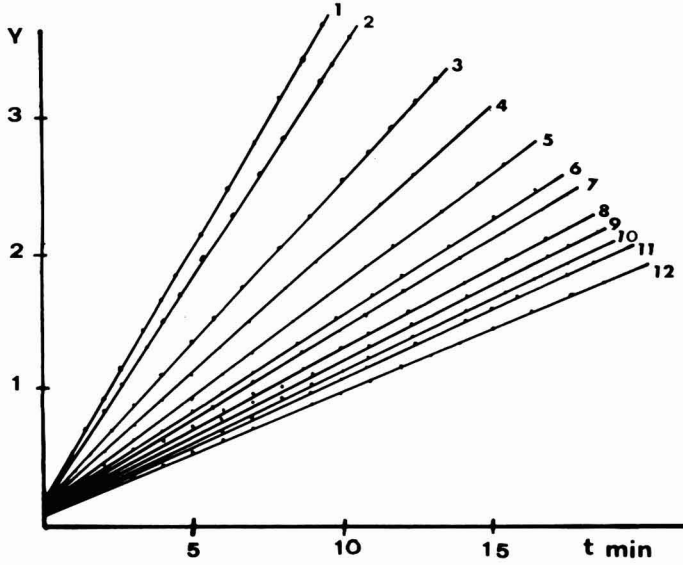

FIG. 1. Plots of reaction rate as a function of time, for different pH values. $C_R = 2.5 \times 10^5$ M; $C_{Zn} = 8.34 \times 10^{-6}$ M; $C_{DCTA} = 1.67 \times 10^{-4}$ M pH = (1) 8.49; (2) 8.53; (3) 8.71; (4) 8.76; (5) 8.85; (6) 8.95; (7) 9.01; (8) 9.05; (10) 9.12; (11) 9.17; and (12) 9.32.

$$-\frac{d[ZnR_2]}{dt} = K_{(H,Y)R}(1/[HR^-])$$

and the rate equation will be

$$-\frac{d[ZnR_2]}{dt} = K_{(H,Y)R}([ZnR_2]/[HR^-]). \quad (1)$$

To study the influence of the pH, the conditional rate constant, $K_{(R,H,Y)}$, was determined at various pH values, at constant concentrations of DCTA and MTAMR. Thus Eq. (1) can be written as

$$K_{(H,Y)R} = K_{(R,H,Y)}[HR^-].$$

Values in Fig. 2-1 comform to a straight line, $Y = 13.68X$ (cc = 0.9984), whose slope gives $K_{(Y)} = 13.68\ sec^{-1}$, and the rate equation will be

$$-\frac{d[ZnR_2]}{dt} = K_{(Y)}([ZnR_2]\ [H^+]/[HR^-]).$$

The reaction rate also increases with increasing DCTA concentration, at constant concentration of MTAMR and hydrogen ion. A procedure similar to those previously described allows the calculation of $K_{(R,H)Y}$, Table II. Since

$$K_{(H,Y)R} = K_{(Y)}[H^+]$$

in Eq. (1) it follows that

$$K_{(R,H,Y)} = K_{(Y)}([H^+]/[HR^-])$$

and then

$$K_{(Y)} = K_{(R,H,Y)}([HR^-]/[H^+]). \quad (2)$$

The values of $K_{(Y)}$ obtained from Eq. (2) are plotted vs. $[Y']$, i.e., the total concentration of DCTA not bound to any metal ion, Fig. 2-3. From the slope of the resulting straight line, $Y = 0.90 + 6.88 \times 10^4 X$ (cc = 0.9998),

TABLE 2

CONDITIONAL RATE CONSTANT, $K_{(R,H,Y)}$, $C_{Zn} = 8.33 \times 10^{-6}\ M$, $\mu = 0.25$, $T = 25°C$

$C_Y \times 10^5$	pH	$K_{(R,H,Y)} \times 10^2\ sec^{-1}$
4.167	8.85	6.72
8.334	8.85	9.72
16.667	8.84	17.69
25.000	8.85	22.49
33.334	8.83	31.98

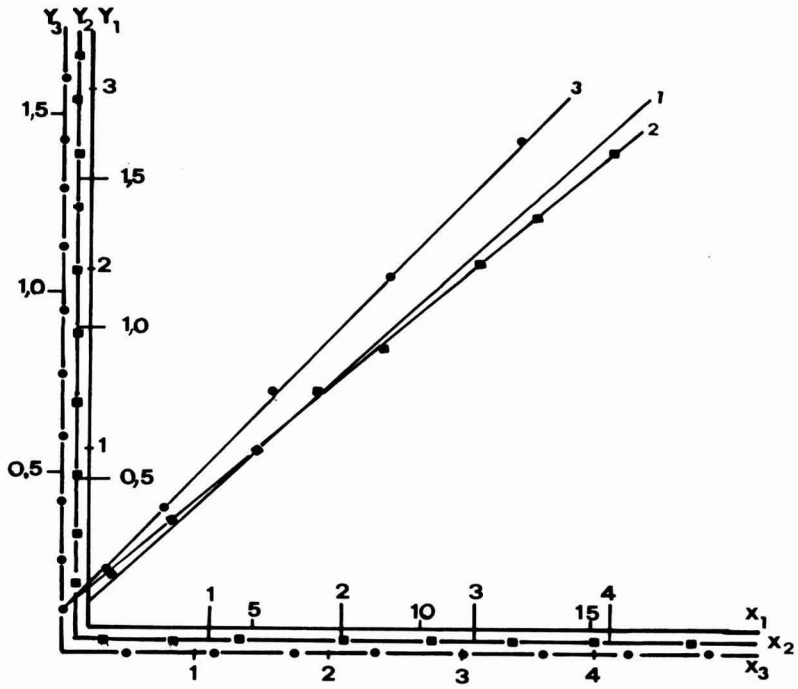

FIG. 2. Plots of the rate constants (1) $Y_1 = K_{(H,Y)R} \times 10^8$, $X_1 = [H^+] \times 10^{10}$; (2) $Y_2 = K_{(R,H,Y)} \times 10^{-1}$, $X_2 = (1/[HR^-]) \times 10^{-4}$; (3) $Y_3 = K_{(Y)} \times 10^{-3}$, $X_3 = [Y'] \times 10^4$.

$K = 6.88 \times 10^4$ liter·mol^{-1}·sec^{-1} was calculated. Thus the rate law for the substitution reaction can be expressed as

$$-\frac{d[ZnR_2^{2-}]}{dt} = K\frac{[ZnR_2^{2-}][H^+][Y']}{[HR^-]}. \qquad (3)$$

To check the validity of Eq. 3, the reaction rates calculated according to Eq. 3 were plotted vs. the reaction rates experimentally determined. The resulting plot conforms to the straight line $Y = 6.18 \times 10^{-9} + 0.9739X$ (cc = 0.9937), which shows the consistence of the proposed rate law. This rate law is in accordance with equations proposed by several authors (*3–5, 7, 8*) for similar displacement reactions.

SUMMARY

Spectrophotometric studies have been carried out on the kinetics of the ligand-substitution reaction of the Zinc(II)-4-(4′-methyl-2′-thiazolylazo)-2-methylresorcinol complex with 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid, in the pH range 8.4 – 9.4 at $\mu = 0.25$ and 25°C. The reaction rate constant was established to be $-(d[ZnR_2]/dt) = K[ZnR_2][H^+][Y']/[HR^-]$, and $K = 6.88 \times 10^4$ liter·mol^{-1}·sec^{-1} was obtained.

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Spectrophotometric Determination of Osmium (VIII) with Promazine Hydrochloride

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INTRODUCTION

Spectrophotometric methods are especially useful for the determination of osmium because the metal is present as a minor constituent in most platinum metal occurrences. Several organic reagents (*1*, *2*) containing the

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group, e.g., 2,4-diphenylthiosemicarbazide (*5*) and *o*-hydroxythiobenzhydrazide (*4*) have been proposed for the spectrophotometry of osmium.

In the present paper promazine hydrochloride (PH) is recommended as a suitable reagent for the spectrophotometric determination of microgram amounts of osmium. It forms the orange oxidation product with osmium (VIII) in acidic media. The optimal conditions for the formation of orange oxidation product of promazine hydrochloride with osmium tetroxide were examined.

Promazine hydrochloride was earlier proposed for the spectrophotometric determination of palladium (*8*) and as a redox indicator in vanadometry (*3*).

EXPERIMENTAL

Reagents. A standard solution of osmium (VIII) was prepared from osmium tetroxide (Feinbiochemica, Heidelberg) in 0.2 *M* sodium hydroxide. The solution was standardized iodometrically (*2*).

Promazine hydrochloride (10-[3-dimethylaminopropyl]-phenothiazine hydrochloride, EGYT-Budapest) was used as aqueous 0.01 *M* solution and stored in an amber bottle in a refrigerator. Standardized gravimetrically with silicotungstic acid (*6*).

All chemicals used were of analytical purity.

Apparatus. Specord UV/VIS and Spekol (Carl Zeiss, Jena, E.Germany) spectrophotometers were used with 1-cm cells.

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RESULTS AND DISCUSSION

We have found that promazine hydrochloride is oxidized reversibly by osmium tetroxide and a number of oxidants (e.g., Ce (IV), VO_3^-, $Cr_2O_7^{2-}$, BrO_3^-, JO_3^-, JO_4^-, NO_2^-, etc.) with the formation of colored oxidation product. Then the oxidation product (free radical) is further oxidized irreversibly by strong oxidizing agents to a colorless sulfoxide (*7*). The reaction may be represented as follows:

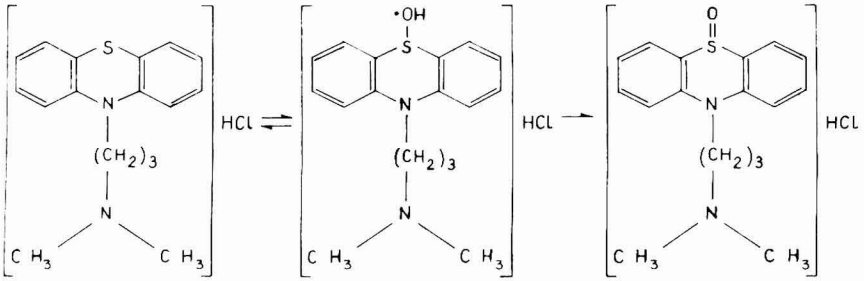

Figure 1 shows the ultraviolet spectra of the nonoxidized and oxidized forms of promazine hydrochloride. The spectrum of the free radical shows a bathochromic shift, whereas the sulfoxide shows a hypsochromic shift with respect to the nonoxidized form of PH in the range 220–280 nm. Figure 2 gives the spectrum of the colored oxidation product of PH in the visible range. Table 1 summarizes the results.

TABLE 1
VALUES OF λ_{max} AND ϵ OF NONOXIDIZED AND OXIDIZED FORMS OF PH

Form of PH	λ_{max} (nm)	ϵ ($l \cdot mole^{-1} \cdot cm^{-1}$)
Nonoxidized	206	2.2×10^4
	252	3.0×10^4
Free radical	224	2.4×10^4
	272	4.5×10^4
	512	9.2×10^3
Sulfoxide	233	2.5×10^4
	272	1.2×10^4
	304	7.4×10^3
	340	5.5×10^3

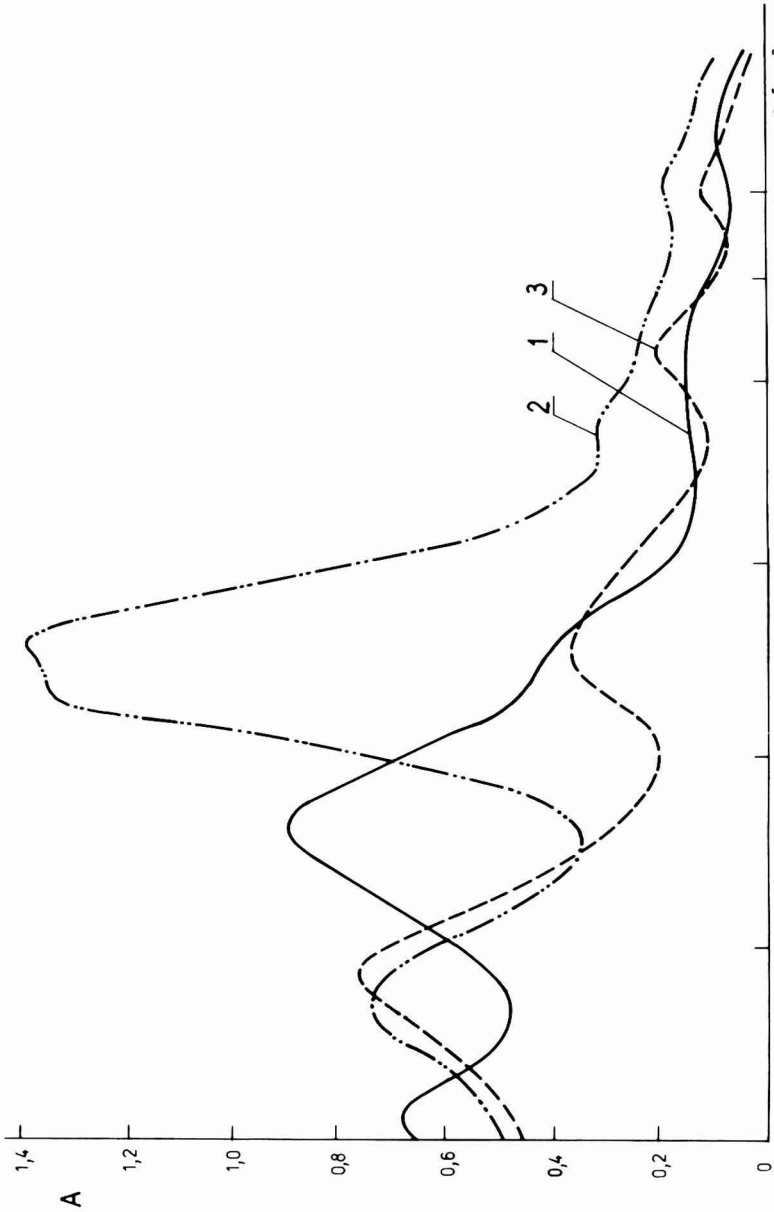

FIG. 1. Ultraviolet-region absorption spectra of aqueous solutions of promazine hydrochloride: 1—Nonoxidized form; 2—Free radical; 3—Sulfoxide. $C_{PM} = 3 \cdot 10^{-5}$ *M*; $C_{HCl} = 3$ *M*; Os:PM = 1:1.

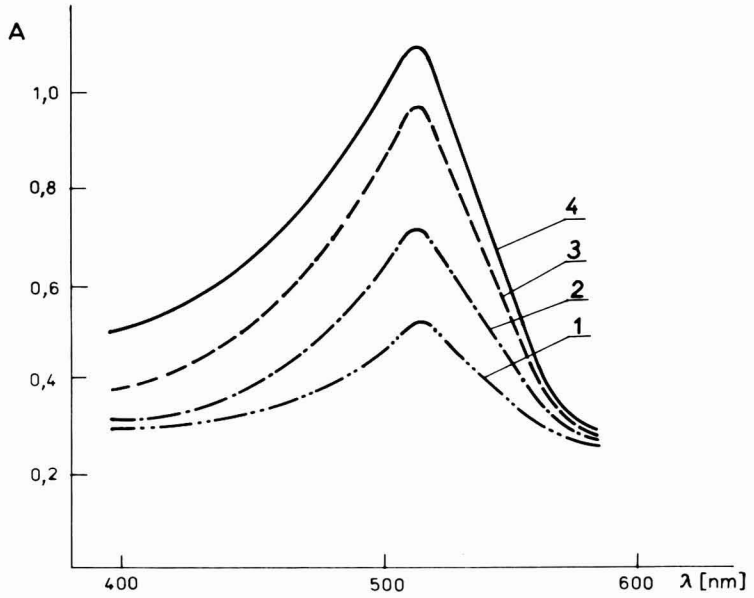

FIG. 2. Visible-region absorption spectra of aqueous solutions of free radical of promazine hydrochloride obtained at various molar ratios of Os:PM. Curves: 1—1:1; 2—1:2; 3—1:3; 4—1:4. $C_{Os} = 3 \cdot 10^{-5}$ *M*; $C_{HCl} = 3$ *M*.

The absorption spectra of the orange oxidation product obtained in various concentrations of sulfuric, hydrochloric, and phosphoric acid were recorded and show that the absorbance is maximal for 2.5–4.0 *N* hydrochloric acid. Nitric acid cannot be used because above a concentration 0.1 *N* it oxidizes promazine hydrochloride to form colored products.

The absorbance in 3 *N* hydrochloric acid medium is maximal when the molar ratio Os:PM is 1:4.

The determination of osmium (VIII). Transfer the sample solution containing 25–250 μg of osmium (VIII), 15 ml of 10 *N* hydrochloric acid and 3 ml of 0.2% PH solution to a 25-ml volumetric flask and dilute to the mark with doubly distilled water. The solution was mixed well and the absorbance was measured at 512 nm after standing 12 min against a reagent blank prepared in the same way.

Beer's law is obeyed over the osmium concentration range 1–10 μg/ml. The errors are, in general, about ±2%.

Effect of temperature. The colored product formed by PH with osmium (VIII) is stable over the temperature range 4–40°C as shown by the constant absorbance readings.

Effect of foreign ions. The effect of cations and anions which often accompany osmium (VIII) are studied. The results are summarized in Table 2. The proposed method offers the advantages of simplicity, rapidity, and sensitivity without the need for extraction or heating.

TABLE 2
EFFECT OF FOREIGN IONS IN THE DETERMINATION OF 5 μg OF OSMIUM (VIII)

Ion added	Amount tolerated (μg)	Ion added	Amount tolerated (μg)
Ru(III)	1	Ni(II)	600
Rh(III)	15	Ag(I)	3
Pd(II)	1	F^-	2200
Ir(III)	12	NO_3^-	2100
Pt(IV)	13	SO_4^{2-}	1100
Au(III)	1	PO_4^{3-}	1300
Cu(II)	40	CH_3COO^-	5400
Fe(III)	6	EDTA	4900
Co(II)	110		

SUMMARY

Promazine hydrochloride is proposed as a new reagent for the spectrophotometric determination of osmium (VIII). The reagent forms orange oxidation product with osmium tetroxide at room temperature in acidic media. The effects of acid concentration, time, temperature, and foreign ions are reported. Beer's law is obeyed in the osmium concentration range 1–10 μg/ml.

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A Comparison of Three Methods for Cholinesterase Analysis

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INTRODUCTION

The use of anticholinesterase insecticides such as organophosphates and carbamates to reduce populations of arthropods that attack animals and plants has made it desirable to monitor the cholinesterase (ChE) level of the blood of persons exposed to these chemicals. Poisonings of animals necessitates measurement of ChE levels to allow treatment and documentation of exposure. Methods employed for estimating ChE inhibition by organophosphates may given erroneously low inhibition values when applied to the estimation of ChE inhibition by carbamate insecticides (*2, 11*). Underestimation of cholinesterase inhibition by carbamate insecticides could have serious consequences, medically and legally. Others have described methods of analysis for acetylcholinesterase using [^{14}C]acetylcholine. These are presented in a paper authored by Johnson and Russell (*4*). They variously use ion exchange, partition, or separation by some means. They also used a reaction vessel within a scintillation vial.

Historically our laboratory has used the modified Michel method as adapted for our needs (*8*). The pH Stat method was compared with the modified Michel method to be certain no bias or lack of sensitivity was being produced in results on diagnostic samples. Carbamate poisoning and exposure to reversible cholinesterase inhibitors was of particular concern which, as stated above, were reputed to produce difficulty for ΔpH or pH Stat methods.

The following work was undertaken to see if differences would be observed with ΔpH, pH Stat, or radiometric methods with carbamate-exposed biological specimens and animals.

MATERIALS AND METHOD

Acetyl[1-^{14}C]choline chloride is available from New England Nuclear, Boston, Mass. (sp act 5–25 mCi/mmole). Chromatography media, 5 × 18-cm strips, Gelman Instruments, Ann Arbor, Mich.; β counter, Chicago

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Nuclear, Chicago, Ill.; micropipets, Becton–Dickinson Company, Research Triangle Park, N.C.; volumetric glassware; timer. Carbofuran (Furadan) was obtained from the FMC Corporation, Middleport, N.Y., and phorate (Thimet) was obtained from American Cyanamid Company, Princeton, N.J.

Method of Test

The pH Stat method, as well as the radiometric method, is described in great detail in the Appendix. The ΔpH test (*8*) for cholinesterase activity has been used for 12 years at the Veterinary Diagnostic Laboratory for suspected cholinesterase inhibitor exposure. To compare the pH Stat to the ΔpH method, routinely submitted samples were run by both methods for a period of 2 months.

The freeze-dried (FD) human blood (used as a laboratory monitor for at least 2 years and therefore very well defined) was amended with phorate and carbofuran, and organophosphorus and carbamate pesticide, respectively, and used as a basis for comparison in the radiometric method.

Thin-layer chromatography was developed to separate acetate and acetylcholine. Reaction times of 20, 60, and 120 sec were compared for acetylcholine chloride and freeze-dried blood reconstituted. Water and methanol developing solvents were investigated.

Animal Experiment

The blood of male mice (6 months old, weighing 36–40 g) was used in the study. Carbofuran was dissolved in 95% ethanol and an injection was made intraperitoneally using a 50-μl syringe (Hamilton Co., Reno, Nev.) with a 27-gauge, 0.5-in. needle. The volume of injection was 5 μl/10 g of body weight. Controls received an appropriate volume of ethanol. Five minutes after injection, single blood samples of 0.5–1 ml were obtained from each mouse by cardiac puncture. In the highest-dose group, animals died within 5 min after dosing. They were bled as soon as their heart stopped. Blood samples were put into heparinized test tubes (12 × 75 mm), and kept at 4°C for 24 hr before the cholinesterase determinations. ΔpH and pH Stat methods were compared on regular diagnostic specimens submitted for analysis. The freeze-dried (FD) normal human blood served as a control sample each time the methods were run.

RESULTS

Acetate Chromatography

No significant differences were found among 20-, 60-, and 120-sec assays in level of β activity of ^{14}C-labeled acetate produced. Therefore, an assay of 60 sec was selected for ease of sequential replications.

TABLE 1
ACETATE CHROMATOGRAPHY: [^{14}C]ACETATE DISTRIBUTION (6-IN. STRIP) WITH FRESH BLOOD

Location (in. below solvent front)	Counts per min
1	800,000
2	11,000
3	300
4	300
5	300
Total activity	812,000
Activity in top inch, 98.5%	

Chromatographic developing solvents compared were methanol (100%) and water:methanol (25:75%). Methanol was selected because it was found to be more consistent in overall total counts and in duplications of each portion of plates counted, whereas the water:methanol (25:75%) developing solvent proved sporadic in total counts and in counts of portions of plates. Results from acetate analysis are shown in Table 1.

Organophosphate and Carbamate Freeze-Dried Blood

The bloods compared in the radiometric analysis were normal freeze-dried reconstituted blood, organophosphorus-spiked freeze-dried blood, and carbamate-spiked freeze-dried blood. For all three bloods the majority of activity remained in the bottom inch of the plate, the differences being in the amount of activity migrating to the middle and top portions of the plate. The next highest levels of activity were in the top inch of the plate and the smallest levels of activity were in the middle inch of the plate.

