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CONTENTS

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Briefs

Organic Polychloride Microanalysis: A Guide to Volatiles and Multichlorinates in the Oxygen Flask. JOHN M. CORLISS AND NATALIE B. SCHOLTZ, *Chemical Laboratory, Edgewood Arsenal, Maryland 21010; and* THOMAS E. WOLLNER, *3M Company, St. Paul, MN 55101.*

Following a rough comparison with other mineralization procedures, techniques are given for sampling and burning solids and liquids, both volatile and nonvolatile, in an oxygen flask, using sample wrappers fabricated from pressure-sensitive tape.

Microchem. J. **17**, 135 (1972).

Some New Data Regarding the Formation of Alkylglycosides. L. SZEKERES AND K. TETTAMANTI, Technologcial Institute, Budapest, Hungary.

The formation of alkylglucosides, at various concentrations, of methyl, ethyl, propyl, and isopropyl alcohol has been investigated. The formation of methyl and ethyl glycosides of D-fructose was also studied.

Microchem. J. **17**, 148 (1972).

Charge-Transfer Complexes and Their Applications, Distinction and Determination of Some Aromatic Amines. J. P. SHARMA AND R. D. TIWARI, *Department of Chemistry, Allahabad University, Allahabad, India.*

Charge-transfer reactions between aromatic amines and *s*-trinitrobenzene have been utilized for the distinction and determination of some primary, secondary, and tertiary aromatic amines. Charge-transfer band maxima of the complexes show red shifts in passing from primary to secondary to tertiary amines.

Microchem. J. **17**, 151 (1972).

A Micromethod for the Determination of Blood Urea Nitrogen in Serum. MARGARET ANN WARWICK, *West Jersey Hospital, Camden, New Jersey 08104.*

A micromethod for the determination of blood urea nitrogen, using a commercial product, is described. Comparisons are made between this method and others. That described has several advantages, including the use of disposable pipettes and the elimination of obtaining the sample by venipuncture.

Microchem. J. **17**, 160 (1972).

Spectrophotometric Determination of Titanium with Tiron. A. V. MOHARIR, V. A. K. SARMA, AND G. S. R. KRISHNA MURTI, *Division of Agricultural Physics, Indian Agricultural Research Institute, New Delhi, India.*

The procedure for the spectrophotometric determination of titanium with Tiron (disodium 1,2-dihydroxybenzene-3,5-disulfonate) was modified by using thioglycolic acid to reduce the Fe(III)–Tiron complex.

Microchem. J. **17**, 167 (1972).

Identification of the Source of the Crude Drug Tu-zhu-ye Using Low-temperature Plasma Ashing. KEIICHIRO HOZUMI, *Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan,* MASAKAZU HUTOH, AND KOUICHIRO UMEMOTO, *Kyoto College of Pharmacy, Kyoto, Japan.*

The source of the crude Korean diuretic drug, "Tu-zhu-ye," has never been established. The present work was done for the purpose of identifying the source and specimens were investigated which were collected at Seoul and Taegu, Korea. The samples were subjected to low-temperature ashing using high-frequency oxygen plasma. The ashes were compared microscopically with those of known specimens. The source from Seoul was identified, but not from Taegu. Further investigation of the latter is under way.

Microchem. J. **17**, 173 (1972).

Spectrophotometric Studies of the Complexes of Quadrivalent Titanium, Zirconium, and Hafnium with Dibromopyrogallol Sulfonphthalein. SUBHASH C. PANDE AND SATENDRA P. SANGAL, *Laxminaryan Institute of Technology, Nagpur University, Nagpur, India.*

The characteristics of the colored chelates of the metals are described, as well as the methods of determination of the molar ratios, range of pH for optimum stability, etc.

Microchem. J. **17**, 186 (1972).

Methods for the Isolation and Characterization of Constituents of Natural Products. XIV. Use of Iodine Monochloride for Detecting Unsaturation in Microgram Quantities of Colored Derivatives. D. P. SCHWARTZ, J. L. WEIHRAUCH, AND C. R. BREWINGTON, *Dairy Products Laboratory, Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D.C. 20250.*

Included in the study were the 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds, the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of alcohols, the α-methyl-2,4,6-trinitrophenylhydrazone derivatives of alkoxyacetaldehyde compounds, the 2,6-dinitrophenylhydrazone derivatives of pyruvamides and the *N*-2,4-dinitrophenylethanolamine esters of fatty acids. Compounds which add iodine monochloride are sufficiently retarded on thin-layer partition plates to be classified as unsaturated. Carbonyl derivatives in which the double bond or bonds are conjugated with the hydrazone linkage and certain carbonyl compounds containing a double bond β,γ to the hydrazone linkage fail to be detected.

Microchem. J. **17**, 193 (1972).

Use of Ditelluratoargentate and Cerium(IV) in Microanalysis: Determination of Mixtures of Citric and Oxalic Acids and of Formic Acid and Methyl Alcohol. P. K. JAISWAL, *Department of Chemistry, M.M.M.V., Bhat Par Rani, Deoria, U.P. India.*

Citric acid is oxidized by Ag(III) to CO_2 and H_2O while oxalic acid is unaffected. Both acids are oxidized by cerium(IV). Silver(III) oxidized formic acid, but not methyl alcohol, while cerium(IV) oxidized both. These preferential oxidations forms the bases of these determinations.

Microchem. J. **17**, 200 (1972).

Simultaneous Determination of Aluminum, Copper, Iron, and Manganese. J. PAUL AND SHARAD M. SHAH, *Chemistry Department, University of Bridgeport, Bridge-Port, Connecticut 06602.*

The method is based on the sequential formation and extraction of tris(1,10-phenanthroline) iron(II), followed by the selective formation of copper diethyldithiocarbamate and its extraction by *n*-butyl acetate in the presence of perchloric acid. The remaining aqueous phase containing manganese and aluminum is neutralized with ammonia, extracted twice with chloroform, and the manganese determined by formation and extraction of manganese diethyldithiocarbamate into isobutyl acetate, in the presence of perchlorate ions. Aluminum is determined in the remaining aqueous phase as its 8-hydroxyquinolinate.

Microchem. J. **17**, 204 (1972).

I. Direct Titrimetric Microdetermination of L-Histidine. II. Microdetermination of L-Histidine and L-Arginine Together in One Solution Without Separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

L-Histidine is determined by titration with lead nitrate using xylenol orange or pyrogallol red as indicator. When L-histidine is present along with L-arginine, the histidine is first determined as above and then the arginine is titrated with ferrous ammonium sulfate using chromazural red S as indicator.

Microchem. J. **17**, 210 (1972).

A Glass Sprayer for Thin-Layer or Paper Chromatography. FERNANDO WALLS, *Instituto de Química de la Universidad Nacional Autónoma de México, México 20, D.F., México.*

A very fine spray is obtained from this simple sprayer.

Microchem. J. **17**, 214 (1972).

New Method for Isolation of Carrier Free Sulfur-35 and Phosphorus-32 from Neutron Activated Potassium Chloride. HISHAM F. ALY AND MOUSTAFA M. ABDEL-HAMID, *Nuclear Chemistry Department, Atomic Energy Establishment, Cairo, U.A.R.*

The distribution of phosphate and sulfur anions on a developed extraction chromatographic column was investigated. A working procedure for separation of carrier free ^{32}P and ^{35}S from neutron activated potassium chloride target was adapted. The procedure proved to be adequate and efficient for production of high chemical and radiochemical pure sulfur-35 and phosphorus-32 radioactivities. The distribution behavior of the studied species was discussed in the light of the different interactions affecting the preferential generation of the anionic species.

Microchem. J. **17**, 215 (1972).