As noted in Table 2, the normal blood had twice the amount of activity migrating to the top of the plate as phorate- and carbofuran-treated blood

TABLE 2
CHOLINESTERASE ACTIVITY OF ORGANOPHOSPHORUS AND CARBAMATE FREEZE-DRIED (FD) BLOOD

Portion of 3-in. plate	Activity averages (%)		
	Normal blood	OP blood	Carbamate blood
Top inch	20	10.3	11
Middle inch	7	5.3	8
Bottom inch	73	84.3	81

samples, which showed little difference in the amount of activity in the top portion of the plate.

Inhibition of Cholinesterase by Carbofuran in Mice

None of the mice that received 0.35 or 0.7 mg/kg of carbofuran died. However, all three mice that were given 1.7 mg/kg died. Clinical signs of toxicity, including ataxia, tremors, central nervous system depression, and tachypnea followed by dyspnea were observed during the study. In the case of the ΔpH values, a less obvious change was observed for the nonlethal doses than for the radiometric method (Table 3).

ΔpH and pH Stat Methods Compared for Blood

Table 4 compares the ΔpH and pH Stat methods on diagnostic cases. The freeze-dried reconstituted blood was used as a control. No significant differences were detected between methods on these samples.

DISCUSSION

Radiometric studies of the kinetics of AChE and its inhibition by insecticidal carbamates have shown that comparatively high acetylcholine concentration and dilution of the carbamylated enzyme cause hydrolytic regeneration of the enzyme (*9*, *14*). Consequently, a radiometric method was developed that overcomes these difficulties and provides a truer picture of ChE inhibition by carbamate insecticides (*1*, *3*, *10*, *12*, *13*).

In this method the effects of dilution of samples containing the enzyme are eliminated by the rapidity of the assay (20 sec) and the use of a very low acetylcholine concentration (5×10^{-5} *M*). Briefly, the method consists of incubating a 10-μl sample of hemolyzed blood with a 5μl drop of 1.5×10^{-5} *M* acetyl-1-^{14}C-choline chloride as substrate on a microscope slide or cover slip. The enzymatic hydrolysis of the acetylcholine is esti-

TABLE 3
INHIBITION OF CHOLINESTERASE BY INTRAPERITONEAL INJECTION OF CARBOFURAN IN MICE[a]

Radiometric TLC ChE method		ΔpH ChE method	Carbofuran dosage (mg/kg)
Acetate activity	Acetylcholine activity		
0.47	0.53	0.54	0.0
0.25	0.75	0.42	0.35
0.33	0.67	0.43	0.70
0.23	0.77	0.17	1.7[b]

[a] These data represent three observations of each dose. The pooled variation of cholinestrase value was ±10% of each mean.

[b] Lethal dose.

TABLE 4
COMPARISON OF THE ΔpH AND pH STAT METHODS ON DIAGNOSTIC CASES

Blood	pH Stat (ml/min)	ΔpH (units/hr)
9643	0.04	0.20
FD[a]	0.06	0.40
13487		
1	0.05	0.38
2	0.06	0.37
3	0.06	0.54
4	0.06	0.48
5	0.05	0.40
6	0.02	0.18
FD	0.10	0.75
13647		
1	0.11	0.68
2	0.04	0.26
3	0.04	0.24
4	0.07	0.55
5	0.04	0.24
FD	0.10	0.96
14248	0.05	0.32
14203	0.11	0.66
FD	0.14	0.86
17529		
1	0.01	0.08
2	0.00	0.09
17402		
A	0.00	0.03
B	0.00	0.03
C	0.00	0.02
D	0.01	0.01
17322	0.06	0.67
FD	0.90	0.86

[a] FD = Freeze-dried normal human blood.

mated from the loss of ^{14}C activity through volatilization of the labeled acetic acid. Twenty seconds after the addition of the acetylcholine, 5 μl of HCl are added to terminate the reaction. The slides are dried to remove all enzymatically formed acetic acid and the radioactivity is estimated by scintillation counting. The ChE activity of the blood is directly proportional to the loss of radioactivity. Procedural details have been described by Winteringham and Disney (1964b, 1964c) and by Doherty (1967). The

coefficient of variation for duplicate estimations is about ±5% which is similar to that obtained from routine analysis by the electrometric method (Disney 1965).

Even though the cholinesterase (ChE) level of lethally affected mice did not become zero, a significant change was observed with administration of carbofuran, a commercial carbamate pesticide. There do not appear to be major differences in results between ΔpH, pH Stat or radiometric AChE analysis of blood from lethally affected animals. Meerdink (*5*) has also shown no significant difference between ΔpH and pH Stat analysis for cholinesterase. At the intermediate level the ΔpH test provided a change of 20% from the normal blood. The radiometric test exhibited a 50% change. This change would be easier to spot on unknown samples. But in all cases, it would be well to have a normal control for the animals being tested just for reference. Moisture balance and other pathological conditions besides cholinesterase inhibiting chemicals can affect cholinesterase activity.

CONCLUSION

ΔpH, pH Stat, or radiometric analysis for cholinesterase levels in blood are equally useful for detecting severely cholinesterase-inhibited animals. The simplest method for routine screening is the ΔpH. The pH Stat method can give a permanent document and may be of special significance to some research needs, but it does not differ materially from the ΔpH method (modified Michel). The radiometric method appears to be equally good for measuring cholinesterase levels. It does require the use of radioactive isotope ^{14}C; but it uses the smallest sample volume (5 μl) and this should be of special value for research purposes. It appears that in exposed, but not in lethally affected, animals, using the radiometric method may enhance the ability to detect decreased enzyme activity.

APPENDIX 1: ACETYLCHOLINESTERASE[1]

This procedure has the advantage of rapid automated sample analysis on a Metrohm pH Stat, making it possible to have two different readouts on one sample.

Reagents and Apparatus

a. Acetylcholine substrate. 0.11 *M* acetylcholine chloride, 2.0 g in 100 ml of distilled water; or 2.48 g acetylcholine bromide or 3.00 g acetylcholine iodide in 100 ml double-distilled water.

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b. Saponin. 0.01%, 0.1 g dissolved in 1000 ml of distilled water.

c. Diluent. Sodium and calcium chloride, 0.05 *M* $NaCl/CaCl_2$ in double-distilled water.

d. Base. 0.005 *N* NaOH.

e. pH meter. Corning research model (or equivalent).

f. Reference electrodes. Glass and Calomel.

g. pH Stat. Metrohm (or equivalent).

Procedure

1. Blood must be drawn in heparinized containers.
2. Hemolyze 1.0 ml whole blood with 9.0 ml saponin solution.
3. Add 1 ml of saponin-treated blood, 1 ml $NaCl/CaCl_2$ solution to pH Stat reservoir. Titrate to pH 7.6 with 0.005 *N* base.
4. Add 1 ml of acetylcholine bromide solution. Titrate to 7.6 pH and time for 3 min at this point.
5. Calculate base added in milliliter per minute. Normal whole blood samples require 0.13 ml NaOH per minute. Normal samples produce a response of 0.6 ΔpH per hour. Fifty-five units activity of acetylcholinesterase produce the same response.

Calculations. This is assuming $\{AChE\} \alpha \Delta pH \alpha \{H^+\}$ ml/min.

$$VMAX \text{ (Velocity Maximum)} \alpha \{S\} \alpha \{AChE\}$$

or

$$\text{Rate} = \{AChE\}\{S\},$$

where $\{S\}$ = substrate, $\{AChE\}$ = enzyme concentration. Since ΔpH and ml base/min are both rates.

$$\{AChE\} = \frac{\text{pH (unknown)}}{\text{pH (normal)}} \times 55 \text{ units}$$

$$= \frac{\text{ml base/min (unknown blood)}}{\text{ml base/min (normal blood)}}.$$

General Considerations

Fluid volume in animal, and other liver or kidney conditions, may affect cholinesterase activity of an animal. Other tissues may be run. Generally a 1:10 dilution of the tissues is made in Ringer's solution and the equivalent of 100 mg of tissue added to the titration vessel. The pH Stat analysis may be done.

Accuracy and Precision

Both ΔpH and pH Stat methods appear to be reproducible. Many determinations on freeze-dried blood have given an average ΔpH of 0.6 ml and 0.13 ml of base means with ±10% variation.

APPENDIX 2: [^{14}C]CHOLINESTERASE TEST

As little as 5 μl of blood may be tested by the use of ^{14}C-labeled acetylcholine chloride. The test involves the use of ion thin-layer chromatography strips and a methanol solvent to separate [^{14}C]acetate enzymatically produced from the unreacted acetylcholine. The presence of cholinesterase is proportional to the quantity of acetate produced.

Reagents and Apparatus

1. ^{14}C acetylcholine chloride (ACC) solution. One microcurie of ACC is dissolved in 10 ml of water and a 1:5 dilution is made for the test reagent substrate.

b. Ion TLC strips. Gelman Instrument Company.

c. Methanol. Nanograde or equivalent, Mallinckrodt Chemical Company.

d. Double-distilled water. Millipore Q (or equivalent).

e. Micropipets. B & D Company (or equivalent).

f. Scissors, tweezers.

g. Glassine paper sheets.

h. β counter. Chicago Nuclear (or equivalent).

Procedure

1. Aliquots of 5 μl blood are placed on duplicate chromatographic strips 3 in. long and 1 in. wide. The fluid is placed in the center of the strip approximately ¼ in. from the bottom. A new strip should be used as a blank each day. A control sample of normal blood is handled in the same way.

2. Add 5 μl of the dilute ACC substitute solution to the blood and allow 60 sec for production of the acetate.

3. Place the strip in a closed glass chamber with ⅛ in. of methanol in the bottom and allow the solvent to migrate to the top of the strip (⅛ in. of the top).

4. The strip is removed and dried in a suitable hood. It is cut in three horizontal 1-in. strips; the 1-in. strip nearest the solvent front should contain 95% of the acetate activity. The strip at the origin should contain 95% of the ACC substrate activity.

5. The strips are counted using the Geiger counter (thin window) for 1 min or more depending on the counting efficiency of the system.

$$\text{Cholinesterase activity} = \frac{\text{acetate cpm (unknown blood)}}{\text{acetate cpm (normal blood)}} \times 50 \text{ units}$$

$$= \text{units activity}$$

General Considerations

Duplicates are always done. Other replicates may be desirable depending on the variation in the technique on normal samples and the magnitude of the variation in the counting technique itself.

An uninhibited blood should produce 20% of activity as acetate. A totally inhibited blood should produce 10% of activity as acetate. Gradients of difference are observed with partially inhibited bloods. In work with inhibitors, no difference has been observed between organophosphorus and carbamate compounds.

To avoid contamination, the following procedures should be considered. Chromatography strips should be handled with tweezers at all times. Use scissors to cut strips into three equal parts for counting. Utensils used for handling strips should be rinsed three times with methanol after each use. The strips to be counted should be placed on glassine paper sheets in the counting chamber. Glassine paper sheets should be changed after each use.

SUMMARY

Three methods of cholinesterase analysis in blood are compared: the ΔpH (modified Michel method), pH Stat, and radiometric methods. The ΔpH method was determined to be the best choice for routine laboratory screeeñing for organophosphate exposure. The methods all agree within experimental variation. The radiometric method uses a thin-layer chromatography (TLC) separation of acetate (^{14}C) activity from acetylcholine (^{14}C) activity with direct β counting or scintillation counting to determine the concentration of acetate activity. The methods were compared on freeze-dried human blood and on experimentally carbamate-inhibited mouse blood. The radiometric analysis may be performed using as little as 5 μl of blood. The radiometric method may enhance the ability to detect sublethal exposure to cholinesterase inhibitors. It should be of particular use where sampling size is of greatest importance.

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Some Analytical Applications of Aromatic Sulfonyl Haloamines: Determination of Thiocyanate and Cyanide Ions in Metal Complexes and Salts and Thiosemicarbazide in Metal Complexes with Bromamine-T

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INTRODUCTION

Considerable attention has recently centered around the chemistry of aromatic sulfonyl haloamines (*4*). These compounds have diverse properties and behave as oxidants and halogenating agents which act both as bases and nucleophiles, and nitrenoids in some cases. They have extensively been employed in our laboratories as oxidimetric reagents in kinetic studies and in the determination of a variety of reductants in aqueous, partially aqueous, and nonaqueous media (*8–20*). A recent addition (*23*) to this class of *N*-haloamines is sodium *N*-bromo-4-methylbenzenesulfonamide or bromamine-T (p-CH_3–C_6H_4–$SO_2NBrNa \cdot 3\ H_2O$; abbreviated as RNBrNa or BAT).

Thiocyanates find a number of industrial applications in photography; in printing and dyeing textiles; in the manufacture of synthetic dye stuffs, sulfocyanides, thioureas, and mustard oil; in medicine and freezing mix-

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tures; and as a reagent in analytical chemistry. The analytical procedures reported (*8*) so far for estimating NCS^- ions include the use of hypohalite, bromine, iodine, iodate, hydrogen peroxide, chloramine-T (CAT) and dichloroamine-T (DCT).

Cyanides have a number of industrial applications (*16*) in the extraction of noble metals, in photography, and in the preparation of insecticides and cyanogen derivatives. Silver nitrate, alkaline permanganate, iodine, CAT, DCT, and lead tetraacetate are some of the reagents used for estimating cyanide in solution (*16*, *17*).

Thiosemicarbazide is employed in the characterization of aldehydes, ketones, and polysaccharides and as a metal complexing agent. The oxidants used for its estimation include alkali metal hypohalites, lead tetraacetate, CAT, and DCT (*19*, *20*).

The present studies report the preparation of BAT and its characterization through mass spectrum and spectral studies (uv, ir, and FT ^{13}C and ^1H NMR) and its applications in determining NCS^- and CN^- ions present in metal salts and complexes and thiosemicarbazide (TSC) in its metal complexes. The methods are simple, elegant, and reproducible under the experimental conditions described. Another interesting feature is that the technique can be employed for computing the number of CN^-, NCS^-, and TSC ligands in the respective complexes.

MATERIALS AND METHODS

Potassium thiocyanate (AR, E'Merck) was dried at 150°C and its purity was checked. Metal thiocyanates LiNCS, NaNCS, $Cd(NCS)_2$, $Zn(NCS)_2$, $Ni(NCS)_2 \cdot 0.5H_2O$, $Ba(NCS)_2 \cdot 2H_2O$, $Pb(NCS)_2$, and $UO_2(NCS)_2 \cdot 3H_2O$, and complexes $K_2Cd(NCS)_4 \cdot 2H_2O$, $K_2Zn(NCS)_4 \cdot 4H_2O$, $K_4Ni(NCS)_6 \cdot 4H_2O$, $KUO_2(NCS)_3 \cdot 2H_2O$, and $K_4Pb(NCS)_6$ were prepared (*8*) and their purity was checked by elemental analyses. AR grade KCN (Reanal, Hungary) and NaCN were used without further purification. The salts AgCN and $Zn(CN)_2$ and complexes $KAg(CN)_2$ and $K_2Zn(CN)_4$ were prepared (*16*). E'Merck thiosemicarbazide was recrystallized from aqueous solution. Platinum and palladium complexes were prepared from $H_2PtCl_6 \cdot xH_2O$ and $PdCl_2$ (Johnson–Mathey, Ltd., London). The following thiosemicarbazide (L) complexes were prepared by methods reported elsewhere (*19–22*): ML_2X_2, where M = Zn, Cd, Ni, or Hg, X = Cl, NO_3, ClO_4, or ½ SO_4; *cis*- and *trans*-PtL_2Cl_2 and PdL_2X_2, where X = Br, I, CN, NCS, or NO_3; *cis*- and *trans*-$PdL_2(NO_3)_2$; PdL_2X_2, where X = Cl, Br, I, or NCS; and neutral complexes[3] $M(L-H)_2$, where M = Ni, Pt, or Pd. The complexes were recrystallized from aqueous solution and characterized by their elemental analyses and ir spectra (*21*, *22*).

[3] (L–H) represents NH_2NCSNH_2.

Preparation of Bromamine-T

BAT was obtained by dissolving DBT in 4 *M* NaOH (*23*). About 20 g of DBT was dissolved with stirring in ~30 ml of 4 *M* NaOH at room temperature and the resultant aqueous solution was cooled in ice. Pale yellow crystals of BAT formed, were filtered under suction, washed quickly with the minimum quantity of cold water, and dried over phosphorus pentoxide. The purity of BAT was checked iodometrically.

Spectral Data of Bromamine-T

Mass spectrometry. The electron impact mass spectrum of BAT was obtained on a DuPont 21-291 mass spectrometer using 70-eV electrons with source and probe temperatures at 285 and 50°C, respectively. The compound has peaks at *m/e* 326 (m^+, weak), 171 ($H_3C-C_6H_4-SO_2NH_2^+$, strong), 155 ($H_3C-C_6H_4-SO_2^+$), and 91 ($H_3C-C_6H_4^+$).

Ultraviolet. The uv spectrum of BAT in aqueous solution was obtained with a Beckman DK-2A ratio-recording dual-beam spectrophotometer. The compound has a λ_{max} at 224 nm ($\log \xi_{max} = 4.2125$).

Infrared. The ir spectrum of the compound was recorded on a Perkin–Elmer 298 grating infrared spectrophotometer. The spectrum (*1–3, 5, 24*) obtained in Nujol shows characteristic bands (cm^{-1}) at 3500 (strong, ν –OH), 2150 (weak, ν C=C), 1656 (medium, δ–OH), 1235 (strong, ν_{asym} $-SO_2$), 1120 (strong, shoulder, ν_{sym} $-SO_2$), 1075, 1015 (medium, aromatic in plane δ–CH), 915 (strong, ν S–N), 802 (strong, 1,4–disubstituted phenyl ring), 665 (medium, ν N–Br), and 615 (medium, out of plane ring deformation). Here ν = stretching.

Nuclear magnetic resonance. NMR spectra of BAT were obtained in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard.

^1H spectrum: δ (relative to TMS); 2.4 (singlet corresponding to $-CH_3$); 7.8 (doublet for *ortho* H); 7.4 (doublet for *meta* H). The coupling constant $J_{o,m}$ is 8.0 Hz.

^{13}C spectrum: (ppm relative to TMS); 145.39 (C–1, carbon attached to heteroatom); 140.50 (C–4); 131.75 (C–2,6); 129.40 (C–3,5); and 23.0 (methyl carbon).

Reductant Solutions

Triply distilled water was used in preparing the solutions. Aqueous solutions (~2 mg/ml) of thiocyanates, soluble cyanides (KCN and NaCN), complex cyanides ($KAg(CN)_2$ and $K_2Zn(CN)_4$), and TSC complexes were prepared, while $Pb(NCS)_2$ was dissolved in 2 *N* acetic acid. Aqueous KCN (0.04 *M* and solution should be standardized) was used as solvent (*17*) for the insoluble cyanides, AgCN and $Zn(CN)_2$.

Bromamine-T Solution

An approximately decinormal solution of BAT was prepared, by dissolving about 8.2 g of the solid in 500 ml of triply distilled water. It was then standardized by the iodometric method and stored in amber-colored bottles.

Buffer Solutions

The following buffer solutions were prepared according to the standard methods reported in literature (*6*): pH 1 and 2 (HCl + KCl); pH 3 (citric acid + Na_2HPO_4); pH 4–6 (acetate + acetic acid); pH 7–9 (borax + boric acid + NaCl); and pH 10 ($NaHCO_3$ + Na_2CO_3).

Compounds of acceptable grades of purity were used in preparing other solutions.

Preliminary Investigations

Known quantities of the reductant solutions were added to a known volume of BAT (50–60% excess) in an iodine flask. The reaction mixture was set aside for various intervals of time at room temperature (27 ± 3°C) with occasional shaking. The excess of BAT left unconsumed was iodometrically determined by back titration with standard thiosulfate.

The results of some of these investigations with KNCS and KCN are shown in Table 1, where the oxidation period is 5 min. It is seen that oxidation of NCS^- ion is slow in 0.1–0.2 *N* HCl, H_2SO_4 and $HClO_4$, and buffers of pH 1–10, but is rapid in presence of NaOH, and an eight-electron stoichiometry is noted in 0.1–0.2 *M* alkali. Slow oxidation of KCN took place in buffer media and a two-electron stoichiometry was noted in 0.1–0.2 *M* NaOH (Table 1). Similarly, stoichiometric oxidation of TSC complexes, with the exception of those of Pt, took place within 5–10 min. The latter were oxidized in 30 min. A 12-electron change per TSC molecule in the complex was observed in all cases.

Recommended Procedure

Add aliquots of NCS^- or CN^- (salt or complex) or TSC complex solutions to a known volume (50–60% excess) of 0.1 *N* BAT in an iodine flask, containing enough NaOH to maintain an overall concentration of 0.1–0.2 *N* alkali. Set aside for 5 min in the case of thiocyanates and cyanides and for 10 min with the TSC complexes (30 min for Pt complexes) with occasional shaking. Add 10 ml of 2 N H_2SO_4 and 20 ml of 10% KI and dilute to 150 ml. Titrate the liberated iodine against 0.1 *N* sodium thiosulfate to a starch endpoint (V_1 ml). Run a blank with BAT alone (V_2 ml).

The amount of the reductant (x, micromoles) in the sample solution is given by $x = 10^3 N (V_2 - V_1)/E$, where N is the normality of thiosulfate

TABLE 1

OBSERVED STOICHIOMETRY OF OXIDATION OF POTASSIUM THIOCYANATE AND POTASSIUM CYANIDE IN DIFFERENT BUFFER AND SOLVENT MEDIA WITH BROMAMINE-T

Medium	μmol of BAT used / μmol of KNCS taken	Number of electrons changing per mole of KNCS	μmol of BAT used / μmol of KCN taken	Number of electrons changing per mole of KCN
0.1 *N* $HClO_4$	3.276	6.56	—	—
0.2 *N* $HClO_4$	3.247	6.56	—	—
0.1 *N* HCl	3.276	6.56	—	—
0.2 *N* HCl	3.248	6.50	—	—
0.1 *N* H_2SO_4	3.109	6.22	—	—
0.2 *N* H_2SO_4	3.167	6.34	—	—
pH 1	3.408	6.82	0.055	0.11
pH 2	3.329	6.66	0.062	0.12
pH 3	3.428	6.86	0.039	0.08
pH 4	3.356	6.72	0.061	0.12
pH 5	3.277	6.56	0.052	0.10
pH 6	3.339	6.68	0.071	0.14
pH 7	3.428	6.86	0.069	0.14
pH 8	3.276	6.56	0.089	0.18
pH 9	3.128	6.26	0.068	0.14
pH 10	3.246	6.49	0.397	0.80
0.0002 *N* NaOH	3.377	6.75	—	—
0.002 *N* NaOH	3.400	6.80	—	—
0.02 *N* NaOH	3.568	7.13	0.851	1.70
0.05 *N* NaOH	3.917	7.83	0.970	1.94
0.10 *N* NaOH	4.013	8.03	1.000	2.00
0.15 *N* NaOH	4.002	8.00	1.001	2.00
0.20 *N* NaOH	4.000	8.00	1.005	2.01

and E is the number of electrons changing per molecule of the reductant. For $KAg(CN)_2$ and $K_2Zn(CN)_4$, E = 4 and 8, respectively. For Cd, Ni, Pb, Zn, Ba, and UO_2 thiocyanates, E = 16, while for complex thiocyanates of U, Zn, Cd, Ni, and Pb, E = 24, 32, 32, 48, and 48, respectively. For TSC complexes of the type ML_2X_2 or $M(L-H)_2$, E = 24. $PtL_2(CN)_2$ is oxidized with a 28-electron change, due to the presence of CN^- ion. For $PtL_2(NCS)_2$ and $PdL_2(NCS)_2$, E = 40, since NCS^- ion is oxidized under these conditions.