The Study of the Electrochemical Oxidation Mechanism of Diphenylamine Derivatives in Dipolar Aprotic Solvents. II. The Electrochemical Oxidation of 4-Aminodiphenylamine in Acetonitrile. T. M. H. SABER, G. FARSANG, AND L. LADÁNYI, *Institute of Inorganic and Analytical Chemistry, L. Eötvös University, Budapest, Hungary.*

Oxidation occurred in two steps, the first involved complete oxidation of one molecule through an ECE mechanism. The ejected proton, in the follow-up reaction, is received by the other molecule, which undergoes oxidation at the potential of the second step. This result was confirmed from the effect of acid and base addition on the oxidation patterns.

Microchem. J. **17**, 220 (1972).

Application of Acid–Base Indicator Character in the Detection of Isomers and Homologous Compounds. Detection of 6-Chloro-*o*-cresol in the Presence of *o*-Cresol and 4-Chloro-*o*-cresol. LÁSZLÓ LÉGRÁDI, *Nitrockémia Ipartelepek (Nitrochemical Industrial Plants), Füzfögyártelep, Hungary.*

The method is based upon the preparation of azo compounds obtained by reacting the corresponding cresol derivative with diazotized *p*-nitroaniline.

Microchem. J. **17**, 231 (1972).

Methods for the Isolation and Characterization of Constituents of Natural Products. XV. Application of a Periodic Acid Column for Locating Double Bond Position. JOHN L. WEIHRAUCH AND DANIEL P. SCHWARTZ, *Dairy Products Laboratory, Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 20250.*

The ability of a microcolumn of periodic acid impregnated on magnesium sulfate to effect oxidation of ethylenic unsaturation was studied. The aldehyde, produced on the hydrocarbon side of the double bond in 30–40% yield, was converted to a 2,4-dinitrophenylhydrazone on a micro-derivatizing column and identified by tlc. The method was applied to acids, their methyl esters, alcohols, aldehydes, and colored derivatives of the last two. Double bonds in various positions were located but several exceptions were noted. Samples as small as 5μg were run successfully.

Microchem. J. **17**, 234 (1972).

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Organic Polychloride Microanalysis: A Guide to Volatiles and Multichlorinates in the Oxygen Flask

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INTRODUCTION

Degree of chlorination of organic materials affects both volatility and resistance to oxidation. Each of these properties makes difficult the selection of a quantitative means of mineralization for elemental microanalysis. Since the analysis for chlorine content invariably is finished with the chlorine in chloride form, a reduction method of mineralization is indicated. The reduction methods (e.g., sodium fusion) have high theoretical promise of rapid and complete conversion of organic chlorine into inorganic chloride; however, in microanalysis the oxidation methods are usually easier in application. Purely oxidative mineralization (e.g., Carius wet combustion, peroxide fusion, oxygen combustion tube) when applied to perchlorinated hydrocarbons, requires careful technique and great attention to detail to achieve precision and accuracy. The Schöniger oxygen-flask combustion (*12, 13*) is undoubtedly simpler in application, but low results have frequently been experienced with multichlorinated organic compounds (*7*).

Two causes are recognized for these low results: (i) the formation of elemental chlorine; and (ii) incomplete combustion. The first may be overcome by selecting proper reducing conditions in the absorbing solution to effect chloride formation (*4, 10*). Furthermore, increasing the organic matter in the sample wrapper has been found to improve results in the analysis of highly chlorinated materials (*2, 3*). With this additional organic matter present in the flame, the intensity of the flame is boosted and the reductive volume is increased, thus enhancing the opportunity for chloride formation. Some authors (*2, 9, 14*) have suggested using an office-type adhesive tape as a sample wrapper for

refractive materials in the oxygen flask. While this substitution in sample-wrapping material does not necessarily increase the organic matter in the flame, a denser material is substituted as sample wrapper. These dense film materials are more resistant than paper to water absorption, and have less occluded moisture at the time of combustion. Because of this, the flame propagates more actively throughout the entire tape wrapper, flaring almost immediately into instant combustion. With this instantaneous and simultaneous combustion, a hotter and larger flame is produced. Furthermore, the sealed tape wrapper offers better containment to sample materials. In the case of sample materials with appreciable vapor pressure, volatilization is suppressed until the moment of combustion, if the tape wrapper has been properly assembled. At the moment of release of volatile materials by the advancing flame, the only outlet is through both the reductive and oxidative volumes of the flame and chloride formation is complete. On the other hand, incomplete combustion can occur when burning perchlorinates in a paper sample wrapper. There can be partial cleavage and loss of organic chloride moieties, which may volatilize away from the hot combustion flame and escape mineralization. Both these errors are prevented by using a properly designed adhesive-tape sample wrapper for samples that burn in this manner. Complete combustion occurs without emission of elemental chlorine, as is consistent with the normal oxygen-flask combustion process (*5*).

Following an initial study involving the mineralization of perchloroethane, the oxygen-flask combustion with tape-wrapped samples appeared to be a method which could be expanded for versatility and improved for accuracy.

Although the technique has been suggested frequently enough to enjoy wide use, it was thought that close control over blanks and other refinements in technique might provide greater applicability. In this work a "best" tape was sought for sample wrapping.

MATERIALS AND METHODS

Perchloroethane was chosen as a typical solid perchlorinate for the initial study on mineralization. It has a melting point of 187°C (777 mm Hg) (*15*) and has an appreciable vapor pressure at room temperature. Thus, it was easily purified by sublimation. The purified perchloroethane was mineralized prior to chloride determination by a number of procedures as outlined in Table 1 with results shown as percentage chlorine for comparison with the calculated value.

The first entry on Table 1 represents results obtained with the unmodified Schöniger flask decomposition; the second, with decomposition

TABLE 1

STUDY WITH HEXACHLOROETHANE $(Cl_3C)_2$
Calc 89.9% Cl

Procedure	Results (% Cl)
Oxygen flask: paper wrapper	72.0
Oxygen combustion tube	85.0, 89.2
Peroxide fusion	87.7, 88.8, 89.1, 89.7
Sodium fusion	89.5, 89.6, 90.0
Oxygen flask: tape wrapper	90.0, 89.6, 90.1, 89.5

in a Pregl-type microcombustion, or spiral, tube (*1*). These results on highly chlorinated materials are some indication of the difficulties with supposedly universal methods. In the table, the two fusion procedures are presented together because of their inherent similiarities: each carried out in a micro-Parr bomb, which implies bomb-cup leaching and washing. In the sodium fusion, it was known from earlier work (*11*) that sodium vapor in the bomb instantly reacts with perhalo compounds to produce sodium halide, while peroxide fusion requires actual melt contact. These facts indicate simpler bombing techniques for sodium fusion, followed by better control over both wash volumes and alkali concentrations in the wash solutions. Furthermore, contamination of wash with metal ions from the bomb itself is always much greater with peroxide fusion; however, sodium fusion is not without difficulty. At its simplest, the bomb cup would be placed in a solution for static leaching, followed by immediate titration; but both the charcoal from the fusion and the presence of the bomb cup cause mercurimetric end-point fading. Therefore, leaching followed by filtration is required. The titration, performed in an essentially alcoholic medium, limited the volume of wash water; while the presence of the carbon required prolonged and thorough leaching. These requirements may be, and were, met by a regimen of alcoholic and aqueous washes performed in conjunction with a shaker (*8*).

Oxygen-flask combustion with the sample wrapped in pressure-sensitive cellophane tape, the final entry in Table 1, gave results paralleling the sodium fusion procedure and with much less effort; although an appreciable blank was introduced due to the tape. This blank was measured by two methods: (i) following empty sample wrapper combustion in which a known chloride titer was present in the absorption solution (*6*); and (ii) following combustion of a known quantity of standard organic chloride material. In each case, 2 sq inches of tape were used and the blank was the gross titer less that due to the amount

of chloride known to be present. Two square inches of tape weighed about 110 mg; larger amounts results in excessively sooty combustions, which utilmately caused obstruction of the visual end point. Burned alone, the tape produced chloride to consume an average of 0.27 ml of 0.005 *N* mercury(II); with chlorobenzoic acid, a net average of 0.29 ml was consumed. In these tests, the overall average of six determinations was 0.28 ml/2 sq inches of cellophane tape. On this basis, the titration blank of cellophane tape was found to be reproducible not only from length to length, but also from roll to roll and even from brand to brand. The tapes initially observed were Scotch Brand Transparent Tape No. 600 made by the 3M Company, Permacel 404, and a now discontinued Texcel cellophane tape, the last two manufactured by the Johnson & Johnson Company.