Calculations for the recovery of insoluble cyanides AgCN and $Zn(CN)_2$ which were dissolved in aqueous KCN (y, molar) are as follows: let a ml of cyanide mixture require V_1 ml of the oxidant. Volume of the oxidant (V_2 ml) required for a ml of y M KCN would be $V_2 = 2ya/N$, where N is the normality of the oxidant. Then oxidation of insoluble cyanide alone would require $(V_1 - V_2)$ ml of the oxidant. The amount (x, micromoles) of insoluble cyanide in the sample solution is $x = 10^3N(V_1 - V_2)/E$. For AgCN and $Zn(CN)_2$, E = 2 and 4, respectively.

RESULTS AND DISCUSSION

Table 1 gives the time dependence of the oxidation of NCS^- and CN^- ions in various buffer and solvent media. It follows from this table that the studied reactions with BAT proceed quantitatively and stoichiometrically with consumption of eight and two equivalents of the oxidant per mole of NCS^- and CN^- ions, respectively. The stoichiometry could be represented as follows:

$$M(NCS)_x + 4x\ RNBrNa + 2x\ OH^- + 3x\ H_2O \rightarrow M(CNO)_x + x\ SO_4^{2-} + 4x\ RNH_2 + 4x\ Na^+ + 4x\ Br^-.$$

For the oxidation of lithium, potassium and sodium thiocyanates, x = 1 while x = 2 for U, Cd, Zn, Ni, Ba, and Pb salts. Oxidation of complex thiocyanates is represented by

$$MyM_1(NCS)_x + 4x\ RNBrNa + 2x\ OH^- + 3x\ H_2O \rightarrow y\ M^+ + M_1^{2+} + x\ CNO^- + x\ SO_4^{2-} + 4x\ RNH_2 + 4x\ Na^+ + 4x\ Br^-. \quad (2)$$

For the cadmium and zinc complexes, y = 2 and x = 4, and y = 4 and x = 6 for Ni and Pb complexes, while for potassium uranyl thiocyanate y = 1 and x = 3. Oxidation of cyanides and cyanide complexes is represented by

$$M(CN)_x + x\ RNBrNa + x\ H_2O \rightarrow M(CNO)_x + x\ RNH_2 + x\ Na^+ + x\ Br^-, \quad (3)$$

$$KAg(CN)_2 + 2\,RNBrNa + 2\,H_2O \rightarrow K^+ + Ag^+ + 2\,CNO^- + 2\,RNH_2 + 2\,Na^+ + 2\,Br^-, \quad (4)$$

$$K_2Zn(CN)_4 + 4\,RNBrNa + 4\,H_2O \rightarrow 2\,K^+ + Zn^{2+} + 4\,CNO^- + 4\,RNH_2 + 4\,Na^+ + 4\,Br^-. \quad (5)$$

The 12-electron stoichiometry per thiosemicarbazide molecule in the complexes may be represented as follows,

$$M(NH_2NHCSNH_2)_2\,X_2 + 12\,RNBrNa + 10\,H_2O + 2\,OH^- \rightarrow MX_2 + 2\,SO_4^{2-} + 12\,RNH_2 + 2\,CO_2 + 2\,N_2 + 12\,Na^+ + 12\,Br^- + 2\,NH_4^+, \quad (6)$$

$$M(NH_2NCSNH_2)_2 + 12\,RNBrNa + 12\,H_2O \rightarrow M^{2+} + 2\,SO_4^{2-} + 12\,RNH_2 + 2\,CO_2 + 2\,N_2 + 12\,Na^+ + 12\,Br^- + 2\,NH_4^+, \quad (7)$$

$$M(NH_2NHCSNH_2)_2(NCS)_2 + 20\,RNBrNa + 16\,H_2O + 6\,OH^- \rightarrow M^{2+} + 4\,SO_4^{2-} + 2\,CNO^- + 20\,RNH_2 + 2\,CO_2 + 2\,N_2 + 20\,Na^+ + 20\,Br^- + 2\,NH_4^+, \quad (8)$$

$$M(NH_2NHCSNH_2)_2(CN)_2 + 14\,RNBrNa + 12\,H_2O + 2\,OH^- \rightarrow M^{2+} + 2\,SO_4^{2-} + 2\,CNO^- + 14\,RNH_2 + 2\,CO_2 + 2\,N_2 + 14\,Na^+ + 14\,Br^- + 2\,NH_4^+, \quad (9)$$

where M = Zn, Cd, Hg, Ni, Pt, or Pd.

TABLE 2
ACCURACY AND REPRODUCIBILITY OF DETERMINATION OF THIOCYANATE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-T

Reductant	Reductant taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)
LiNCS	118.6	0.052	0.67
KNCS	102.9	0.066	0.66
NaNCS	133.3	0.011	0.20
$Cd(NCS)_2$	43.8	0.016	0.46
$Ni(NCS)_2 \cdot 0.5\,H_2O$	53.9	0.016	0.47
$Pb(NCS)_2$	27.5	0.052	0.58
$Zn(NCS)_2$	53.7	0.016	0.47
$Ba(NCS)_2 \cdot 2\,H_2O$	49.1	0.028	0.40
$UO_2(NCS)_2 \cdot 3\,H_2O$	26.9	0.039	0.49
$K_2Zn(NCS)_4 \cdot 4\,H_2O$	25.0	0.021	0.19
$K_2Cd(NCS)_4 \cdot 2\,H_2O$	20.8	0.022	0.19
$K_4Ni(NCS)_6 \cdot 4\,H_2O$	15.0	0.021	0.22
$K_4Pb(NCS)_6$	15.0	0.021	0.20
$KUO_2(NCS)_3 \cdot 2\,H_2O$	25.0	0.025	0.19

[a] Calculated for six trials.

TABLE 3
DETERMINATION OF THIOCYANATE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-T

Reductant	Range studied (μmol)	Error in recovery (%)
LiNCS	23.71–593.01	0.52–0.00
KNCS	20.60–514.42	0.50–0.27
NaNCS	26.66–671.60	0.47–0.00
$Cd(NCS)_2$	8.80–221.32	0.67–0.00
$Ni(NCS)_2 \cdot 0.5\ H_2O$	10.78–269.46	0.30–0.00
$Pb(NCS)_2$	5.51–137.69	0.60–0.00
$Zn(NCS)_2$	10.75–268.74	0.62–0.00
$Ba(NCS)_2 \cdot 2\ H_2O$	9.82–245.42	0.49–0.00
$UO_2(NCS)_2 \cdot 3\ H_2O$	5.39–134.66	0.51–0.00
$K_2Zn(NCS)_4 \cdot 4\ H_2O$	5.00–125.11	0.09–0.00
$K_2Cd(NCS)_4 \cdot 2\ H_2O$	4.99–124.84	0.44–0.03
$K_4Ni(NCS)_6 \cdot 4\ H_2O$	1.57–75.19	0.53–0.07
$K_4Pb(NCS)_6$	3.01–75.18	0.47–0.05
$KUO_2(NCS)_3 \cdot 2\ H_2O$	5.01–125.22	0.38–0.06

TABLE 4
ACCURACY AND REPRODUCIBILITY OF DETERMINATION OF CYANIDE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-T

Reductant	Reductant taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)
KCN	152.8	0.082	0.08
NaCN	201.2	0.031	0.31
AgCN	100.0	0.080	0.60
$Zn(CN)_2$	99.7	0.016	0.27
$KAg(CN)_2$	52.2	0.053	0.26
$K_2Zn(CN)_4$	37.2	0.036	0.39

[a] Calculated for six trials.

TABLE 5
DETERMINATION OF CYANIDE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-T

Reductant	Range studied (μmol)	Error in recovery (%)
KCN	30.57–764.21	0.50–0.02
NaCN	40.20–1005.71	0.25–0.01
AgCN	20.01–504.11	0.38–0.05
$Zn(CN)_2$	19.93–498.30	0.68–0.00
$KAg(CN)_2$	10.45–260.65	0.50–0.02
$K_2Zn(CN)_4$	7.43–185.78	0.53–0.00

TABLE 6
ACCURACY AND REPRODUCIBILITY OF DETERMINATION OF THIOSEMICARBAZIDE IN ITS METAL COMPLEXES WITH BROMAMINE-T

Complex	Reductant taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)
ZnL_2SO_4	42.2	0.017	0.17
ZnL_2Cl_2	45.9	0.023	0.23
$ZnL_2(NO_3)_2$	27.2	0.030	0.30
$ZnL_2(ClO_4)_2$	23.0	0.056	0.54
CdL_2SO_4	31.1	0.022	0.18
CdL_2Cl_2	27.4	0.022	0.22
HgL_2Cl_2	21.9	0.036	0.37
$NiL_2SO_4 \cdot 3H_2O$	26.5	0.016	0.16
$NiL_2(NO_3)_2$	27.4	0.021	0.21
NiL_2Cl_2	32.3	0.017	0.17
$Ni(L-H)_2$	42.1	0.011	0.11
PdL_2Cl_2	27.7	0.021	0.21
PdL_2Br_2	22.8	0.020	0.20
PdL_2I_2	18.8	0.031	0.30
$PdL_2(NCS)_2$	24.8	0.017	0.17
cis-$PdL_2(NO_3)_2$	24.2	0.028	0.28
trans-$PdL_2(NO_3)_2$	18.6	0.039	0.39
$Pd(L-H)_2$	32.7	0.016	0.16
cis-PtL_2Cl_2	22.3	0.000	0.00
trans-PtL_2Cl_2	22.3	0.000	0.00
PtL_2Br_2	18.8	0.033	0.33
PtL_2I_2	16.1	0.033	0.32
$PtL_2(CN)_2$	23.5	0.022	0.22
$PtL_2(NCS)_2$	20.4	0.016	0.15
$PtL_2(NO_3)_2$	20.1	0.026	0.26
$Pt(L-H)_2$	25.5	0.021	0.21

[a] Calculated for six trials.

In each reaction the oxidant undergoes a two-electron change. The products formed in the reactions do not undergo further oxidation. Some typical results of analyses of thiocyanate and cyanide ions present in salts and complexes and thiosemicarbazide in its complexes are given in Tables 2–7. The tables show the range of reductants employed, standard deviation, percentage coefficient of variance, and percentage error in recovery. Each range covers the sample sizes present in 8–10 different aliquots of the reductant solution. It is seen that the results are accurate within an error of about $\pm 0.7\%$.

Interference

Common anions such as SO_4^{2-}, PO_4^{3-}, NO_3^-, ClO_4^-, F^-, Cl^-, and Br^- do not interfere but hydrazine, urea, and thiourea interfere in the estimation.

TABLE 7
DETERMINATION OF THIOSEMICARBAZIDE IN ITS METAL COMPLEXES WITH BROMAMINE-T

Complex	Range studied (μmol)	Error in recovery (%)
ZnL_2SO_4	8.46–211.00	0.68–0.14
ZnL_2Cl_2	9.19–229.55	0.50–0.00
$ZnL_2(NO_3)_2$	5.44–135.90	0.50–0.10
$ZnL_2(ClO_4)_2$	4.61–115.32	0.49–0.00
CdL_2SO_4	6.22–155.53	0.41–0.00
CdL_2Cl_2	5.47–136.76	0.55–0.14
HgL_2Cl_2	4.39–109.63	0.51–0.08
$NiL_2SO_4 \cdot 3H_2O$	5.30–132.39	0.58–0.00
$NiL_2(NO_3)_2$	5.48–137.02	0.67–0.10
NiL_2Cl_2	6.48–161.91	0.50–0.02
$Ni(L-H)_2$	8.41–210.34	0.50–0.00
PdL_2Cl_2	5.53–138.35	0.50–0.00
PdL_2Br_2	4.56–114.05	0.50–0.00
PdL_2I_2	3.76–94.03	0.50–0.00
$PdL_2(NCS)_2$	4.97–124.20	0.50–0.00
cis-$PdL_2(NO_3)_2$	4.85–121.18	0.50–0.08
trans-$PdL_2(NO_3)_2$	3.73–93.18	0.50–0.00
$Pd(L-H)_2$	6.55–163.72	0.50–0.00
cis-PtL_2Cl_2	4.46–111.53	0.70–0.00
trans-PtL_2Cl_2	4.46–111.53	0.50–0.00
PtL_2Br_2	3.76–94.02	0.50–0.00
PtL_2I_2	3.22–80.42	0.67–0.06
$PtL_2(CN)_2$	4.71–117.63	0.50–0.02
$PtL_2(NCS)_2$	4.09–102.28	0.20–0.10
$PtL_2(NO_3)_2$	4.03–100.74	0.59–0.00
$Pt(L-H)_2$	5.10–127.51	0.50–0.05

Product Analyses

The presence of sulfate in the reaction mixture was detected (*7*) using sodium rhodizonate and barium chloride. Cyanate was identified by the Werner test (*25*) as follows: a few drops of pyridine and 2–3 drops of 1% solution of copper sulfate were added to 10 ml of water. Then 2 ml of chloroform were added, followed by the test solution. On shaking the mixture briskly, a lilac-blue color appears in the chloroform layer due to the formation of the complex, $Cu(CNO)_2(C_5H_5N)_2$. The reduction product of BAT, *p*-toluenesulfonamide, was detected (*9*) by paper chromatography with benzyl alcohol saturated with water as the solvent and 0.5% vanillin in 1% HCl in ethanol as the spray reagent (R_f = 0.91).

It can be concluded that the proposed analytical technique is simple, rapid, reproducible, and accurate and is useful for estimating the reduc-

tants in solution and for computing the number of ligands present in the complexes.

SUMMARY

A simple, rapid, and accurate method for the determination of thiocyanate and cyanide ions in metal complexes and salts, and thiosemicarbazide (TSC) in Zn, Cd, Hg, Ni, Pt, and Pd metal complexes with excess of bromamine-T has been developed. The oxidation involves eight- and two-electron changes, respectively, with NCS^- and CN^- ions and a 12-electron stoichiometry per TSC molecule, in 0.1–0.2 *N* NaOH medium. The proposed method could be employed for computing the number of thiocyanate, cyanide, and TSC ligands in the respective complexes. The aromatic sulfonyl haloamine, bromamine-T, has been prepared and characterized by uv, ir, and FT NMR ^1H and ^{13}C spectral data and its mass spectrum.

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Degradation of Uric Acid and 3-Ribosyluric Acid during Treatment with Charcoal[1]

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INTRODUCTION

Activated charcoal is used to selectively adsorb nucleotides from cold acid extracts of tissues (*10*). This treatment separates the nucleotides from other intermediates and salts that are not adsorbed and avoids the step needed to remove the acid used in the extraction. 3-Ribosyluric acid is the nucleoside or nucleotide present in the highest concentration in the erythrocytes of most cattle (*7*). When cold trichloroacetic acid or perchloric acid extracts of bovine erythrocytes were treated with charcoal and eluted, and the nucleotides separated by column and paper chromatography, it was found that the 3-ribosyluric acid was preferentially lost from the extracts. This paper is a report of the effect of charcoal treatment on uric acid and 3-ribosyluric acid.

MATERIALS AND METHODS

All of the common bases and nucleosides used in this study were purchased from commerical dealers. The 3-ribosyluric acid was prepared as described previously (*6*). The [2-^{14}C]uric acid (specific activity, 57 mCi/mmol) was purchased from Amersham, Arlington Heights, Ill. Their analysis of the labeled uric acid showed a radiochemical purity of 97–98%. When used in the present study, the radiochemical purity was 83%. The major contaminant was allantoin, which accounted for about 12% of the radioactivity, and there were at least 2 other minor radioactive components. The charcoal was purchased from Baker and Adamson, Morristown, N.J. (decolorizing carbon); Sigma Chemical Co., St. Louis, Mo. (activated charcoal); and Matheson, Coleman, and Bell, Norwood, Ohio (Darco G-60). The charcoal (30 g) was suspended in 300 ml of 6 *N* HCl and degassed under vacuum for 5 hr and then washed with deionized water until the pH was 4–5. The charcoal was stored in water and 1 ml of the suspension (about 100 mg) added to 10 ml of the solutions of the

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compounds to be adsorbed. The mixture was shaken and after 10–15 min the charcoal was filtered on Whatman No. 1 filter paper. The charcoal was washed with 10 ml of water and the compounds eluted with 40 ml of ethanol:water:NH_4OH (60:36:4). The ethanol/NH_4OH solution was blown to dryness under a stream of air. The dried residue was dissolved in water for subsequent chromatographic or spectrophotometric analysis.

The ultraviolet absorption spectra of the original solution, the filtrate, the water wash, and the ethanol/NH_4OH eluate were all determined with a Beckman model 25 recording spectrophotometer. When [2-^{14}C]uric acid was used, 0.2 ml of the solution from each step was mixed with 10 ml of a toluene:Triton-X100 (2:1) mixture with 6 g/liter of a mixture of 98% 2,5-diphenyloxazole and 2% *p*-bis-(o-methylstryl)benzene and counted for 10 min in an Isocap/300, 6868 liquid scintillation system from Searle Analytic, Inc. Paper chromatography was done ascending on Whatman No. 3 MM paper in butanol:pyridine:water (1:1:1); *t*-butanol:methylethylketone:formic acid:water (8:6:3:3); ethanol:1 *M* ammonium acetate, pH 3.8 (7:3); ethanol:1 *M* ammonium acetate, pH 7.5 (7:3); 4% trisodium citrate, and 5% $Na_2HPO_4 \cdot 7H_2O$ saturated with isoamyl alcohol. Uric acid and 3-ribosyluric acid were detected with a mineralight ultraviolet lamp and by reaction with 0.2% 2,6-dichloroquinone-4-chloroimide in 95% ethanol (*5*). Allantoin was detected with 1% dimethylaminobenzaldehyde in ethanol:HCl (100:10) (*1*). When [2-^{14}C]uric acid was used the radioactivity was detected by exposing Kodak no-screen X-ray film to the chromatograms for 1–4 weeks. The radioactive areas of the chromatograms were cut out and eluted in 5 ml of water for 2 hr. The solution was filtered and 0.2 ml of the filtrate counted as described above.

The blood used as collected, washed, extracted with cold trichloroacetic acid and chromatographed on a Dowex-1 column as described previously (*8*).

RESULTS

A cold trichloroacetic acid extract of 40 ml of washed bovine erythrocytes was divided into two portions. One half was treated with charcoal and the ethanol/NH_4OH eluate dried; the other half was lyophilized. The dried samples were dissolved in water, diluted, and the ultraviolet absorbance spectra determined (Fig. 1). Curve 1 which is the extract not treated with carbon had the usual spectrum for bovine erythrocytes with a maximum at 297 nm, because of the high concentration of 3-ribosyluric acid present, and a second broader maximum at 256–259 nm from the other nucleotides present. The ultraviolet absorption spectrum of the sample treated with carbon (curve 2) had a marked decrease in the absorbance at 297 nm when compared to the untreated extract. This suggested that the 3-ribosyluric acid was adsorbed to the charcoal and either was not eluted or

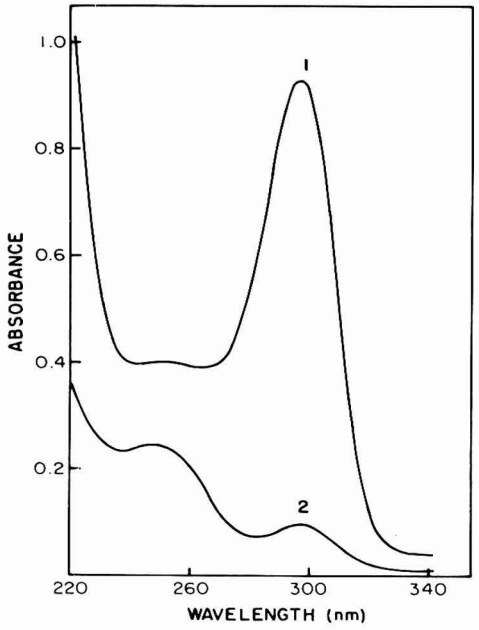

FIG. 1. The ultraviolet absorption spectra of a cold trichloroacetic acid-soluble fraction of washed bovine red cells. Curve 1 is the spectrum of the original extract. Curve 2 is the spectrum of the same extract after treatment with charcoal.

was degraded during the treatment. When the trichloroacetic acid extracts were chromatographed on a Dowex-1 column, the fractions containing the nucleotides were similar in both extracts. For instance, the ATP in the charcoal-treated extract was over 80% that of the untreated extract. However, less than 5% of the 3-ribosyluric acid was recovered in the charcoal-treated extract.

When 1.5×10^{-4} *M* solutions of pure uric acid or 3-ribosyluric acid were treated with charcoal, the compounds were completely adsorbed onto the charcoal, but could not be recovered in the ethanol/NH_4OH eluate. Other bases and nucleosides similar to uric acid were also treated with the charcoal. All of these compounds were quantitatively adsorbed to the charcoal and, with the exception of 1,3-dimethyluric acid, they were recovered in the ethanol/NH_4OH filtrate (Table 1). All of these studies were carried out with the Baker and Adamson decolorizing carbon. Charcoal was obtained from two other sources and the same result was obtained with uric acid.

Since both uric acid and 3-ribosyluric acid are labile in alkali, both compounds were incubated in the ethanol/NH_4OH solvent without charcoal for 30 min and were then evaporated to dryness. The dried residue was dissolved in the original volume of ethanol/NH_4OH and the ul-

TABLE 1
ADSORPTION TO CHARCOAL AND SUBSEQUENT RECOVERY OF BASES AND NUCLEOSIDES

Compound	Concentration (m*M*)	Percentage adsorbed	Percentage recovered
Adenine	0.14	97	80
1,3-Dimethyluric acid	0.15	98	0
Guanine	0.16	97	57
Hypoxanthine	0.15	96	93
Inosine	0.13	96	100
3-Ribosyluric acid	0.15	97	0
Uric acid	0.15	100	0
Xanthine	0.17	98	70
Xanthosine	0.18	97	91

traviolet absorption spectrum determined. The spectra obtained were almost identical to the original ones indicating that the solvent was not degrading the uric acid and 3-ribosyluric acid.

When [2-^{14}C]uric acid (1.5×10^{-4} *M*) was treated with carbon, over 92% of the radioactivity and over 97% of the ultraviolet-absorbing material was adsorbed to the charcoal. After the charcoal was washed with water followed by ethanol/NH_4OH, over 78% of the radioactivity was recovered in the ethanol/NH_4OH. After chromatography of the ethanol/NH_4OH eluate in six solvents and preparation of radioautograms, it was found that there were at least four radioactive compounds, none of which were uric acid. The major radioactive compound recovered was allantoin which accounted for over 85% of the radioactivity.

Since solvents other than the ethanol/NH_4OH mixture used in this study have been used to elute bases and nucleosides from charcoal, the above experiment was repeated using five other solvents to elute the carbon. These solvents were ethanol:water:NH_4OH (60:39:1), ethanol:water:NH_4OH (60:39.9:0.1), ethanol:water:pyridine (50:40:10), 7% phenol, and 0.1 *N* NaOH. With each of these solvents 70–85% of the radioactivity adsorbed to the charcoal was recovered. When these eluates were chromatographed on paper in six different solvents and radioautograms prepared, none of the radioactivity was present in uric acid and over 85% of the label was in allantoin. A radioautogram prepared from a chromatogram run in *t*-butanol:methylethylketone:formic acid:water (8:6:3:3) is shown in Fig. 2.

DISCUSSION

Charcoal is widely used for the adsorption of aromatic compounds to separate them from salts and other compounds that do not adsorb. Nucleic acid bases and nucleosides have been shown to adsorb to and elute

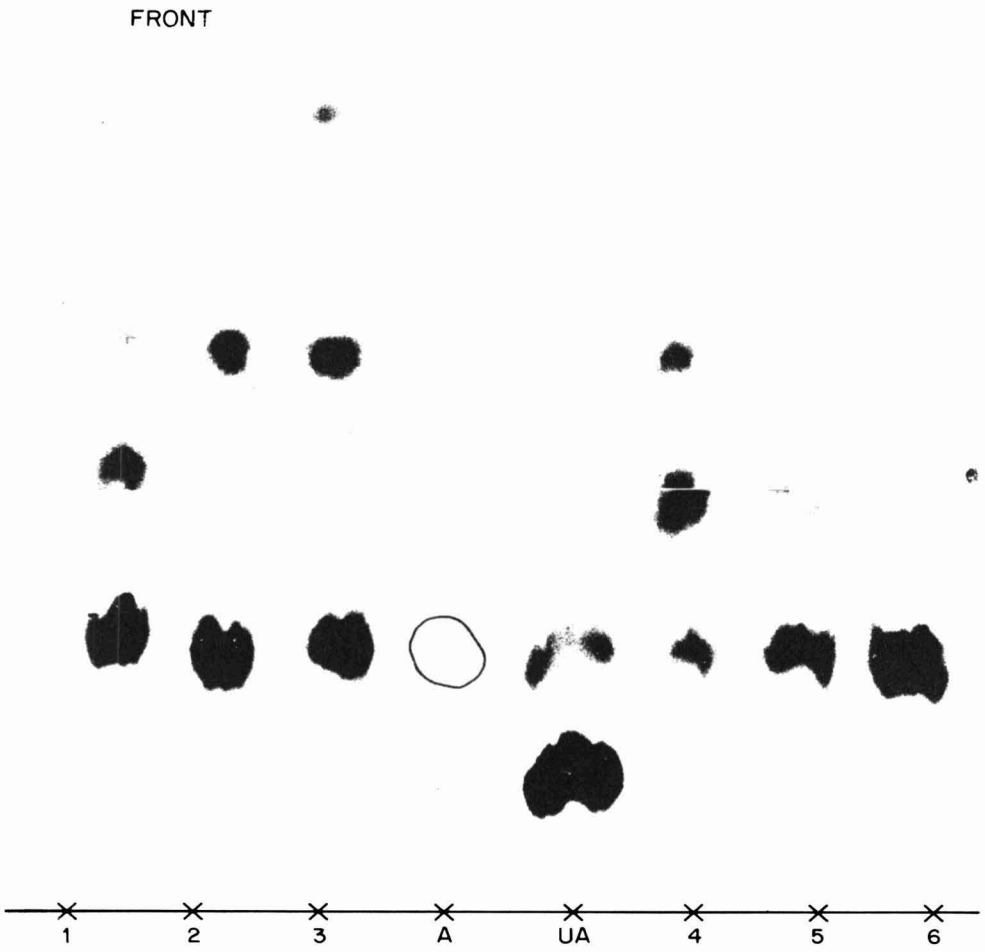

FIG. 2. Radioautogram prepared from a paper chromatogram of [2-^{14}C]uric acid that had been adsorbed to charcoal and eluted with (1) ethanol:water:NH_4OH (60:36:4); (2) ethanol:water:NH_4OH (60:39:1); (3) ethanol:water:NH_4OH (60:39.9:0.1); (4) ethanol:water:pyridine (50:40:10); (5) 7% phenol; and (6) 0.1 *N* NaOH. [2-^{14}C]Uric acid (UA) and allantoin (A) were used as standards. The solvent used for chromatography was *t*-butanol:methylethylketone:formic acid:water (8:6:3:3).

from charcoal by a number of workers. In the present study it was observed that when dilute solutions were used, neither uric acid nor 3-ribosyluric acid could be recovered from charcoal after they were adsorbed. With [2-^{14}C]uric acid over 85% of the radioactivity was recovered as allantoin. It is likely that the 3-ribosyluric acid was converted to a ribosylallantoin.

Dedrick *et al.* (*3*) reported that uric acid treated with activated carbon was partially converted to other products that were not identified. Tijssen

et al. (*9*) reported that creatinine in the presence of active charcoal and oxygen or air was converted to at least two products, one of which was identified as 2-amino-4,5-dihydro-1-methylimidazol-4-one. Brooks and Lant (*2*) reported that [2-^{14}C]uric acid was chemically unstable when stored in strongly alkaline solutions at +4 or −20°C. Allantoin and allantoic acid were the two major degradation products. Even in 0.1 *M* Na_2HPO_4, pH 9.5, uric acid was degraded although the rate was lower. In the present study we did not detect any [^{14}C]allantoic acid after elution of [2-^{14}C]uric acid from charcoal. Although [^{14}C]allantoin was the major product isolated, there were at least two other radioactive compounds detected.

Recently Edwards *et al.* (*4*) determined the concentration of a number of purines in the urine of normal subjects and subjects with a deficiency of hypoxanthine–guanine phosphoribosyltransferase. In these studies when uric acid was determined spectrophotometrically directly on the urine, the concentration of uric acid was much higher than inosine or hypoxanthine. However, when the urine samples were first adsorbed on charcoal, eluted with ethanol:NH_4OH, and then fractionated on a Bio-Gel P-2 column, the concentration of inosine and hypoxanthine markedly exceeded that of uric acid in some of the treatments. On the basis of what was observed in the present study, it is possible that this difference in quantitation may be because of high losses of uric acid during the charcoal treatment.

SUMMARY

Uric acid and 3-ribosyluric acid at a concentration of 1.5×10^{-4} *M* were quantitatively adsorbed to charcoal, but were not recovered when the charcoal was washed with ethanol:water:NH_4OH (60:36:4), a solvent which readily eluted a number of other bases and nucleosides. With [2-^{14}C]uric acid it was shown that the radioactivity was adsorbed to the charcoal and that [^{14}C]allantoin was the primary product recovered after elution. Incubation of uric acid or 3-ribosyluric acid in the ethanol:water:NH_4OH did not result in any degradation. The elution of uric acid from charcoal with other eluents such as 7% phenol, 0.1 *M* NaOH, or ethanol:water:pyridine (50:40:10) also resulted in the conversion of uric acid to allantoin. It was concluded that when uric acid and 3-ribosyluric acid are adsorbed to charcoal and then eluted, there is a substantial conversion of these compounds to the corresponding allantoin.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 26, 394–398 (1981)

Metallo-Amino Acid Complexes

IV. Copper Complexes

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INTRODUCTION

Earlier studies (*1*, *2*, *3*, *4*) have been continued. Limitations of space and material confine this report to complexes of $CuCl_2 \cdot 2H_2O$ with tryptophan, lysine, and aspartic acid.

PROCEDURE

The procedure was the same as that in the previous paper (*4*) except that the copper-complex solutions were evaporated to dryness at room temperature and the dry granules recrystallized from 95% ethanol. In-

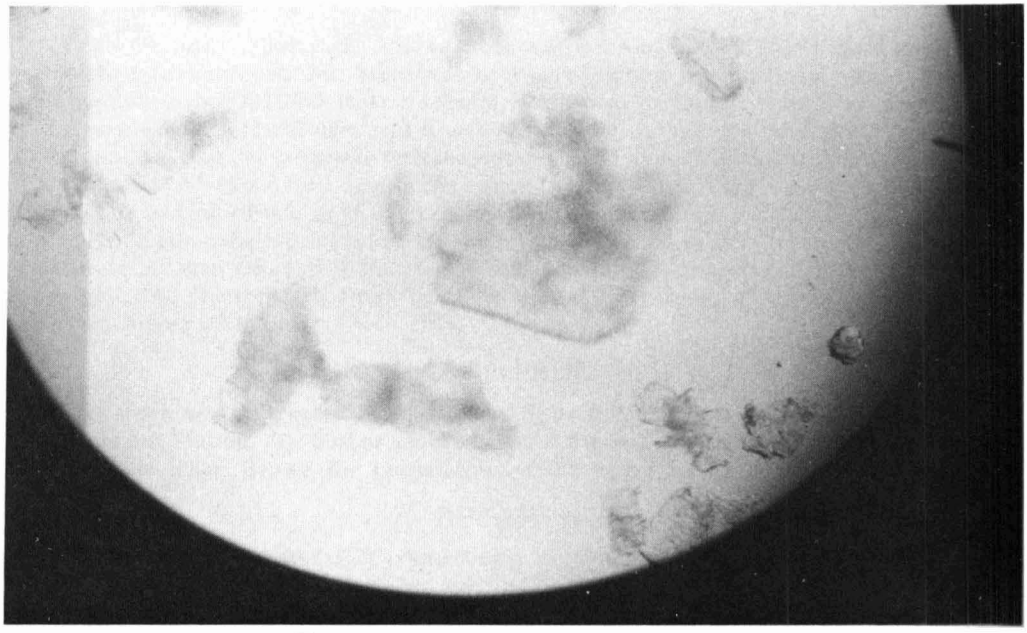

FIG. 1. Crystals of aspartic acid. ×80.

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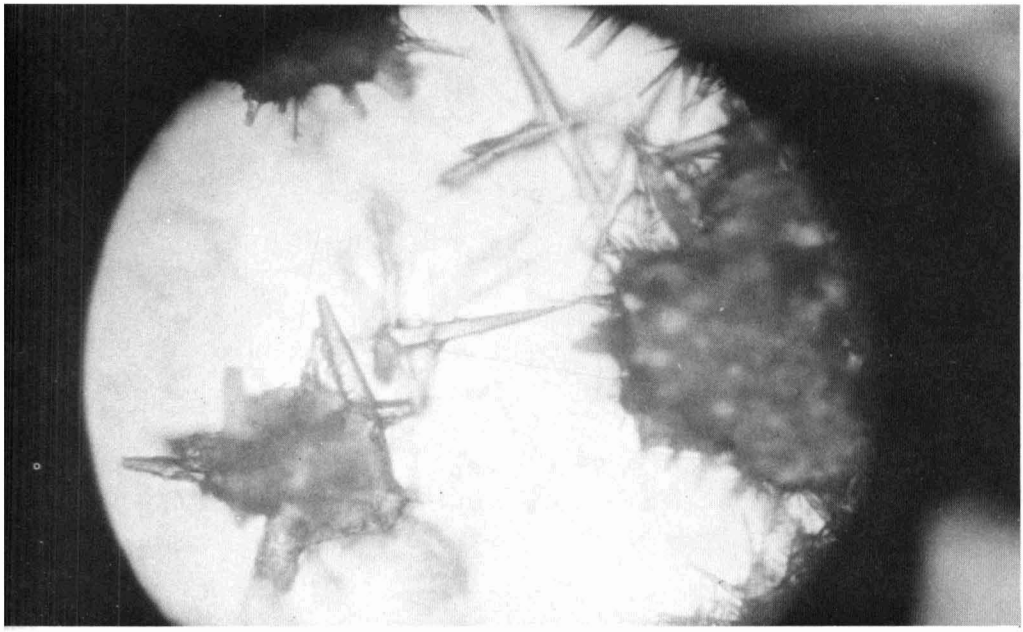

FIG. 2. Crystals of aspartic acid–Cu complex. ×80.

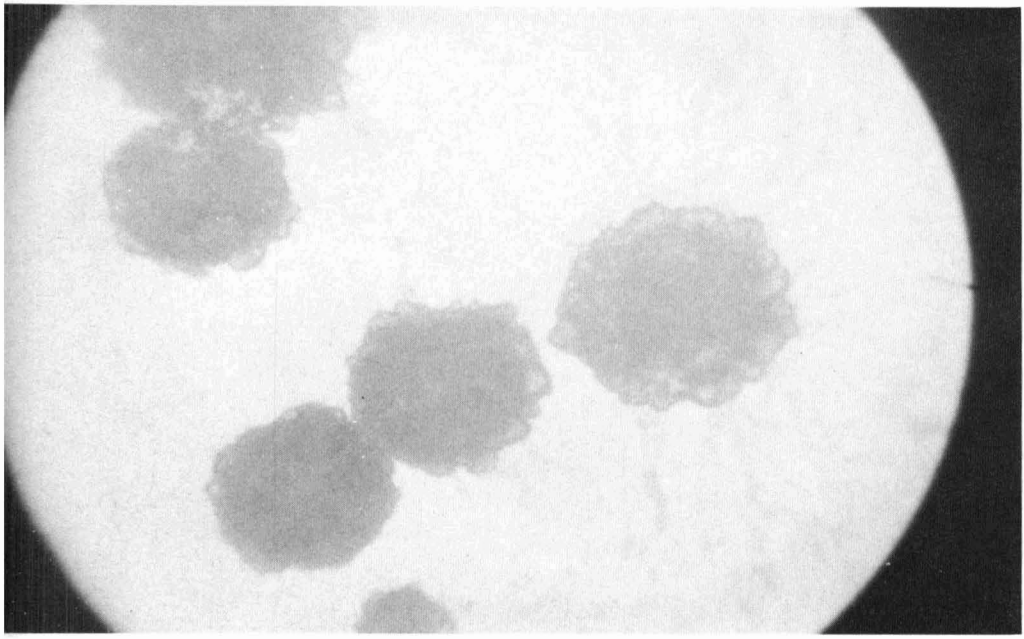

FIG. 3. Crystals of lysine. ×900.

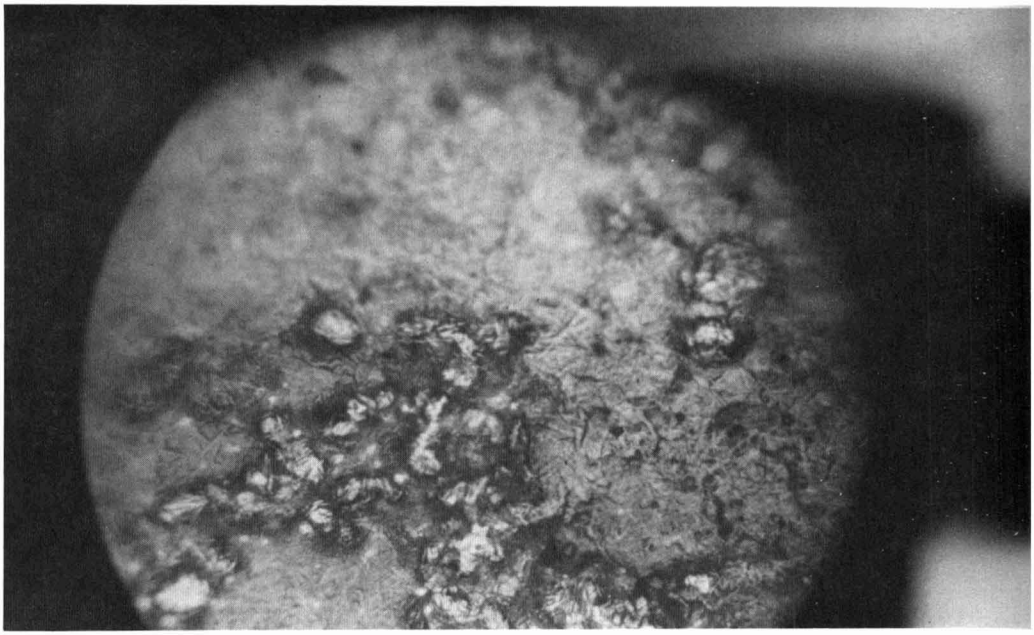

FIG. 4. Crystals of lysine–Cu complex. ×80.

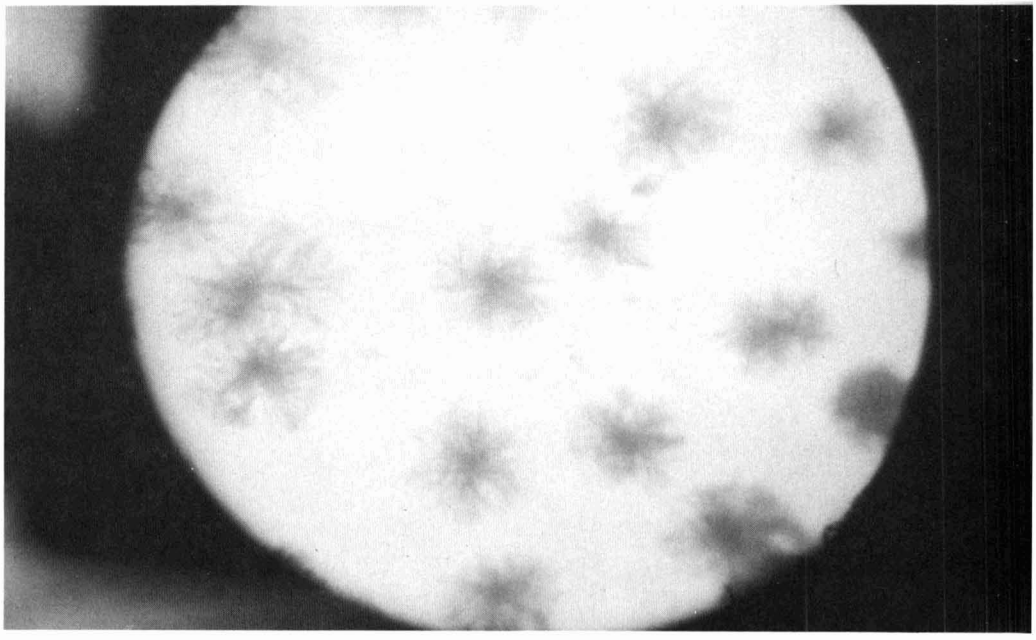

FIG. 5. Crystals of tryptophan. ×80.

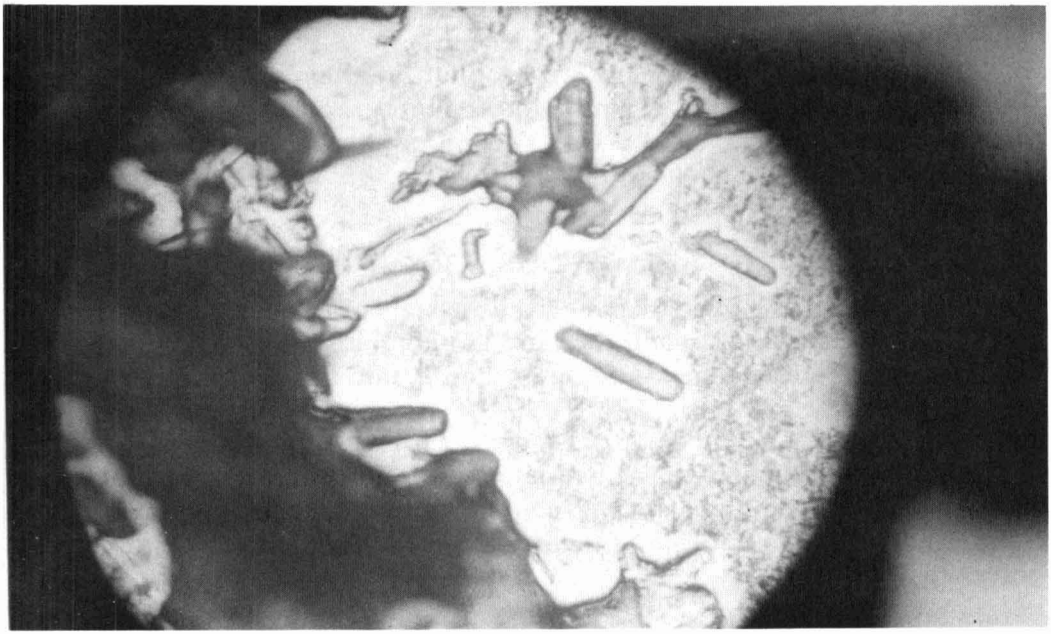

FIG. 6. Crystals of tryptophan–Cu complex. ×80.

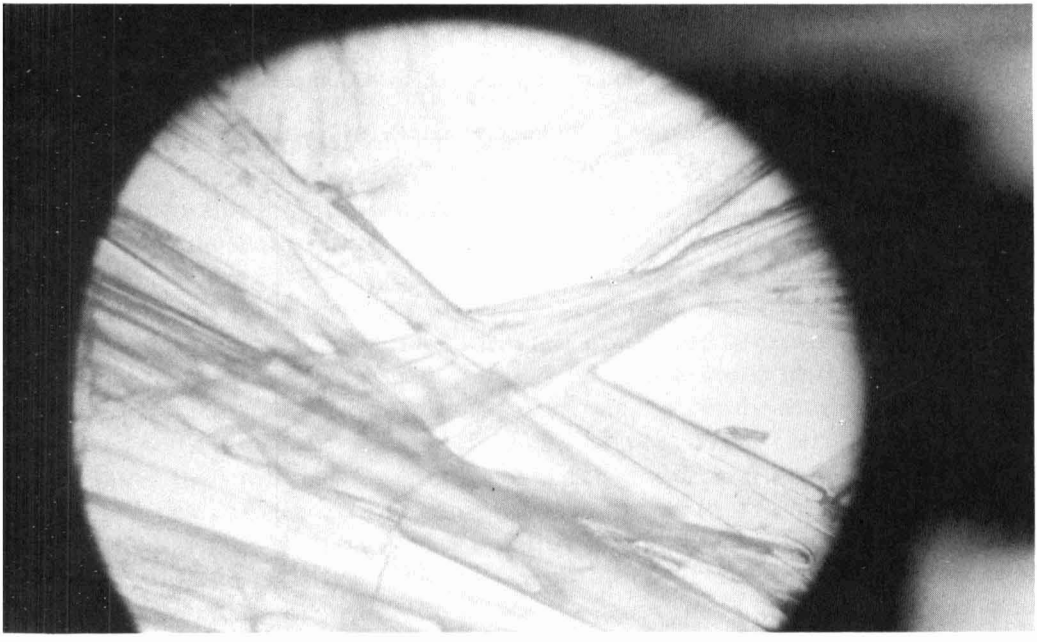

FIG. 7. Crystals of $CuCl_2 \cdot 2H_2O$. ×80.

stead of the expected blue shown by the lysine and aspartic acid complexes, the tryptophan complex was green.

For comparison, pure lysine, tryptophan, and aspartic acid were also recrystallized from 95% ethanol. Photomicrographs were taken by the method previously described (*5*). The photomicrographs of pure lysine were taken at 900× because at 80× the crystals appeared to be empty circles.