In Table 1, the chlorine was determined by a mercurimetric finish (*6*), except for the peroxide fusion, which was finished by silver nitrate titration.

Following these initial studies, two improvements in the use of the tape wrapper were suggested: (i) mastering an exactly suited technique; and (ii) precise control of blank. The photograph (Fig. 1) and brief description are introductory to the technique deemed suitable. Shown is an oblique view of a sample-loading setup. Following attachment of an approximate 6-inch length of tape to the 3 × 5 inch scratch pad as shown in the lower portion of the photograph, the center length of the

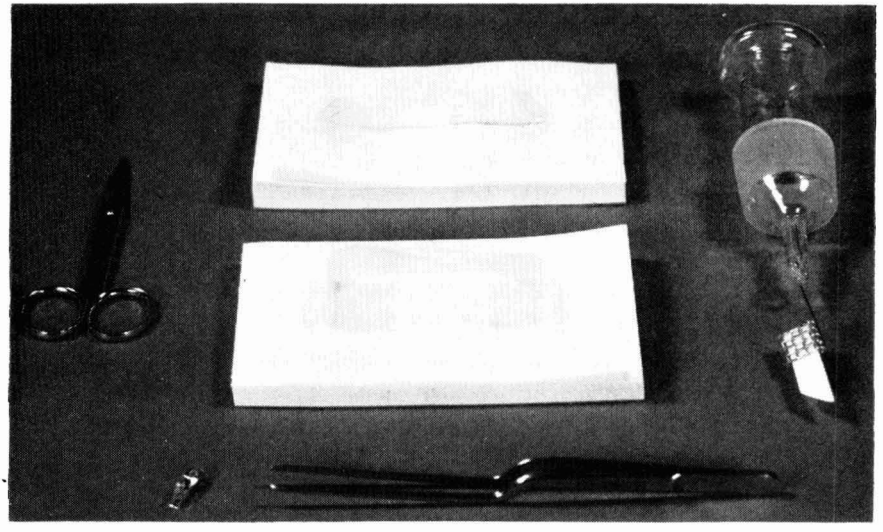

FIG. 1. Sample-loading Setup.

tape is pulled taut so that each end curl is lightly creased to curve the tape and stabilize the center length of the tape. In this manner, the center length is cupped for ready reception of solid sample. Sample is weighed and distributed along the center inch of the tape which is then folded in half lengthwise by manipulating the two lightly creased curls of tape. The upper portion of the photograph shows this fold executed with filter paper in place, and with a nonvolatile liquid sample which has been absorbed on a 10 × 15 cm filter-paper rectangle. To complete the sample wrapper, the center inch is cut out for later trimming, if necessary. At this point, care is exerted to police the tape wrapper for tight sealing, especially along the crease of the fold. The tape used in the wrapper is weighed to calculate blank from previous blank determinations which have now been calculated on the basis of the weight of tape burned. Assembled sample wrappers are burned in 500-ml flasks using paper fuses cut from sample wrappers.

Volatile liquids are sampled in tapered and sealed 1-mm glass coagulation tubes. An idealized volatile-liquid sample wrapper appears in the drawing (Fig. 2) with the sample absorber encased in the tape and the ampoule protruding through the two facing adhesive surfaces. Care needs to be exerted at two points to insure tight sealing: (i) along the crease of the fold, as before; and (ii) where the ampoule protrudes from the sample wrapper. This point is more difficult to seal and is done in two steps; first, by tightly fitting the tape around the ampoule, especially at the point where the two adhesive faces part to admit the ampoule; next, by rolling the 5-cm length of ampoule which passes through the adhesive faces between thumb and forefinger back and forth several times. After proper sealing, the finished sample wrapper is weighed to obtain a tape weight for blank calculation. The sample wrapper is folded (as in the drawing) along the fold line, breaking off the tip of the ampoule. Immediately, the paper fuse is laid along the length of the