DISCUSSION

The marked differences between the crystals of the copper–amino acid complexes (Figs. 2, 4, 6) and the pure amino acids (Figs. 1, 3, 5) indicate complex formation. Their stabilities could not be determined.

CONCLUSIONS

Photomicrographs of pure lysine, tryptophan, and aspartic acid are compared with complexes of these amino acids formed with $CuCl_2 \cdot 2H_2O$ (Fig. 7). The unusual forms of the crystals of pure tryptophan are worthy of note.

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MICROCHEMICAL JOURNAL 26, 399–401 (1981)

Chromatographic Identification of Lipids from the Aqueous Eye Humor

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INTRODUCTION

Lipids used to be intensely studied due to their pronounced biochemical importance. They are valuable components of living organisms, employed as structural and store materials, and they actively participate in cell metabolism. Among the most frequently applied analytical techniques, successful in the investigation of lipids, are thin-layer and gas chromatography. Various authors (*1–3, 5, 6*) suggest ways of extraction and chromatographic determination of different lipid groups in biological materials. The aim of this paper is to make an attempt toward establishing of conditions enabling separation as well as identification of lipids from the aqueous eye humor. In our experiment we used the aqueous humor taken from pig eye.

EXPERIMENTAL

A sample of aqueous humor, its volume approximately 0.3 cm^3, was taken from the anterior eye chamber by means of a syringe. An aqueous humor from three single eyes was extracted with chloroform. Thus the humor sample was treated with a double volume of chloroform (i.e., 1.8 cm^3) and shaken for 30 min, then centrifugated at 3500 rpm. We obtained three separate fractions: the water, the peptide, and the chloroform one. The bottom chloroform layer contained lipids and therefore it underwent further procedure. First it was condensed to a volume of 0.2 cm^3. Then it was chromatographically investigated, using ready-made glass plates covered with silica gel Kieselgel 60 (E. Merck, West Germany), the sorbent layer 0.25 mm thick, which were activated for 10 min at 110°C. The obtained chloroform extract sample was applied in a volume of 50 μl, and those of standards dissolved in chloroform in a volume of 5 μl.

After having experimented with a number of mobile phases the best results proved to be obtained with one composed of cyclohexane and ethyl acetate in a volume ratio of 4:1. After development of a chromato-

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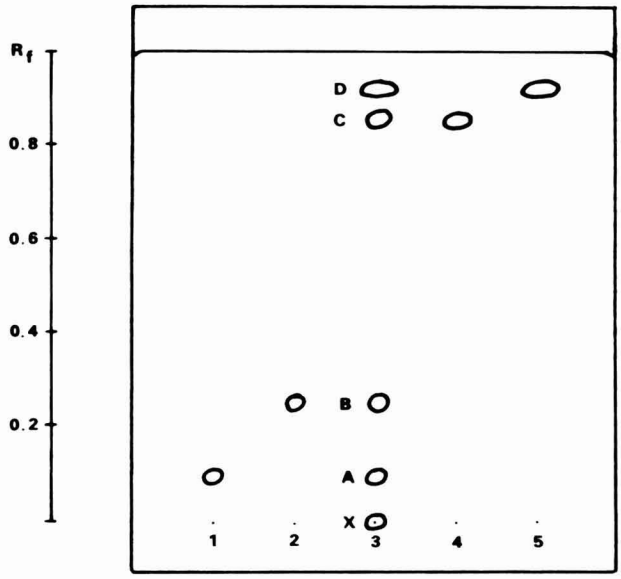

FIG. 1. Chromatographic separation of lipids from the aqueous eye humor; mobile phase: cyclohexaneethyl acetate = 4:1 (v/v).

gram it was dried and visualized in iodine vapors as well as by means of phosphomolybdic acid (*4*). The obtained separation is shown in Fig. 1.

At the starting points one spotted: (1) oleic acid, (2) cholesterol, (3) the investigated sample, (4) triglyceride, and (5) cholesterol palmitate.

On the basis of the performed investigations it was established that the aqueous eye humor contained the following fractions: (A) unsaturated fatty acids, (B) cholesterol, (C) triglycerides, (D) esters of cholesterol and higher fatty acids, and (X) unidentified fraction.

It seems highly probable that the fraction denoted at the chromatogram as the X one contains phospholipids. Identification of the chromatographically separated substances was performed comparing the R_f number values of lipids appearing in a sample and those taken as standards. The above mentioned data are given in Table 1.

TABLE 1
THE R_f VALUES FOR LIPIDS FROM THE AQUEOUS EYE HUMOR

Lipids	R_f values
(A) Unsaturated fatty acids	0.08–0.13
(B) Cholesterol	0.20–0.27
(C) Triglycerides	0.81–0.88
(D) Esters of cholesterol and higher fatty acids	0.92–0.95

DISCUSSION

An investigation was performed of lipids from the liquid eye humor, applying TLC. It is determined from the obtained results that to identify those lipids in the described analytical conditions one needs eye humor from at least three single eyes. The established separation conditions concerning lipids from the aqueous eye humor as well as their identification would enable quantitative determination of those compounds.

SUMMARY

An effort was undertaken to establish the TLC analytical conditions enabling separation and identification of lipids from the aqueous eye humor.

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MICROCHEMICAL JOURNAL 26, 402–410 (1981)

The Determination of Sulfide in the Aqueous Environment

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INTRODUCTION

The combustion of the fossil fuels can potentially mobilize many elements into the environment at rates, in general less than, but comparable to, their rates of flow through water during the weathering cycle (*5*). Since the principal sites of coal combustion are point sources, changes in the composition of the natural environment become most evident relatively close to the source. In particular, the burning of coal for electric power generation is the principal source of SO_2 pollution. In the United States, about 50% of our electricity is generated by coal and another 12% by oil (*10*). From Table 1, it can be seen that in 1974, about 24.3 million tons of sulfur oxides were produced by stationary sources (electric generating plants); most of this was from the combustion of coal (*9*). In 1979, 542 million tons of coal were used for electric power generation which accounts for about 65% of our total coal production. About 95% of the sulfur compounds mobilized is in the form of SO_2 (*12*).

The total generating capacity of Texas is about 40,000 MW (megawatts). This value is expected to increase to 60,000 MW by 1990 with coal accounting for about 30% (*4*). A typical 1000 MW generating plant consumes about 3.6 million tons of coal per year, or about 10,000 tons per day. Thus, as electric companies begin to use more coal and less oil, there will be an increase in the pollutant SO_2 in the environment of these areas (*6*). Houston Lighting and Power Company (HL & P), which provides electrical energy for the area under study, released their projected fuel requirements for 1977–1990 (see Table 2). It can be seen that HL & P foresees an almost total conversion from natural gas to other fuels by 1990 with coal and oil accounting for almost 75% of the total (*11*).

Sulfur dioxide as a major species in the atmosphere is either oxidized to the sulfates or reduced to the sulfides (H_2S, HS^-, or S^{2-}). Since many of these sulfides are soluble in water, they end up in our aqueous environment. Therefore, it is essential that a simple and rapid method be devel-

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TABLE 1
EMISSIONS OF AIR POLLUTANTS IN THE UNITED STATES, BY SOURCE (MILLION TONS/YEAR, 1974)

Source	Sulfur oxides	Nitrogen oxides	Hydro-carbons	Carbon monoxide	Partic-ulates
Transportation	0.8	10.7	12.8	73.5	1.3
Stationary fuel combustion[a]	24.3	11.0	1.7	0.9	5.9
Industrial processes	6.2	0.6	3.1	12.7	11.0
Solid waste disposal	0.0	0.1	0.6	2.4	0.5
Miscellaneous[b]	0.1	0.1	12.2	5.1	0.8
Total	31.4	22.5	30.4	94.6	19.5

[a] Fuel combustion for stationary sources is essentially electric generating plants which are the largest producers of sulfur oxide pollutants.
[b] Includes oil and gasoline production.
Source: Environmental Protection Agency, in "Council on Environmental Quality, 1975" (reproduced from reference 9).

oped which will determine the total sulfide concentration in water and wastewater. The principle objective of this study is to develop a field electrode method for the determination of the sulfide ion in the aqueous environment. Since the sulfide-selective electrode appeared to be well suited for routine determination of sulfide in water because of the simplicity of the measurement, an ion-selective method was used (*1–3, 7*).

EXPERIMENTAL

Apparatus

An Orion Model 94-16 A silver/sulfide ion-selective electrode and an Orion Model 90-02 double-junction reference electrode with Orion filling solution in the inner (Orion's 90-00-02) and outer (10% KNO_3) chambers were used to detect the sulfide ion. An Orion Model 901 digital ionalyzer was used for precision laboratory titration and known addition measurements. An Orion Model 407 A/F specific ion meter was used for direct

TABLE 2
PROJECTED FUEL REQUIREMENTS FOR HOUSTON LIGHTING AND POWER COMPANY FOR 1977, 1980, AND 1990 (HL & P 1980)

Fuel source	1977	1980	1990
Natural gas	100%	77%	11%
Oil	0%	5%	31%
Coal & lignite	0%	18%	43%
Nuclear	0%	0%	15%

Source: Reproduced from reference *11*.

measurement in the laboratory and in the field. The pHs of the samples were obtained by using an Orion Model 91-95-00 combination pH electrode. A magnetic stirrer was used during all measurements (except where stated).

Reagents

A stock solution of saturated sodium sulfide was prepared by dissolving approximately 100 g of reagent grade $Na_2S \cdot 9\ H_2O$ in 100 ml of deaerated water. After vigorously shaking the solution, it was allowed to stand overnight and was stored in a tightly capped bottle. A sulfide antioxidant buffer (SAOB II) was prepared by dissolving 35 g of ascorbic acid, 67 g of disodium EDTA, 200 ml of 10 *M* NaOH in 600 ml of deaerated water in a 1-liter volumetric flask. The mixture was swirled to dissolve the solutes and then diluted to 1 liter. Fresh SAOB II ranged in color from colorless to pale yellow-brown. When SAOB II solution turns dark brown, it has become oxidized, and must be discarded. New SAOB II solutions were stored in tightly capped bottles. All solutions and standards were prepared with deaerated deionized water. A 0.1 *M* lead perchlorate standard solution (obtained from Orion Research) was used to standardize the sulfide solution by titration using the silver/sulfide electrode as an end-point indicator. A weekly sulfide standard was prepared by pipetting 10 ml of the stock solution into a 1-liter volumetric flask. 500 ml of the SAOB II solution was added and the flask was diluted to the mark with deaerated water. The exact concentration was determined by titrating 25 ml of the standard solution with 0.1 *M* lead perchlorate using the silver/sulfide ion-selective electrode as the end-point indicator.

Sampling

Samples of surface waters were collected from lakes in the vicinity of the first local coal-fired generating plant and from the Houston Ship Channel (SE Texas). The samples were collected in precleaned plastic containers (polyethylene) and the containers were capped immediately. Samples were divided into two groups, one for laboratory analysis, and the other for field analysis which was buffered with SAOB II. The measurements were done by use of silver/sulfide electrode immediately. The other samples were transferred back to the laboratory for further study.

RESULTS AND DISCUSSION

The determination of sulfide samples in the field has proven to be very difficult because of the rapid oxidation of the sulfide ions in the standards and samples by oxygen from the air. This oxidation makes it almost impossible to standardize a sulfide solution under field conditions. Therefore, our standard solutions were prepared in the laboratory just before leaving for the field.

A weekly sulfide standard was prepared by pipetting 10 ml of a saturated sulfide solution into a 1-liter volumetric flask, adding 500 ml of the SAOB II solution, and diluting the flask to the liter mark with deaerated deionized water. The exact concentration of the weekly standard was determined by titration with a 0.1 *M* lead perchlorate standard solution using the silver/sulfide ion-selective electrode as the end-point indicator. Standards for use in the field were then prepared by serial dilutions of the weekly standard. A tenfold dilution, which was prepared by pipetting 10 ml of the weekly standard into a 100 ml plastic volumetric flask with screw cap, adding 45 ml of SAOB II, and diluting to the mark with deaerated deionized water, resulted in a solution concentration on the order of 10^{-3} *M*. A 10^{-4} *M* solution was prepared by pipetting 10 ml of the 10^{-3} *M* solution and following the above procedure. Solutions with a concentration of 10^{-5}, 10^{-6}, and 10^{-7} *M* were prepared in a similar manner.

These standards were all tightly capped in the plastic volumetric flasks and transported to the field sites. Once in the field, the standards were used to calibrate the field instrument (Orion Model 407 A/F Specific Ion Meter). In calibrating the meter, two standards were needed which bracketed the concentration of the unknown samples. The calibration process is described in detail by Orion Research (*13*).

All samples were buffered 1:1 with SAOB II before each measurement. The SAOB II buffer contains ascorbic acid which retards air oxidation of sulfide ions in solution. The buffer also fixes the pH of the solution at a highly alkaline level (pH $>$ 12) which insures that the sulfide present occurs chiefly as S^{2-} rather than as HS^- or H_2S which is present at lower pH levels. The electrode only sensed S^{2-}.

Retardation Effect of SAOB II

In this study, we needed to know the effect of oxygen in the atmosphere upon our samples with respect to time. Cromie and co-workers observed an oxidative consumption of sulfide ions when they replaced the nitrogen stream in their isolated sulfide system with oxygen (*8*). In order to investigate this effect, we made four solutions of the same concentration. The sulfide concentration of the four solutions (W, X, Y, and Z) were measured and recorded immediately. Two of the four samples were exposed to the atmosphere (sample W which contained SAOB II and sample X which did not) and the other two samples, Y and Z were placed in tightly closed containers (SAOB II was added to sample Y while sample Z contained no SAOB II). Each sample was tested for sulfide every 24 hours for a period of 5 days. The solutions were tested again on the 7th day and on the 14th day. The results of these tests are shown in Tables 3, 4, 5, and 6. It can be seen from the tables that the rate of oxidation appears to double for those samples without SAOB II in both the exposed and unexposed

TABLE 3
CONCENTRATION, AS A FUNCTION OF TIME, OF S^{2-} IN A STANDARD SULFIDE SOLUTION CONTAINING SAOB II AND EXPOSED TO AIR AT ROOM TEMPERATURE (SAMPLE W)

Time (hr)	Concn. (ppm)	Rate[a] (per hr)
0	749	
24	713	1.5
48	665	2.0
72	641	1.0
96	605	1.5
144	461	3.0

[a] Rate = Disappearance of S^{2-} in ppm per hr.
Note. All measurements were done at pH = 12.7.

TABLE 4
CONCENTRATION, AS A FUNCTION OF TIME, OF S^{2-} IN A STANDARD SULFIDE SOLUTION CONTAINING SAOB II AND STORED IN TIGHTLY CAPPED CONTAINERS AT ROOM TEMPERATURE (SAMPLE Y)

Time (hr)	Concn. (ppm)	Rate[a] (per hr)
0	749	
24	734	0.6
48	720	0.6
72	706	0.6
96	677	1.2
144	590	1.8

[a] Rate = Disappearance of S^{2-} in ppm per hr.
Note. All measurements were done at pH = 12.7.

TABLE 5
CONCENTRATION, AS A FUNCTION OF TIME, OF S^{2-} IN A STANDARD SULFIDE SOLUTION WITHOUT SAOB II AND STORED IN TIGHTLY CAPPED CONTAINERS AT ROOM TEMPERATURE (SAMPLE Z)

Time (hr)	Concn. (ppm)	Rate[a] (per hr)
0	744	
24	713	1.5
48	691	0.9
72	662	1.2
96	648	0.6
144	590	1.2

[a] Rate = Disappearance of S^{2-} in ppm per hr.
Note. All measurements were done at pH = 12.7.

TABLE 6
CONCENTRATION AS A FUNCTION OF TIME OF S^{2-} IN A STANDARD SULFIDE SOLUTION WITHOUT SAOB II AND EXPOSED TO AIR AT ROOM TEMPERATURE (SAMPLE X)

Time (hr)	Concn. (ppm)	Rate[a] (per hr)
0	749	
24	676	3.0
48	605	3.0
72	576	1.2
96	504	3.0
144	204	6.2

[a] Rate = Disappearance of S^{2-} in ppm per hour.
Note. All measurements were done at pH = 12.7.

cases for the first 2 days. They also show that the rate of oxidation is about three times faster for the samples with SAOB II added when exposed to the air but only two times faster when samples not containing SAOB II are exposed for the first 2 days.

Presently, this portion of the study is under reinvestigation and expansion. The initial concentration and laboratory conditions seem to greatly effect the results. We also plan to investigate this portion under field conditions.

Measurement of Unknown Samples

Unknown samples were collected from a series of lakes in Fort Bend County, Texas, at three different times and once from the Houston Ship Channel, near LaPorte, Texas. The sulfide ion concentration of these samples were determined by direct measurement by use of the Orion Model 407 A/F specific ion meter. The potential of each solution was read directly from the instrument in millivolts and converted to ppb by the use of the following equation:

$$C = A \cdot W,$$

where:

A equals the potential in millivolts,
W equals the concentration of the more concentrated standard in ppb, and
C equals concentration of the sulfide ion in the sample in ppb.

As shown in Table 7, the sulfide ion concentration of the unknown samples ranged from a low of 4 ppb to a high of 18 ppb for those samples collected in Fort Bend County. The samples collected from the Houston Ship Channel contained more sulfide with a range of 13 ppb to 42 ppb. We

TABLE 7
MEASUREMENTS OF THE CONCENTRATIONS S^{2-} IN UNKNOWN SAMPLES AT ROOM TEMPERATURE

Samples	Concentration (ppb)
SL 1-3	6
SL 2-2	5
SL 4-1	6
SL 1-2	4
MC 5-2	5
MC 6-2	4
MC 15-2	8
MC 2-2	18
MC 1-2	6
MC 3-2	5
A	42
B	13
C	19
D	16

Note. All measurements were done using SAOB II solutions. Samples A, B, C, and D were collected from the Houston Ship Channel (all others from Fort Bend County).

did not experience any interferences from the other ions which may have been present in these samples but dissolved oxygen and oxygen from the air were always a problem during these measurements. However, this problem was eliminated by use of the SAOB II.

The concentration of S^{2-} in unknown samples in the laboratory and field (Table 8) are significantly different because the magnetic stirrer was not used in the field determination. However, this probelm seems to have been eliminated at the present time by operating the magnetic stirrer with current from the automobile battery.

TABLE 8
MEASUREMENTS OF THE S^{2-} CONCENTRATION IN THE UNKNOWN SAMPLES IN THE FIELD AND IN THE LABORATORY

Samples	Concn.[a] (ppb)	Concn.[b] (ppb)
A	28	43
B	8	13
C	5	19
D	9	16

[a] Concentrations were obtained in the field.
[b] Concentrations were obtained under laboratory conditions.
Note. Field measurements were done without the aid of a magnetic stirrer.

TABLE 9
MEASUREMENTS OF THE S^{2-} CONCENTRATION IN THE UNKNOWN SAMPLES IN THE FIELD AND IN THE LABORATORY

Samples	Concn.[a] (ppb)	Concn.[b] (ppb)
MC 1-1	5	2
SL 3-3	3	0
SI 1-4	0	0
SI 4-2	0	0

[a] Concentrations were obtained in the field.
[b] Concentrations were obtained under laboratory conditions.

The discrepancy of sulfide concentration shown in Table 9 may be credited to the air oxidation of the sulfide ions. By comparing the concentration of S^{2-} in the field and in the laboratory (measurements after 24 hours) the rate of oxidation is approximately 3 ppb per 24 hours.

It may be concluded that sulfide ion concentrations (as low as 4 ppb) can be determined directly in the laboratory or in the field by use of this electrode method.

It was observed that the sulfide ion concentration of the unknown field samples changed as shown in Table 9 when returned to the laboratory in tightly capped plastic containers. Therefore, it seems as if it might be possible for one to actually make their measurements after the return trip back to the laboratory without measurable loss of sulfide ions due to air oxidation within a 4-hour period since the change in the table is observed to be small.

SUMMARY

Direct measurements were made by use of a sulfide ion-selective electrode in conjunction with a double-junction reference electrode and an Orion Model 407 A/F specific ion meter. This method enables simple and rapid determinations of the total sulfide ion concentration in water. Total sulfide was determined in samples collected from lakes in Fort Bend County, Texas, which ranged from 4 ppb to 18 ppb and from the Houston Ship Channel which ranged from 13 ppb to 42 ppb.

A series of experiments shows that (1) the rate of oxidation of S^{2-} exposed to air is about three times slower for solutions containing the sulfide antioxidant buffer (SAOB II) and (2) the rate of oxidation of S^{2-} in tightly capped containers is about twice as fast for solutions not containing SAOB II than for solutions containing SAOB II.

It was concluded that the use of SAOB II almost eliminates the problem of air oxidation of sulfide ions during direct measurements and that sulfide ion concentrations (as low as 4 ppb) can be determined directly in the laboratory and in the field by use of this electrode method.

ACKNOWLEDGMENTS

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Critical Study of the Interference of Silicic Derivatives on Fluoride Determination

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INTRODUCTION

The methods used for fluoride determination in some complex pharmaceutical dosage forms containing, for example, trace metals and vitamins, must be accurate, specific, and sensitive. Potentiometry with a fluoride-selective electrode (*12*) and spectrophotometry of complexes with zirconium(IV)–sodium alizarine sulfonate or cerium(III)–alizarine complexon (*2*, *3*) are among the more commonly used methods.

In almost all cases, previous separation of the fluoride anions from the interfering matrix is necessary: this step is usually performed by microdiffusion as hydrofluoric acid (*4–6*) or by codistillation of hexafluorosilicic acid with superheated steam (*12*) after reaction of fluoride with a strong inorganic acid (perchloric, sulfuric, or phosphoric) in the presence of silica.

Very few interferences are observed with the last separation procedure, which makes it specially attractive. Indeed, the main interferences produced by chloride anions and aluminum(III) are easily eliminated by addition of the appropriate reagent: silver sulfate and phosphoric acid, respectively (*8*).

Until recently, no interference has been reported in the presence of a silicic derivative; on the contrary, the addition of a small quantity of silica (washed sand or glass beads) is usually recommended in order to promote the hexafluorosilicic acid formation.

During fluoride determinations in pharmaceutical dosage forms, we recently discovered that silicic acid present in a studied form was indubitably responsible for an interference during hexafluorosilicic acid distillation as well as hydrofluoric acid microdiffusion (*7*).

As this phenomenon could not be explained according to the chemical nature of the interfering derivatives, we decided to investigate some of their physical properties in order to get a logical explanation of the interference. For this purpose, the influence of particle size, surface area, and

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pore distribution of various samples of silica, silicic acid, and silica gel were examined concerning the recovery of added fluoride anions after hexafluorosilicic acid distillation. We describe hereafter the results of this study.

MATERIALS AND METHODS

Apparatus and reagents

Apparatus for distillation with superheated steam according to Hanocq *et al.* (*12*, *8*):

Beckman Acta V ultraviolet-visible spectrophotometer.

Quantasorb (Quantachrome Corp.) surface-area analyzer (*11*).

Orr (Micrometrics Corp. Model 2100 A) surface-area and pore-volume analyzer (*9*).

Standard fluoride solutions (250 μg F^-/5 ml and 10 μg F^-/10 ml).

Concentrated $HClO_4$, 70%, analytical grade.

0.02 *N* NaOH.

CH_3COOH/CH_3COONa, buffer solution, pH 3.5.

0.001 *M* alizarine complexon.

0.001 *M* $Ce(NO_3)_3$.

50% (v/v) DMSO.

The preparation of these solutions was described elsewhere (*2*, *3*). Silicic derivatives: their nature is mentioned in Table 1.

Procedures

Distillation with superheated steam (*12*, *8*). A known amount (100, 200, or 300 mg) of a silicic derivative is introduced in the appropriate flask of the apparatus as well as three glass beads; 25 ml $HClO_4$ (70%) and 250 μg fluoride (5 ml of a standard solution) are then added and the codistillation of hexafluorosilicic acid with superheated steam, which is generated in another flask, is initiated. The temperature of the reaction mixture is kept constant by means of a solvent (tetrachlorethane) distilling in a closed vessel surrounding the first flask.

The superheated steam is regulated in such a way that 200 ml water distils in 1 hr. The distillate is collected in a 250 ml volumetric flask containing 10 ml of 0.02 *N* NaOH solution and the fluoride determination is achieved after diluting to the mark.

The calibration assay is performed in the absence of silicic derivative whereas, in the reagent blank, fluoride as well as silicic derivative are absent. These assays are performed in the same way as the main assay.

Spectrophotometry with alizarine complexon (*2*, *3*). The distillate (10 ml) or 10 ml of a standard solution (containing 10 μg F^-/10 ml) are introduced in a 50-ml volumetric flask. Then, with agitation after each

TABLE 1

SPECTROPHOTOMETRIC DETERMINATION OF FLUORIDE ADDED TO VARIOUS SILICIC DERIVATIVES AFTER HEXAFLUOROSILICIC ACID DISTILLATION AND DETERMINATION OF SOME PHYSICAL PROPERTIES OF THE SAMPLES

Sample	Determination of 250 μg F			Physical properties		
	Silicic derivative (mg)	Percentage recovery	Number of analyses	Granulometry acc. to the supplier (μm)	Specific surface area (m^2/g)	
					Quantasorb	Orr
Silicic acids						
Sample 1	100	90.1 ± 1.5	3			
	200	87.6 ± 1.4	2	—	53	31.3
	100″	96.6	1			
Sample 2	100	98.6	1	—	204	181.6
	200	100.0	1			
Silica gels						
Sample 3	100	92.2 ± 0.4	2			
	300	83.7	1	200–500	416	—
	100″	98.6	1			
Sample 4	100	93.6 ± 1.2	4			
	300	86.2	1	10–40	482	—
	100″	98.6	1			
Sample 5	100	95.0 ± 1.0	3	63–200	636	—
	300	89.6 ± 2.3	2			
Sample 6	100	95.6 ± 1.0	2	—	56	34.5
	300	91.2	1			
Silica						
Sample 7	100	98.6 ± 0.7	2	—	13	—
	100″	99.3	1			
Sample 8	100	99.3	1	—	0.6	—
	300	99.3	1			

″ Determination of a new amount of fluoride added to a sample after having achieved a first assay, without washing the distillation apparatus between both analyses.

addition, 2 ml pH 3.5 buffer, 5 ml Ce $(NO_3)_3$, 25 ml DMSO, and 5 ml alizarine complexon solutions are successively added and the flask is filled with water to the mark. The absorbance of these solutions is measured after 10 min at 625 nm against the reagent blank.

Surface area, adsorption and desorption isotherms, and pore-volume distribution (*9*, *11*). Degassing of the sample is accomplished by nitrogen flowing through the powder for either 5 hr at 150°C (for measurements with "Quantasorb") or 15 hr at room temperature (for measurements with "Orr").

A dynamic, continuous flow system is used in the first instrument (Quantasorb); BET surface area was obtained by using two gaseous mixtures (10 and 30% nitrogen in helium). A static volumetric system is used in the second instrument (Orr); BET surface area, adsorption and desorption isotherms, and pore-volume distribution were obtained by using different nitrogen equilibrium pressures.

All measurements were achieved at liquid nitrogen temperature. The calculations were performed by following the standard procedures recommended by the suppliers (*9*, *11*).

RESULTS AND DISCUSSION

Recoveries of 250 μg fluoride added to various quantities of silicic derivatives (100, 200, and 300 mg) are reported in Table 1: two silica samples (7 and 8) as well as one silicic acid (sample 2) do not interfere during hexafluorosilicic acid distillation, even when they are present in large quantities. (The recovery of a known amount of fluoride determined in the same manner, in the absence of a silicic derivative, is 99% $\pm$ 1% with $n = 5$.) On the other hand, the second studied silicic acid (sample 1) and all silica gels cause interferences to a certain extent.

Two experimental findings must be underlined: first, the magnitude of the interference increases by addition of a larger amount of interfering derivative; second, nearly quantitative recoveries are obtained for each studied substance if a second determination is performed in the presence of a silicic derivative when a new addition of fluoride is made subsequently to a first assay (footnote *a* in Table 1). It therefore seems that the phenomenon responsible for the stated interference is neutralized during the first part of this last experiment.

These results incited us to determine the adsorption characteristics of the silicic derivatives by means of the analysis of their specific surface area. We first examined some supplier's data about granulometry (see Table 1) but quickly found that the interference could not be explained by this physical parameter. The precise surface area determination by two different instruments (see Experimental) led to the results reported in Table 1. It appears clear that no logical explanation of the interference can

be given by the collected data; indeed, no relation can be established between the importance of the interference and the magnitude of the surface area.

Nevertheless, the adsorption–desorption isotherms (Fig. 1) of three selected derivatives (two silicic acids and one silica gel) gave an interesting piece of information: considering the presence of a hysteresis loop in the region of low relative pressure, we observed that a microporous character was common to all adsorbents (*10*).

Moreover, it seemed to us that the shapes of the isotherms were slightly different from one derivative to another, which would imply a difference in the pore-volume distribution.

In order to get a more precise representation of the pore structure of the three selected samples, we drew graphs of the pore-volume distribution by calculating them from desorption isotherm data using standard procedures (*1*).

In Fig. 2, each experimental point corresponds to the pore volume (as

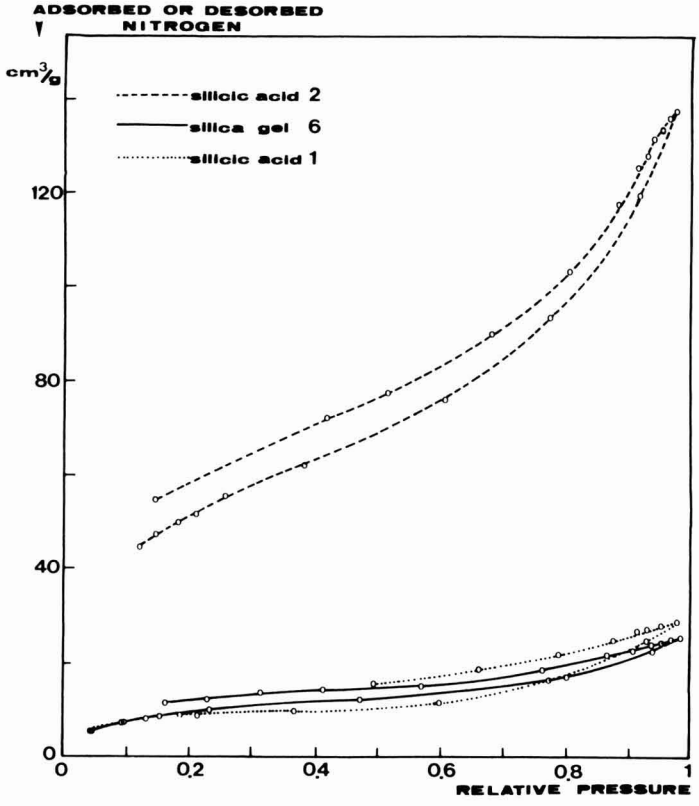

FIG. 1. Adsorption and desorption isotherms of three silicic derivatives.

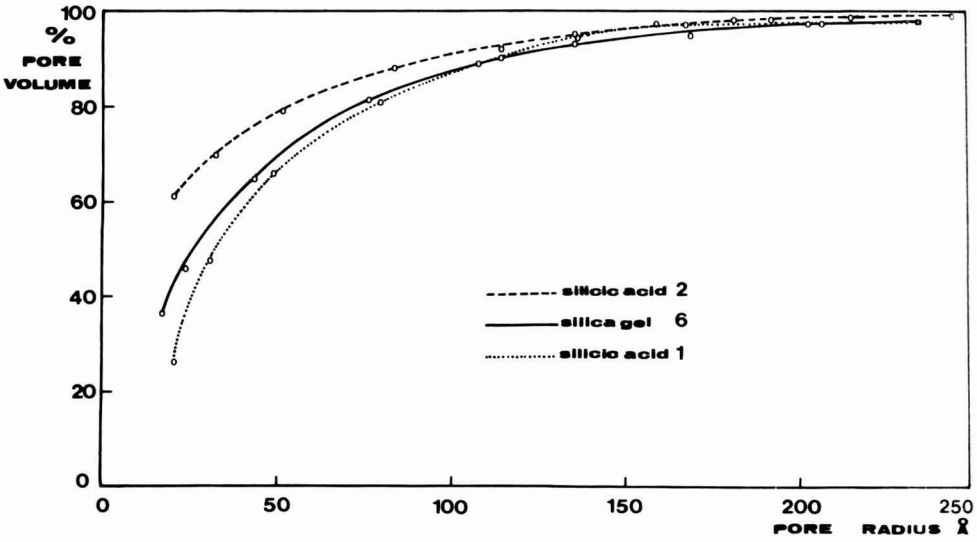

FIG. 2. Pore-volume distribution of three silicic derivatives.

percentage of total volume) with a radius ≤ to the corresponding value of the abscissa (Å). This figure indicates the existence of appreciable differences among derivatives; moreover, this last physical parameter can directly be correlated with the magnitude of the stated interference: the derivative with very small pores (silicic acid, sample 2) does not interfere during hexafluorosilicic acid distillation whereas the increase of pore size can be put in parallel with the increase of the importance of the studied interference (respectively, silica gel sample 6 and silicic acid sample 1).

On the grounds of these experimental results, we can give a plausible explanation of the reported interferences by attributing them to some porous structure characteristics of the silicic derivatives. Indeed, typically nonporous silicic derivatives (*10*)—such as the two studied silicas—do not interfere during hexafluorosilicic acid distillation; on the other hand, porous adsorbants—such as silicic acids and silica gels—are liable to interfere when their pores reach a definite size that is approximately 50 Å in diameter.

In conclusion, we think that an imprisonment of some fluoride anions (or one of their derivatives) occurs in the pores of the interfering silicic derivatives and that the interference is affected by larger pore dimensions.

In our opinion, the reported results are likely to be of interest not only for the analyst whose duty is to check the conformity of pharmaceutical dosage forms but also for the supplier who has to consider the risk of a decrease in bioavailability of fluoride in complex preparations including silicic derivatives.

SUMMARY

Several physical properties of various silicic derivatives are examined in order to elucidate an interference with fluoride during codistillation of hexafluorosilicic acid with superheated steam.

The stated interference cannot be explained by the granulometry or by the specific surface area of the interfering derivatives but their porous structure: a nonporous adsorbent (silica) does not interfere; a porous adsorbent (silicic acid and silica gel) is liable to interfere when its pores reach a definite size, approximately a diameter of 50 Å.

The occurrence of imprisonment of some fluoride anions in the pores of the interfering silicic derivatives is suggested.

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Stoichiometry, Ringbom Optimal Range, and Other Parameters for the Copper(I)–Bathocuproine Complex

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INTRODUCTION

The spectrophotometric determination of microamounts of copper is made at present with several reagents (*3,5–12,18*), most of them connected with phenantroline. A book has been published on this subject (*17*).

Since Smith and Wilkins (*19*) described in 1953 the new reagent bathocuproine (2,9-dimethyl 4,7-diphenyl 1,10-phenantroline) several workers have used it in different materials (*1, 2, 22*).

The author of the present paper, in collaboration with J. Henriet (*15*), used it for the determination of soluble copper in insoluble copper fungicides. The method was applied as described by Merck (*13*) and the results were not good. After some modifications, the method was applied by CIPAC in a collaborative study (*16*) and accepted as a full CIPAC method (*4*).

The reaction is the following: Copper is reduced to the Cu^+ state with ascorbic acid. After adding the bathocuproine reagent, the colored complex is extracted with chloroform, its absorbance measured at 465 nm, and compared to a standard calibration curve.

Some of the best conditions for the formation of the complex and some factors which affect it are known, but until now the stoichiometry of the complex and the Ringbom optimal range have never been studied, although they have been presumed.

EXPERIMENTAL

Reagents

All the solvents and reagents are the same as those used in a previous work (*16*).

Apparatus

The apparatus was as described in a previous paper (*16*).

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Procedure

a. Preparation of the calibration curve. The methodology is the same as that employed in the cited previous paper (*16*).

b. Determination of copper. Pipet 20 ml of the solution, containing $10-40 \times 10^{-6}$ g of copper to a 100-ml separating funnel. Add 1 ml ascorbic acid solution and shake. After 1 min add 2.5 ml of sodium acetate solution and shake. After another minute develop the color by pipetting 10.0 ml of the bathocuproine solution in chloroform. Extract the complex by shaking vigorously for 1 min. Allow the layers to separate, filter the chloroform extract through the filter paper into a clean and dry stoppered tube, and measure the absorbance at 465 nm, about 15 min after the extraction, using chloroform as reference.

If the extinction is higher than the extinction of the latest point of the calibration curve, dilute the solution with the buffered solution in such a way that the extinction falls on the calibration curve. Read off the quantity of copper ($Q \times 10^{-6}$ g) corresponding to the extinction found:

$$\text{Percentage of copper} = 0.05\ Q \text{ mg liter}^{-1}.$$

If the solution has been diluted, correct the formula accordingly.

Carry out a blank determination on reagents, using 20 ml of the sodium acetate–acetic buffer solution. The extinction must be of the same order as that for the extinction of the point 0 on the calibration curve.

RESULTS AND DISCUSSION

Effect of the bathocuproine solvent. Merck (*13*) proposed ethanol as solvent for bathocuproine. We demonstrated that using ethanol the decomposition of the complex was greater than 10% in 10 min, whereas using chloroform (with ethanol as stabilizer of course) as solvent for the bathocuproine, the complex obtained was three times more stable, although in both cases the stability was not complete, at least during the first hour.

Effect of light, temperature, and time. The reaction was developed in sunlight and in the dark, at 4 and 25°C, and measures of absorbance taken for 1 hr from 5 to 5 min.

The complex was almost stable during the first hour and no effects were observed because of the variation of these factors.

Adsorption of the copper on the glass. The use of the buffered solution of sodium acetate–acetic acid was introduced after checking that some quantities of copper were adsorbed on the glass wall, when very diluted solutions were used, at neutral pH and very low ionic strength.

Order of addition of reagents. Although it was demonstrated that this is not an important matter, we always add the reagents in the following order: ascorbic acid, sodium acetate, bathocuproine.

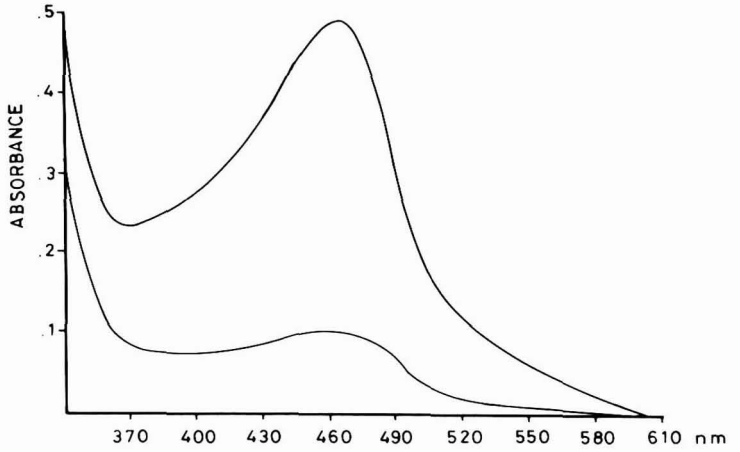

FIG. 1. Absorption spectra of copper(I)–bathocuproine complex solutions at pH 6.5 and concentrations of 1.25 and 0.1325 × 10^{-6} g ml^{-1}.

Adsorption spectra. It was studied from 340 to 600 nm, every 5 nm, with two copper solutions at 1.25 and 0.1325 × 10^{-6} g ml^{-1}, respectively. Results are shown on Fig. 1 and it can be seen that there is formation of only one complex with a maximum of absorbance at 465 nm.

Stoichiometry of the reaction. The continuous-variations method (*20*) and the molar ratio method (*21*) were applied and represented in Figs. 2 and 3, respectively. The initial concentrations of copper were 1.387 and 0.4 × 10^{-4} *M*, respectively. In both cases the conclusion is that the forma-

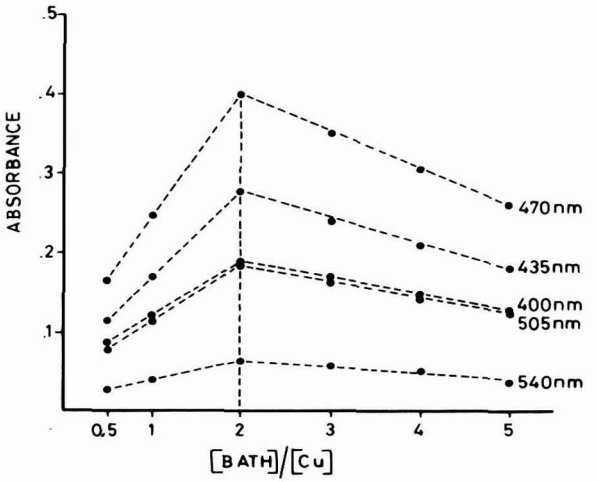

FIG. 2. Stoichiometry of copper(I)–bathocuproine complex (continuous variations method). The initial concentration of copper was 1.387 × 10^{-4} *M*.

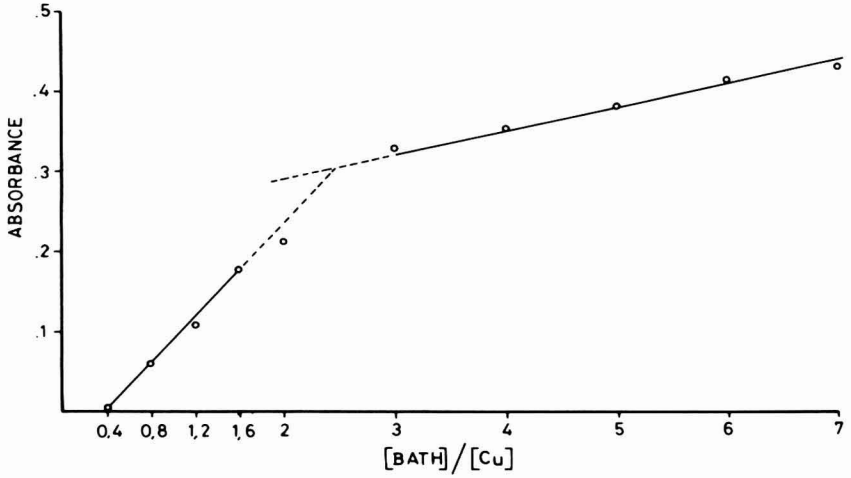

FIG. 3. Stoichiometry of copper(I)–bathocuproine complex (molar ratio method). The initial concentration of copper was 0.4×10^{-4} *M*.

tion of the complex takes place in the ratio copper(I) to bathocuproine of 1:2.

Figure 2 shows at the same time that only one complex is formed, being all the curves, at different wavelengths, of the same shape.

Minimum error space and Beer's law. Figure 4 shows the representation of concentrations/absorbances. Beer's law is followed between 0.2 and 4.4×10^{-6} g of copper ml^{-1}. The molar absorptivity is 1.3×10^4 liters mol^{-1} cm^{-1}.

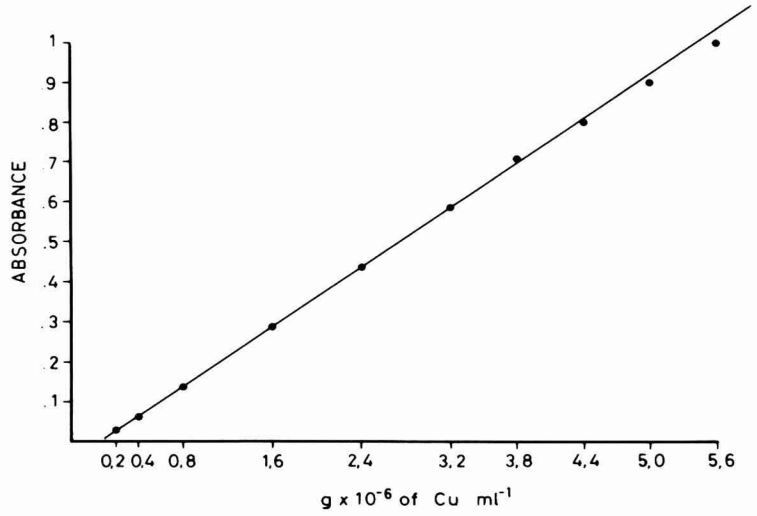

FIG. 4. Verification of the Beer's law by the complex copper(I)–bathocuproine.

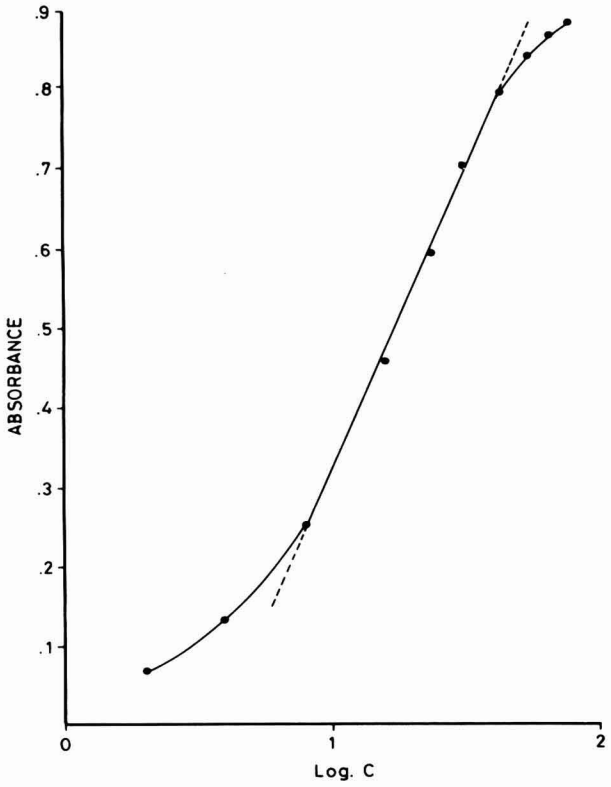

FIG. 5. Ringbom's plot for the copper(I)–bathocuproine complex solutions.

Ringbom's plot (*14*) for the copper(I)–bathocuproine complex solution is on Fig. 5 and the optimum range was found to be from 0.7 to 4.0 $\times$ 10^{-6} g of copper ml^{-1}.

Reproducibility. Ten determinations were made over a solution prepared at 2 $\times$ 10^{-6} g of copper ml^{-1}. Results were the following: 1.980, 2.005, 1.985, 2.005, 2.008, 2.015, 1.980, 2.035, 2.032, and 2.020. Results are quite good, with a standard deviation of 0.020 and a relative standard deviation of 0.995 for a mean concentration of 2.0065 $\times$ 10^{-6} g ml^{-1}.

Influence of foreign ions. Although this subject has been widely studied, we have checked it and our results, summarized in Table 1, are not, in fact, in complete accordance with those recognized by Smith and Wilkins (*19*) who do not find any interference, which they justify by saying that it is normal, because phenantrolinic complexes, in general, do not present any interference. Our results are not even in accordance with those given by Merck (*13*); so Cd, Ag, Hg, and Fe do not interfere, in our work conditions, at concentrations 100 times that of the copper. On the contrary, our results coincide with regard to Co and Sn, which show

TABLE 1
INFLUENCE OF FOREIGN IONS ON THE DETERMINATION OF COPPER WITH BATHOCUPROINE

Ions	(Cu) (g ml^{-1})	(Ion)/Cu	Relative error (%)	(Ion)/(Cu)	Relative error (%)
Fe(II)	1.25×10^{-6}	10	−1.5	100	0
Fe(III)	1.25×10^{-6}	10	−1.5	100	+2.0
Mn(II)	1.25×10^{-6}	10	−3.5	100	+3.2
Pb(II)	1.25×10^{-6}	10	+0.4	100	+3.6
Ba(II)	1.25×10^{-6}	10	−1.5	100	+3.2
Sn(II)	1.25×10^{-6}	10	−2.7	100	−59.9
Cd(II)	1.25×10^{-6}	10	−3.5	100	+3.2
Mg(II)	1.25×10^{-6}	10	−3.5	100	0
Co(II)	1.25×10^{-6}	10	−1.5	100	+10.1
Ca(II)	1.25×10^{-6}	10	−1.5	100	+1.2
Hg(II)	1.25×10^{-6}	10	−0.4	100	+1.2
K(I)	1.25×10^{-6}	10	−0.4	100	−1.0
NH_4(I)	1.25×10^{-6}	10	−1.5	100	+1.3
Al(III)	1.25×10^{-6}	10	−0.8	100	−3.6
Ag(I)	1.25×10^{-6}	10	−0.8	100	+1.2
F^-	1.25×10^{-6}	10	−1.5	100	+1.2
Br^-	1.25×10^{-6}	10	−1.5	100	+0.4
$C_2O_4^{2-}$	1.25×10^{-6}	10	+0.4	100	+2.4
NO_3^-	1.25×10^{-6}	20	−1.5	200	−3.2
Cl^-	1.25×10^{-6}	30	−3.5	300	0
SO_4^{2-}	1.25×10^{-6}	50	−0.8	500	−2.5

interference at the cited concentration, although that interference is not appreciable at concentrations 10 times that of the copper. The interference of these two elements (Sn and Co) is probably due to the reducing power of Sn(II) and the colored nature of Co(II) salts.

CONCLUSIONS

With the low copper concentration which we have used in this research, the stability of the copper—bathocuproine complex has been increased three times, by changing the solvent from ethanol to chloroform.

The use of a buffered solution has suppressed the adsorption of copper on the glass walls, eliminating this source of error.

The stoichiometry and the optimum range of the reaction have been studied for the first time, the former being in accordance with what had already been assumed.

The influence of foreign ions has been studied and found not to be in complete accordance with other already published results.

SUMMARY

The stoichiometry of the copper(I)–bathocuproine complex is studied and encountered to be in a 1:2 molar ratio.

The Ringbom optimal range falls between 0.7 and 4.0 × 10^{-6} g of copper ml^{-1}.

Beer's law is obeyed over the range of 0.2–4.4 × 10^{-6} g of copper ml^{-1}, and the molar absorptivity is 1.3 × 10^{4} liters mol^{-1} cm^{-1}.

The standard deviation, calculated from 10 determinations on a solution containing 2 × 10^{-6} g of copper ml^{-1}, is 0.020.

The bathocuproine solvent is changed from ethanol to chloroform.

The influence of foreign ions is studied and compared with the results of other authors.

ACKNOWLEDGMENT

Some helpful suggestions from Dr. M. Lachica are gratefully acknowledged.

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MICROCHEMICAL JOURNAL 26, 426–435 (1981)

Microdetermination and pK_a Measurement of Some Aliphatic Amines Using the Copper-Ion-Selective Electrode[1]

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INTRODUCTION

Many methods are available for the determination of amines. Aqueous and nonaqueous neutralization and nitrosation titrations are the most common methods used (*14*). However, these procedures suffer from a lack of selectivity and instability of the titrants. Selective reactions based on the ability of the amines to coordinate metal ions have been reported. The reaction of aliphatic amines with a mixture of KCNS and cobalt(II) to give a blue complex extractable into organic solvents (*11*), and that based on the reaction of iron(III) in the presence of acetyl chloride to give a greenish violet color (*13*) have been investigated spectrophotometrically.

The reaction of aliphatic amines with copper(II) in nonaqueous media produces a measurable colored complex (*6*). This reaction has also been applied in aqueous acidic media to the spectrophotometric determination of aliphatic and aromatic mono- and diamines (*8*, *12*). The effect of amines on diminishing the absorbance of the Cu–EDTA complex in aqueous media has also been used for the spectrophotometric determination of primary amines singly (*2*) or simultaneously with tertiary amines in mixtures (*3*).

The reaction of secondary amines with CS_2 yields dithiocarbamic acid which, in turn, can react with either copper to give a measurable colored complex (*1*) or with nickel to form a precipitate which can be measured by atomic absorption spectrometry after dissolution (*10*). Atomic absorption spectrometric methods based on the reaction of copper with aromatic and aliphatic amines in the presence of acetaldehyde and nitrosalicylic acid have also been described (*9*).

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All of the reported reactions of amines involving metals have been monitored by spectroscopic techniques, and most of these methods require a time-consuming separation step resulting in poor precision. There are only two previous reports on the use of ion-selective electrodes for the determination of amines. In one (*5*) the amines were reacted with hydrogen chloride gas in anhydrous ether. After ether evaporation they were titrated with silver nitrate using the chloride electrode. The other dealt with the preparation of an air-gap electrode selective for methylamine with some interferences by ammonia and dimethylamine (*7*). This investigation describes a simple and accurate method for the microdetermination of aliphatic amines by direct titration with copper. The equivalence points are potentiometrically monitored using the copper-ion-selective electrode. The method is also used for the measurement of the pK_a of some aliphatic primary amines.

EXPERIMENTAL

Reagents

All reagents used were of analytical reagent grade. Double-distilled water was used throughout.

Apparatus

The potentiometric measurements were made with a Pye–Unicam pH-meter (Model 290) using an Orion copper-ion-selective electrode (94-29) or a graphite–[Ag_2S–CuS] electrode in conjunction with an Orion double-junction reference electrode (90-02) with 10% KNO_3 in the outer compartment. Some measurements were made with a titration system controlled by a Tektronix 4051 graphics system. The pK_a measurements were conducted in a 50-ml double-jacketed cell and the temperature was maintained at 25 ± 0.1°C.

Procedure

Preparation of graphite–[Ag_2S–CuS] electrode. Cut a 3-cm-long rod of spectroscopically pure graphite (~5 mm diameter), place in a clean test tube, add 5 ml of a saturated solution of sodium sulfide and leave for 1 hr. Transfer the rod to another test tube containing a saturated aqueous solution of silver nitrate and leave for another hour. Remove the rod, wash several times with twice-distilled water and ethanol until no silver ions can be detected in the washing solutions. Repeat the same steps using copper sulfate instead of silver nitrate to precipitate CuS on the same rod. Electrical contact is effected by wrapping one end of the rod with a small piece of pure copper metal sheet attached to a copper wire. Insert the electrode in a PVC sleeve leaving about 2 cm of the electrode protruding

as a measuring surface. When the electrode is not in use, store in double-distilled water. The life span of the electrode is at least 8 months.

Procedure for determination of aliphatic amines. Weigh accurately a sample containing 3–20 mg of amine and then dissolve in water, or transfer a 10-ml aliquot of an aqueous solution containing the same weight to a 50-ml beaker. Insert into the solution either a solid-state copper-ion-selective electrode or a graphite–[Ag_2S–CuS] electrode, together with a double-junction reference electrode. Titrate aliquots immediately after sample preparation with 0.01 *M* copper sulfate solution.

Measurement of the dissociation constant of aliphatic amines. Transfer a 10.0-ml aliquot of 0.01 *M* aqueous isopropanolamine to a 50-ml double-jacketed cell thermostated at 25°C. Immerse the copper-ion selective electrode and the double-junction reference electrode and slowly titrate with 0.01 *M* copper sulfate solution. Repeat three times and record the average potential reading at half complexation, $(HCP)_s$. Follow the same procedure with 10.0-ml aliquots of 0.01 *M* of the test primary aliphatic amine sample. Record the average potential at half complexation, $(HCP)_t$. Calculate the dissociation constant of the test amine according to Eq. 1 (*15*).

$$pK_a(aq) = 10.55 - 0.02151\left[(HCP)_s - (HCP)_t\right] \quad (1)$$

RESULTS AND DISCUSSION

Potentiometric Determination of Aliphatic Amines

The potentiometric titration of aliphatic monoamines with copper ions using the copper-ion-selective electrode shows stoichiometric, reproducible, and sharp inflections at points corresponding to a 2:1 molar reaction (Eq. 2). With diamines, if the two amino groups are separated by no more than three methylene groups (e.g., 1,2- and 1,3-diamines) they act as a single amino group (Eq. 3). However, if the amino functions are separated by more than four methylene groups (e.g., 1,4- and 1,5-diamines), they react as two separate amino groups (Eq. 4). This stoichiometry is reproducible in aqueous and water/alcohol media. The stoichiometry obtained by other workers was: respectively, 2Cu:1Am:4Cl (*6*), 1Cu:1Am and 1Cu:2Am (*8,12*) (where Am = amine).

$$Cu^{2+} + 2\ H_2NR + 2\ H_2O \rightleftharpoons \left[(H_2O)_2Cu(H_2NR)_2\right]^{2+} \quad (2)$$

$$Cu^{2+} + 2(H_2N)_2R' \rightleftharpoons \left[R'(NH_2)_2Cu(H_2N)_2R'\right]^{2+} \quad (3)$$

$$Cu^{2+} + (H_2N)_2R'' + 2\ H_2O \rightleftharpoons \left[(H_2O)_2Cu(H_2N)_2R''\right]^{2+} \quad (4)$$

Other metal ions such as Hg(II), Pb(II), and Cd(II) also form complexes with aliphatic amines but in no case were the titration curves and

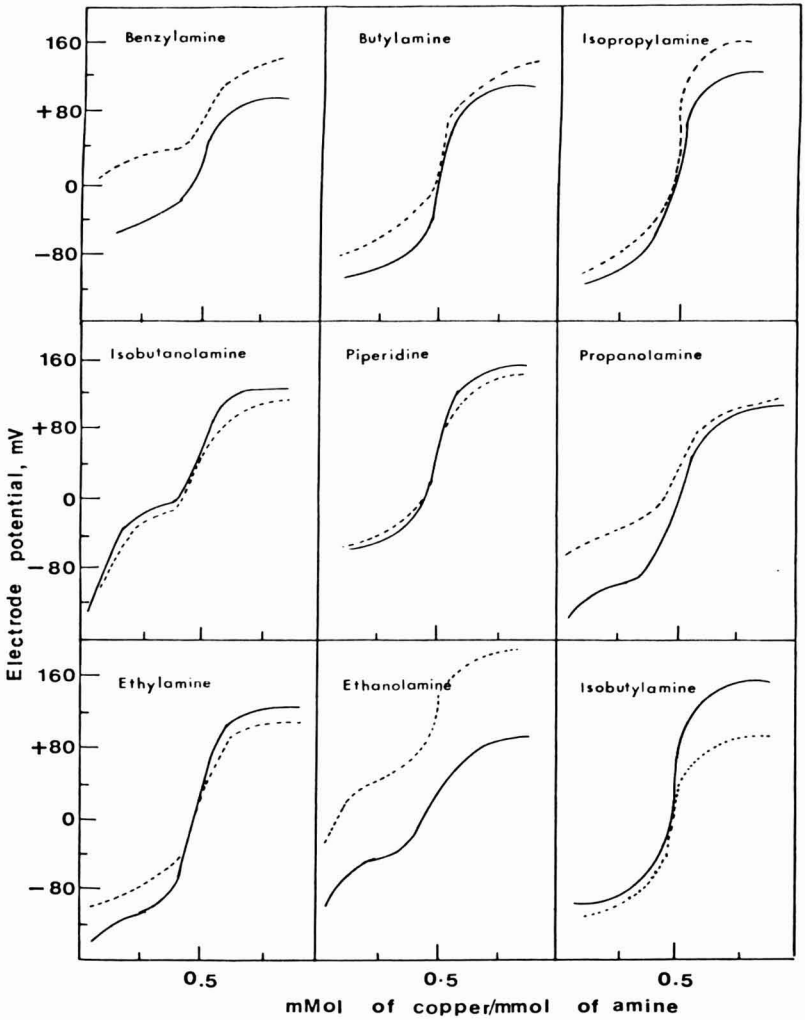

FIG. 1. Typical titration curves of some aliphatic amines using copper sulfate as a titrant and (——) the solid-state copper-ion-selective electrode; (– – –) graphite–Ag_2S–Cus electrode.

recoveries superior to those obtained by using copper. In working with piperidine, we have found that 1 mole of Cd(II) reacts with 2 moles of the amine, like Cu(II). However, for Pb(II) and Hg(II) the stoichiometry is different: 1 mole of Pb(II) reacts with 1.5 moles of amine while 1 mole of Hg(II) reacts with 2.5 moles of the amine. Moreover, unless solutions are immediately titrated with Hg(II), two potentiometric breaks are obtained, and the distance between the two breaks increases with time.

TABLE 1
MICRODETERMINATION OF SOME ALIPHATIC MONOAMINES BY POTENTIOMETRIC TITRATION WITH COPPER SULFATE USING THE SOLID-STATE COPPER-ION-SELECTIVE ELECTRODE

Amine	Weight (mg)		Percentage recovery
	Taken	Found	
Piperidine	7.41	7.20	97.2
	9.32	9.11	97.7
Butylamine	6.11	6.01	98.4
	7.71	7.62	98.8
Benzylamine	10.20	10.11	99.1
	12.20	12.20	100.0
Ethanolamine	13.64	13.53	99.2
	18.15	18.04	99.4
Propanolamine	9.34	9.24	98.9
	11.72	11.63	99.2
Ethylamine	6.02	5.91	98.2
	7.12	7.02	98.6
Isopropylamine	7.14	7.05	98.7
	9.52	9.41	98.8
Isobutanolamine	14.43	14.22	98.5
	16.82	16.41	97.6
Isobutylamine	5.81	5.73	98.6
	11.73	11.62	99.1

Titrations with the various cations were carried out using the corresponding cation-selective electrodes.

The potentiometric titration of some aliphatic amines with standard copper sulfate solution in aqueous media was monitored with the solid-state copper-ion-selective electrode (Ag_2S-CuS membrane) or a simple electrode sensitive to copper ions prepared by precipitation of Ag_2S and CuS onto a graphite rod. Titration curves were obtained with sharp inflections at the equivalence points in the range of 80–200 and 80–140 mV with the solid-state and graphite electrodes, respectively (Fig. 1).

Determination of as little as 0.1 m*M* of various aliphatic monoamines using both electrodes shows an average recovery of 98.5% and a mean standard deviation of 0.5% (Tables 1 and 2). The differences between the results obtained with both electrodes never exceeded 1%. Table 3 lists the results obtained with some aliphatic diamines: the mean standard devia-

TABLE 2
MICRODETERMINATION OF SOME ALIPHATIC MONOAMINES BY POTENTIOMETRIC TITRATION WITH COPPER SULFATE USING GRAPHITE–CuS–Ag_2S ELECTRODE

Amine	Weight (mg)		Percentage recovery
	Taken	Found	
Piperidine	13.03	12.82	98.4
	14.51	14.32	98.7
Butylamine	7.33	7.25	98.9
	10.21	10.11	99.0
Benzylamine	8.90	8.71	97.9
	15.71	15.32	97.5
Ethanolamine	8.13	7.94	97.7
	11.01	10.80	98.1
Propanolamine	8.42	8.34	99.0
	9.90	9.82	99.1
Ethylamine	4.91	4.81	98.0
	6.90	6.80	98.6
Isopropylamine	7.61	7.51	98.7
	10.41	10.22	98.2
Isobutanolamine	11.73	11.54	98.4
	13.74	13.53	98.5
Isobutylamine	6.51	6.40	98.3
	13.52	13.23	97.8

tion here is also 0.5%. The high recoveries of some diamine samples are probably caused by the presence of small amounts of low molecular weight amine impurities.

The method is applicable to the determination of primary, secondary, and tertiary aliphatic amines of $pK_a > 9$. Aliphatic amines of pK_a less than 9 (e.g., triethanolamine and hexamethylenetetramine) show no inflection. Titration of binary and tertiary mixtures of aliphatic monoamines show only one inflection that is equivalent to the total amine nitrogen. The presence of a 100-fold molar excess of amides (e.g., acetamide and benzamide) and aromatic amines (e.g., pyridine and toluidine) does not cause an interference.

Potentiometric Measurement of the pK_a *of Some Aliphatic Amines*

A linear relationship was obtained between the electrode potential at half complexation (HCP) and the pK_a of the aliphatic primary amines.

TABLE 3
MICRODETERMINATION OF SOME ALIPHATIC DIAMINES BY POTENTIOMETRIC TITRATION WITH COPPER SULFATE USING THE SOLID-STATE COPPER-ION-SELECTIVE ELECTRODE

Amine	Weight (mg)		Percentage recovery
	Taken	Found	
1,2-Diaminoethane	5.0	4.9	98.0
	7.0	6.9	98.6
	6.0	5.9	98.3
1,2-Diaminopropane	2.7375	2.501	94.7
	2.7375	2.590	94.6
	2.7375	2.592	94.7
1,3-Diaminopropane	3.800	3.671	96.6
	3.800	3.673	96.7
	3.800	3.673	96.7
1,4-Diaminobutane	3.825	4.175	109.2
	3.825	4.174	109.1
	3.825	4.177	109.2
1,5-Diaminopentane	4.205	4.548	108.2
	4.205	4.567	108.7
	4.205	4.576	108.9
1,7-Diaminoheptane	4.700	4.858	103.4
	4.700	4.874	103.7
	4.700	4.878	103.8

This relation can be used for pK_a measurement of any member of the primary amine series. A more accurate relation was obtained by following a method suggested by Streuli (*15*). Thus, propylamine was chosen as a reference compound and was titrated with copper sulfate using the copper-ion-selective electrode under the same conditions and concentrations used with other amines. The electrode potential at half complexation of propylamine (HCP) was arbitrarily assigned a value of zero and the HCP values of the other amines were referred to it, by algebraic subtraction, to obtain ΔHCP. A linear relationship between the ΔHCP values and the pK_a(aq) of the amines at 25°C was obtained with a slope of 45 mV/pK_a unit (Fig. 2).

The ΔHCP values of various amines are in correct relation to one another and are independent of the day-to-day shift in the liquid junction potential of the electrode system or the measurement conditions. Equation 1, based on the data presented in Fig. 2, represents a mathematical relation between pK_a(aq) of primary aliphatic amines and ΔHCP values

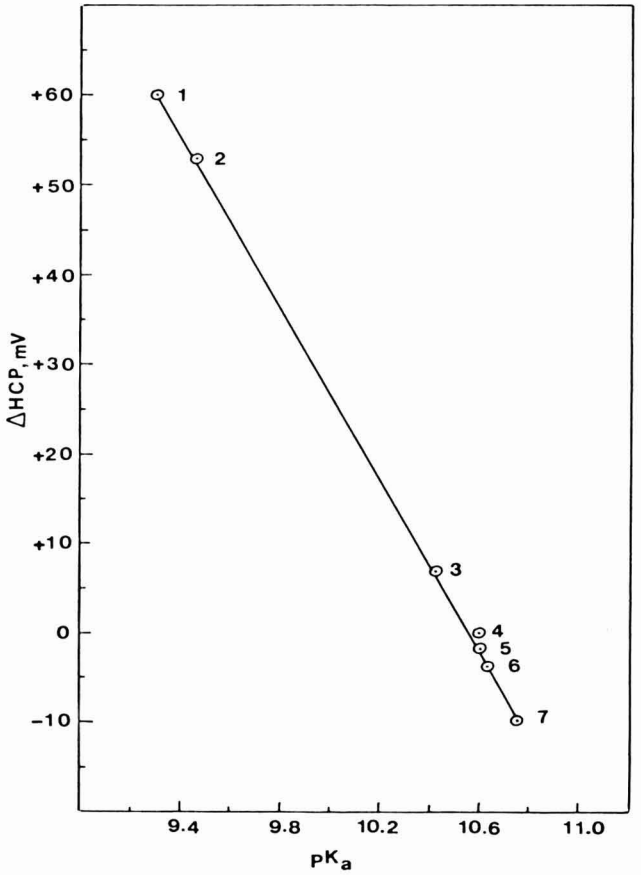

FIG. 2. Relation between the HCP and pK_a of some aliphatic amines: (1) benzylamine; (2) ethanolamine; (3) isobutylamine; (4) propylamine; (5) butylamine; (6) isopropylamine; and (7) ethylamine.

and can be directly used for calculation of the pK_a of any amine in this series. The pK_a values of some amines were calculated using this equation and the results are listed in Table 4. Good agreement was obtained (within ± 0.03 pK_a unit) between the literature and the calculated values for pK_a.

The HCP values can also be used to compare the stability of copper-amine complexes. The higher the HCP values, the greater the dissociation of the complex, and the lower the stability. Thus, the stability of the copper complexes of the aliphatic amines investigated in this study is in the order: ethylamine > isopropylamine> butylamine> propylamine > isobutylamine > ethanolamine > benzylamine, which is the sequence of decreasing pK_a values of the corresponding amines.

TABLE 4
POTENTIOMETRIC MEASUREMENT OF THE pK_a OF SOME ALIPHATIC AMINES USING THE COPPER-ION-SELECTIVE ELECTRODE

Amine	ΔHCP (mV)	pK_a(aq) Literature[a]	pK_a(aq) Calculated	ΔpK_a
Ethylamine	−10	10.75	10.76	+0.01
Isopropylamine	−4	10.63	10.65	+0.02
Butylamine	−2	10.61	10.59	−0.02
Propylamine	0	10.59	10.55	−0.04
Isobutylamine	+7	10.42	10.47	+0.05
Ethanolamine	+53	9.45	9.41	−0.04
Benzylamine	+60	9.30	9.26	−0.04

[a] According to Reference (*4*).

The present method for amine determination offers many advantages over the procedures in current use. Besides the high accuracy and simplicity, it is free from interferences from aromatic amines and amides, and the titrant is very stable. Moreover, the method provides a simple procedure for measurement of the pK_a of aliphatic amines.

SUMMARY

Aliphatic amines were determined on the microscale by potentiometric titration with standard cupric sulfate solution in aqueous or partially aqueous (methanolic) medium. Electromotive forces were monitored with a copper-ion-selective electrode or a graphite rod impregnated with silver sulfide/cupric sulfide, and a double-junction reference electrode. Monoamines and diamines up to 1,3-diaminopropane reacted on a 2:1 amine:Cu basis. Diamines from 1,4-diaminobutane to at least 1,7-diaminoheptane reacted on an equimolar basis. The dissociation constants of some of the amines were determined. A linear relationship exists between the emf at half complexation and the dissociation constants of the amines tested.

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MICROCHEMICAL JOURNAL 26, 436–439 (1981)

A Simple, Rapid, and Accurate Fluorometric Analysis of Epinephrine in Local Anesthetic Solutions

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INTRODUCTION

The fluorometric measurement of epinephrine concentration in local anesthetic solutions has been reported to be somewhat tedious. The reason for the difficulty is that these solutions contain other ingredients including a paraben (usually methylparaben) and sodium bisulfite. For instance Schroeter *et al.* (*7*), Higuchi and Schroeter (*3*), and James (*4*) reported that sodium bisulfite interferes with the development of the epinephrine fluorophor. In addition, James (*4*) suggested that the presence of the local anesthetic will interfere with fluorescence development. Moreover, James (*4*) reported that elaborate elution and separation techniques are required to measure epinephrine fluorometrically and that fluorometric techniques require tedious and complex manipulations resulting in an analysis that is limited in specificity.

In 1977, Parker (*5*) reported that local anesthetic dental cartridges containing epinephrine could be autoclaved without a significant loss of epinephrine concentration despite one report to the contrary (*6*). In 1980 our laboratory reported (*2*) a significant loss of epinephrine content when dental cartridges were exposed to sterilizing ultraviolet irradiation for a 3-month period. The two latter studies utilized a fluorometric analysis for epinephrine.

The present study was designed to show that epinephrine can be measured fluorometrically and in a manner that is simple, rapid, and accurate, and not requiring separation techniques.

MATERIALS AND METHODS

Dental cartridges containing 2% lidocaine hydrochloride and 1:100,000 epinephrine (10 μg/ml)[2] were used in this study. The cartridges had the

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[2] Xylocaine Hydrochloride, Astra Pharmaceutical, Inc., Worcester, Mass. 01606.

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same lot number and expiration date and the analysis was done 8 months before the expiration date.

Six dental cartridges were autoclaved for 10 min at 270°F and 27 psig. Six cartridges were placed on a V-shaped rack such that the cartridges were 2 in. from and at right angles to a line parallel to the long axis of a Sylvania G15 T8 ultraviolet lamp. The cartridges were irradiated for 3 months. A matched set of six cartridges was left in the original container and stored on a laboratory shelf and served as controls. The autoclave and irradiation treatments were chosen because prior studies (*3*, *5*) had shown that autoclave treatment resulted in a slight decrease of epinephrine concentration whereas irradiation exposure caused a marked decrease of epinephrine, i.e., the treatments represent two extremes.

On the day of analysis, 20-μl samples (containing approximately 0.2 μg of epinephrine) were taken from each cartridge of the control, autoclaved, and ultraviolet irradiated groups and placed in test tubes. A second 20-μl sample was taken from all cartridges, placed in test tubes, and 10 μl of a 10 μg/ml epinephrine standard (containing approximately 0.1 μg epinephrine) was added. A third group consisted of a 20-μl sample from each cartridge plus a 20-μl aliquot of the epinephrine standard. All samples were made up to 1.0 ml with 0.2 *N* acetic acid.

The samples and epinephrine standards were oxidized to a trihydroxyindole fluorescent derivative according to the method for norepinephrine described by Ciarlone (*1*). The fluorophors were measured on an Aminco-Bowman Spectrophotofluorometer. The excitation and emission settings were 420 and 500 nm, respectively (uncorrected). The excitation and emission spectra of the epinephrine standard were derived at an earlier time.

RESULTS

As shown in Table 1, the control cartridges averaged 12.1 μg/ml epinephrine; the sample values ranged from 11.5 to 12.8; the addition of 10 μl of standard gave an average value of 17.2 μg/ml epinephrine with a range of 16.9 to 17.4; and, the addition of 20 μl of standard gave an average value of 22.2 μg/ml epinephrine with a range of 21.7 to 22.7. The average incremental increases between the three control lots were 5.1 and 10.1, respectively.

The autoclaved cartridges averaged 11.8 μg/ml epinephrine and the sample values ranged from 11.1 to 12.5; the addition of 10 μl of standard gave an average value of 16.7 μg/ml epinephrine with a range of 16.2 to 18.1; and the addition of 20 μl of standard gave an average value of 21.3 μg/ml epinephrine with a range of 20.7 to 21.7. The average incremental increases between the three autoclaved lots were 4.9 and 9.5, respectively.

TABLE 1
ANALYSIS AND RECOVERY OF EPINEPHRINE[a] CONTAINED IN LOCAL ANESTHETIC DENTAL CARTRIDGES

	20-μl sample	20-μl sample + 10-μl standard	20-μl sample + 20-μl standard
Control (N = 6)	12.1 ± 0.23	17.2 ± 0.10	22.2 ± 0.14
Autoclaved (N = 6)	11.8 ± 0.20	16.7 ± 0.29	21.3 ± 0.15
Irradiated (N = 6)	7.3 ± 0.31	12.2 ± 0.33	17.4 ± 0.26

[a] Listed as the mean concentration in μg/ml ± SEM.

The irradiated cartridges averaged 7.3 μg/ml epinephrine and the sample values ranged from 6.0 to 8.4; the addition of 10 μl of standard gave an average value of 12.2 μg/ml epinephrine with a range of 10.8 to 13.2; and the addition of 20 μl of standard gave an average value of 17.4 μg/ml epinephrine with a range of 16.2 to 18.1. The average incremental increases between the three irradiated lots were 4.9 and 10.1, respectively.

DISCUSSION

The data from the recovery portion of the experiment, i.e., the 10 and 20 μl addition of epinephrine standard, indicate that fluormetric analysis accurately measures the epinephrine concentration in local anesthetic solutions.

In the present study, autoclaving dental cartridges resulted in a 2% decrease of epinephrine concentration and these data correspond to the report by Parker (*5*). Ultraviolet irradiation caused a 40% decrease in epinephrine concentration and these data were higher than that reported in an earlier study (*2*). However, we have found (unpublished observations) that irradiating dental cartridges with ultraviolet light results in an unpredictable decrease in epinephrine content. In fact the higher standard error of the mean (SEM) of the irradiated group also reflects the individually variable response to that treatment.

Lastly, the manufacturer of dental cartridges suggest that these solutions contain 1:100,000 (10 μg/ml) epinephrine. In addition, the United States Pharmacopeia (*8*) suggests that the approved range of concentration of epinephrine in local anesthetic solutions be 9.0 to 11.5 μg/ml. In the present study we report a control value of 12.1 μg/ml and believe the reported value to be correct because of the recovery experiments that were done in this study.

SUMMARY

A method is presented for the fluorometric analysis of epinephrine contained in local anesthetic solutions. The method is simple, rapid, and accurate, and does not require separation techniques.

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BOOK REVIEWS

The Chemical Applications of Transmission Electron Microscopy. By J. R. FRYER. Academic Press, New York, 1979. *X* + 286 pp., $38.00.

It is indeed remarkable for a book of less than 300 pages to cover the material in this active field of solid-state chemistry with such depth, clarity, and critical assessments. The book has been written by a leading investigator in this area with the interest of the serious novice at heart as well as the practicing chemical microscopist. The text is well documented with more than 700 references, many of them dated within the past few years.

The theory and practice of the instrument in addition to specimen preparation are considered in the first part of the book; the second part deals with chemical applications. The scope and organization of the book may be seen from a list of its chapters: **1**, Image Formation and Image Contrast; **2**, Ancillary Apparatus; **3**, Specimen Preparation; **4**, High-Resolution Microscopy and Phase Contrast Image Formation; **5**, Surface Chemistry; **6**, Crystal Chemistry; **7**, Precipitation and Solution Chemistry; **8**, Chemical Reactivity and in Situ Microscopy.

Chemists in general and material scientists should find this volume a useful practical guide in the interpretation of high-resolution micrographs.

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Kirk–Othmer Encyclopedia of Chemical Technology, Vol. 7, 3rd ed. Editorial Board, HERMAN F. MARK, DONALD F. OTHMER, CHARLES G. OVERBERGER, AND GLENN T. SEABORG; Executive Editor, MARTIN GRAYSON; Associate Editor, DAVID ECKROTH. Wiley–Interscience, New York, 1979, xxvi + 891 pp., $120.00, subscription price, $95.00 per volume.

This is the continuation of the volumes reviewed earlier in this journal (*Microchem. J* **24**, 389 (1979); **25**, 141 (1980); **26**, 146 (1981)). Volume 7 continues the subjects beginning with the letter "C" and part of the subjects beginning with the letter "D." There are 52 contributors and 40 subjects covered. Like the other volumes in the series, the subjects covered are of utmost importance and are well done. Certainly, no chemical library will be complete unless it has this complete series.

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Gasohol for Energy Production. By NICHOLAS P. CHEREMISINOFF. Ann Arbor Science Pub., Ann Arbor, Michigan, 1979. x + 140 pp., $14.95.

This volume, part of the Energy Technology Series by this publisher, concerns itself with the conversion of biomass to alcohol fuel production. Of the 140 pages, which include the

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pages for the index, 20 pages are committed to references and bibliography. Covered in the book is material on ethanol and methanol (including their properties and synthesis), automotive use of alcohol fuels, economic predictions on fuel costs, biomass production, and other uses of biomass fuels such as electrical generation. Unfortunately, apparently the author did not peruse the bibliography which he has so nicely compiled (possibly by a computer search) for so much of the material in the book is of a trivial nature and even this is either miscopied or misunderstood. As an example, in Chapter 2, "The Chemistry of Alcohols," any introductory text does a better job and certainly would not list in physicochemical properties the specific gravity of alcohols as "Reference to Air (°C/°C)," when, reference to water is meant. Chapter 3 concerns itself with methanol synthesis. Again, the material is presented unclearly—the reader is left to check the literature if he wishes to find out what arithmetic justifies this statement for the production of methanol from wood, "For hardwood species a very low yield is obtained,. producing only 1–2% methanol (or roughly 2.3 L/tonne)." Ethanol producers will be most pleased to know that according to the author the denaturant criteria of the Bureau of Alcohol, Tobacco, and Firearms for ethanol state that, "The ethanol from the formulated substance be extracted easily for the purpose of human consumption." Other errors are present.

Consequently, the reviewer feels that little faith can be put in this book and he hopes fervently that future books in the series will be of better quality.

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Topics in Automatic Chemical Analysis 1. Edited by J. K. FOREMAN AND P. B. STOCKWELL. Wiley, New York, 1979. 313 pp., $52.50.

This volume is the first in a new series forthcoming from the Ellis Horwood, Ltd., division of Wiley which will survey the current status of laboratory automation and presents reviews of recent developments both in techniques and areas of application. The contributors to the volume are experienced in the work, and this is reflected in the coverage of the material. Both experienced users of automatic systems as well as chemists starting work in adapting automatic analysis to their routine problems will find useful information. Chapter 1 covers the philosophy of automatic analysis and presents information not only on the advantages (including economic factors) but also the limitations of automatic analysis, the type of systems, and the ramifications that automatic analysis have on laboratory personnel. Chapter 2 concerns itself with the problems of designing and building automatic analyzers "in house" when commercial instruments may be unsuitable for the purpose. Obviously in such a chapter only the general considerations of design can be considered, and not the mechanical skills and design of the construction. (Excellent literature references are provided here, as well as at the end of each chapter throughout the book.) Chapter 3 unfortunately reflects in part a repetition of the first two chapters; it provides information on automation in industrial laboratories for individual analysis. The treatment is not as specific as in other chapters in the book. Chapter 4 gives considerable information on automated rate (kinetic) methods with particular information on clinical systems, and is extremely beneficial. Chapter 5 presents information on the application of the Technicon Auto Analyzer II to the analysis of water-soluble vitamins in foodstuffs and covers the subject in excellent detail. It will be most helpful to chemists performing such analyses. Chapter 6 covers imaging detectors for analytical instrumentation. This includes material on optics for absorption spectros-

copy, fluorescence, atomic emission, and Raman Spectroscopy, and on the uses of photodiodes, image dissectors, vidicons, etc. Applications are given to analytical problems, particularly those of biochemical and clinical interest. Chapter 7 critiques certain commercially available clinical analyzers. Chapter 8 applies automation to quantitative gas chromatography with particular emphasis on petrochemical analysis. Subjects such as backflushing, flow switching, etc., as well as sample injection and data processing are included. Chapter 9 concludes the book by presenting an unusual problem—the application of automation to cigarette testing and how this is done with smoking machines to obtain not only conventional expected types of information such as low tars, but also puff profiles and puff frequencies.

The book concludes with a short index. In summary, the book presents considerable information of great interest to chemists concerned with automated analytical procedures. Certain of the contributors treat the material more extensively than others. Irrespective, the net result is a volume of overall merit and value.

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Probing Polymer Structures. Edited by JACK. L. KOENIG. American Chemical Society, Washington, D.C., 1979. x + 277 pp., $33.00.

This volume, "Probing Polymer Structures," compiles 13 papers presented at a symposium held at the 174th National Meeting of the American Chemical Society at Chicago, Illinois, August 29–September 2, 1977. The symposium was sponsored by the Divisions of Polymer Chemistry and Analytical Chemistry. All the papers discuss recent developments in new analytical and instrumental techniques and their applications for improved performance and understanding of the relationships between structure and properties of the commercial polymers that are presently being produced. The book provides both theoretical and experimental bases of the various techniques for the polymer chemists to understand the microstructural variables and the behavior of polymers. It constitutes the "Advances in Chemistry Series 174" of the American Chemical Society. Each paper appears as a separate chapter in the text. A great deal of updated relevant literature and a complete index are included.

Measurements of the electrical noise phenomena associated with thermal transitions and flow in polystyrene and HD–polyethylene systems were conducted by C. Klason and J. Kubat and are discussed in the first paper. In the second chapter, the acoustic emission of polymers during metal deformation (e.g., Zn, steel, Al, Cu, and Pb) under tensile load is reviewed by A. Peterlin.

Chapter 3 by C. G. Andeen and R. W. Hoffman reports the development of a new, accurate, and sensitive instrument, "The Nanotensilometer," for measuring force-elongation data of thin films and fibers with cross-sectional areas as small as 10^{-14} m^2. The nanotensilometer can measure forces of 1×10^{-8} to 5×10^{-1} N. In the following paper, Y. D. Kwon *et al.* describe an apparatus and method for measuring nonlinear viscoelastic properties of polymers in cyclic deformations under relatively high strain amplitude (± 0.1 to 2%).

A critical review on the instrumental aspects and applications of stress mass spectrometry to polymeric materials is presented by M. A. Grayson and C. J. Wolf in Chapter 5. In principle, polymeric materials such as polystyrene and fiber–epoxy composites are subjected to mechanical deformation, and the volatile compounds evolved from the sample are analyzed by a time-of-flight mass spectrometer. It is involved in a mechanochemistry. On the other hand, Chapter 6 by W. L. Truett is concerned with the characterization of organic

polymers via pyrolysis–infrared. By this technique, a substance is pyrolyzed at temperatures ranging from 500 to 1000°C and then the decomposed products are characterized by ir spectroscopy to determine the microstructure of the original polymers. Analysis of polybutadiene is given.

In Chapter 7, a new method, "Inelastic electron tunneling spectroscopy," for investigating the vibrational spectra of organic molecules absorbed onto solid surfaces is demonstrated by H. T. Chu *et al.* Technically, it uses "quantum-effect tunneling of electrons" through a thin insulating layer sandwiched between two metal films. It gives results analogous to ir and Raman spectroscopy. Similarly, Fourier transform ir spectroscopic studies of transitions in polyethylene were conducted by W. W. Hart and J. Koenig and are illustrated in Chapter 8. Several well-reproduced spectra are presented and critically reviewed.

The ninth paper, by G. D. Patterson, describes the theory and experimental procedures used in Brillouin scattering. This technique measures the velocity and attenuation of hypersonic thermal acoustic phonons using light scattering. It is a versatile technique for studying the physical properties of polymers. Both thermodynamic and kinetic information can be obtained.

The scattering of photons and neutrons by internal molecular motion or by external molecular diffusion processes can be detected by quasielastic laser light scattering techniques (Chap. 10 by A. M. Jamieson and M. E. McDonnell) or with a multipass Fabry–Perot spectrometer (S. M. Lindsay and I. W. Shepherd, Chap. 11). These measurements give us new insights into the size, shape, and motion of polymer molecules in solution and in the solid state. Detailed structural analyses by these methods are described.

In Chapter 12, G. G. A. Bohm and K. R. Lucas discuss the use of the "Ion Recombination Luminescence technique" for studying the structure of heterogeneous polymer systems. The results of analyses of polymer blends and block copolymers are demonstrated.

The diffusion coefficient is a valuable parameter which provides structural information at a level that no other technique can offer. Measurement of the diffusion coefficient is an excellent tool for investigating the effect of processing on polymer surface structure. The last paper by M. V. Sefton and K. T. Chiang deals with the structural analysis of SBS block copolymers and polyethylene by diffusion measurements. The mathematical treatment of the diffusion process in a thin film and the concentrance, position, time, and stress-dependence of diffusion coefficient are concisely discussed.

Generally, this book presents current active areas of research in the instrumental methods of characterizing polymers. It is intended to bring the polymer scientist up-to-date regarding instrumentation, developing techniques, and new applications and to help him select suitable and effective methodology from the instrumentation and techniques presently available. Thus, the polymer scientist with the aid of this excellent reference book is able to understand the microvariables of polymers and to develop improved performance through an understanding of the structure–properties relationships on commercial polymers.

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Electrophoresis. A Survey of Techniques and Applications, Part A: Techniques. By Z. DEYL, F. M. EVERAERTS, Z. PRUSIK, AND P. J. SVENDSEN. Elsevier, New York, 1979. 390 pp., $83.00.

A continuing problem in analytical chemistry has been the separation and characterization of the components of complex mixtures. Of the many techniques developed to meet this

problem, electrophoresis has long been of key importance. This book is the first of a two-volume set devoted to electromigration techniques and their applications. It is also Volume 18 of a series on analytical procedures called the "Journal of Chromatography Library."

The volume includes 17 chapters, a list of frequently occurring symbols and a subject index. The first two chapters discuss the theoretical basis of electrophoresis and the difference between various types of electrophoresis. Chapters 3 and 4 discuss some of the factors affecting electrophoretic analysis such as molecular size and shape. The remaining chapters focus on specific electrophoretic techniques such as: zone electrophoresis, gel-type techniques, moving boundary electrophoresis, isoelectric focusing, analytical isotachophoresis, continuous flow-through electrophoresis, continuous flow deviation electrophoresis, and preparative electrophoresis.

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Auger Electron Spectroscopy Reference Manual. By G. E. MCGUIRE. Plenum, New York/London, 1979. IX unpaginated, $39.50.

Auger electron spectroscopy has come to play a dominant role in probing the elemental composition of solid surfaces involving films of atomic depth. The analyst in a surface science laboratory should find this volume useful indeed for the identification and interpretation of Auger electron spectra. The manual covers all the most frequently encountered elements, beginning with beryllium and ending with bismuth, there being the graphically displayed data for some 43 elements.

The Auger results in this volume were obtained with a single-pass cylindrical mirror analyzer and a coaxial gun, the samples being mounted at an angle of 30°. The secondary electron energy distribution, $N(E)$ was obtained in the derivative mode, $dN(E)/dE$ and plotted against the energy, E. All spectra were recorded using a silver standard under a constant set of conditions.

The data include a general survey scan from 0–2000 eV taken with a 5-keV primary beam, 5-μA beam current, and a 6-eV peak-to-peak modulation voltage. Selected characteristic transitions are displayed at higher resolution taken with a 1-eV peak-to-peak modulation voltage. Auger transitions are also displayed as a function of modulation voltage while maintaining a 5-μA beam current and a 5-keV primary beam energy.

The printing is very good and the data are clearly displayed. Though the data will not universally match data from other analyzers, the information can guide the analyst to key instrumental parameters used in this rapidly developing field.

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Radioelement Analysis: Progress and Problems, Proceedings on the Twenty-Third Conference on Analytical Chemistry in Energy Technology. Edited by W. S. LYON, Ann Arbor Science Pub., Ann Arbor, Michigan, 1980. xii + 424 pp., $29.95.

This volume contains 45 papers in addition to R. E. Brooksbank's plenary lecture on the contamination problems in the recovery of the Three Mile Island nuclear plant which was

given in October 1979. The book represents a broad cross section of research efforts in a field whose problems will be with us for a long time. The chemist in the nuclear technology area will find the book excellent for keeping up with many of the analytical and instrumental advances as applied to the many problems created by the nuclear energy industry.

The book is divided into seven sections: Radiochemical Separations and Measurements, Radiochemical Determinations and Measurements, Gamma Spectrometry and Activation Analysis, Environmental Analysis, On Line Monitoring and Facilities, Mass Spectrometry, and Quality Assurance and Standards. The 45 papers include single contributions from Canada, Holland, and Sweden and are approximately evenly divided among the sections.

Analyses of trace contaminants of an increasing number of radionuclides are assuming greater importance as soil, water, and air are continuously being probed. The range of environmental problems facing the analytical chemist may involve the mining and milling of uranium for nuclear power production to the assessment of global fallout. Not only new analytical methods are considered in this volume but also improvements of old methods are considered, as for example, the case of ^{14}C which is produced in the reactor coolant system of a nuclear power plant as a result of a (n,p) reaction. These waters have a potential impact on the environment. The "improved" procedure more completely oxidizes organics, resulting in one or two orders of magnitude higher ^{14}C values than those obtained with the former method.

A number of instrumental and procedural innovations are described. There is the report on the optimization of ZnS alpha counting for low-level alpha fluxes where detection limits substantially less than 0.01 α/cm^2-hr are attained for typical samples. Proton activation analysis allowing for the determination of many toxic elements such as Cr, As, Cd, Sb, and Pb in airborne particles is reported. There is also a description of the analysis of radioactive samples by inductively coupled plasma spectroscopy. A comparison of the performance of several types of instruments for the analysis of low-energy beta-emitters is discussed.

The task of the analytical chemist in this area involves problems concerning the immediate environment of the individual as well as the analytical assessment of global fallout and its effect on general population. The sample matrices include air, soil, water, vegetation, and tissue. The tracing and modeling of global transport and estimating radiation doses to the general population are additional tasks requiring analytical data of highest quality. The range of problems for the analytical chemist is indeed staggering. The release of the radioactive material to the environment as a result of the TMI accident dramatically underscores the position of the analytical chemist in the era of nuclear technology and the need for books such as "Radioelement Analysis."

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