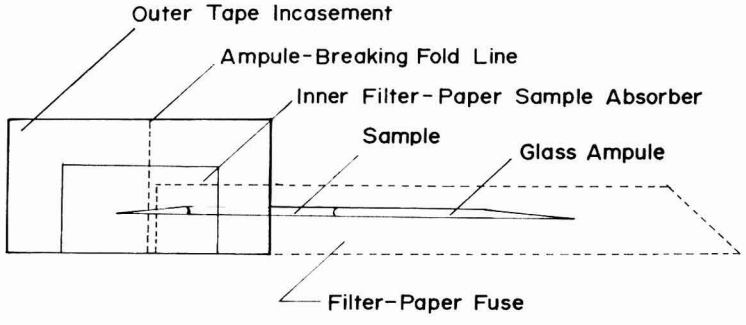

FIG. 2. Volatile-liquid Sample Wrapper.

ampoule; the wrapper assembly is mounted on the flask stopper, fired, and plunged into the flask for combustion in a matter of seconds. In the flask, the burning fuse drives the volatile liquid from the ampoule onto the sample absorber where it is quickly mineralized.

In all the analyses presented here as tabulated results, 3M No. 600 tape was used as sample wrapper. 3M No. 600 tape is cellophane backed with a natural rubber adhesive and resin tackifier. It burns well and is usually the type of tape used by other authors for this application. As may be calculated from the earlier discussion, the blank runs between 27 and 33 μ of chloride/sq. inch (55 mg). Excellent blank control was obtained by reserving one roll for analytical use, determining the blank accurately on a weight basis, and checking this blank about every 10 yards. The weight of tape used in an analysis is obtained just before combustion, as noted; a blank is calculated for this weight and deducted from the titer.

RESULTS

The results presented in Table 2 were obtained on materials considered pure by reason of their source, or because of the agreement of other elemental analyses with calculated values. The oxygen results presented were obtained by difference, and, hence, show greater deviation from calculated values than usual found values. Samples of compounds solid at room temperature [(s) in Table 2] were encased in tape alone; samples of compounds liquid at room temperature [(l) in Table 2] were absorbed on filter paper and encased in tape. The compounds listed, other than the first, were research materials.

In these oxygen-flask combustions, the election to decompose with a tape wrapper was made on the basis of the presence of a plurality of chlorine atoms or volatility sufficient to interfere with normal oxygen-flask combustion, but this information is sometimes insufficient to predict the requirement for a tape wrapper. For example, the second compound and all preparations of the third compound of Table 2 were also analyzed following combustion in paper wrappers, with results of 50.7, 44.5, 44.6, and 44.5% chlorine, respectively. These results are in agreement with percentages in Table 2, where tape wrappers were used in all cases.

The sulfur compound is presented for several reasons. It was one of the few compounds run having intermediate volatility which could not be run by the usual oxygen-flask method; a low 18.2% sulfur was obtained following paper-wrapper combustion. It illustrates that tape wrappers are applicable to the analysis for elements other than the halogens. The sulfur blank produced in burning a sample in a tape wrapper was the

TABLE 2

ANALYTICAL RESULTS ON SOLIDS AND LESS VOLATILE LIQUIDS [a]

Calculated percentages presented parenthetically.

Compound	Basis for purity				Cl (%)	
					Calcd	Found
$Cl(C_6H_4)COOH$ (s)	British Drug Houses, Ltd. standard				(22.6)	22.6, 22.5, 22.7
	C (%)	H (%)	N (%)	O [b] (%)		
$((C_6H_2Cl_3)NH)_2$ (s)	(37.3) 37.2	(1.4) 1.4	(6.7) 6.7	(3.8) 4.0	(50.8)	50.7, 50.6
$(C_6H_2Cl_3)NHC(CH_3)O$ (s)						
1st prep.	(40.3) 39.8	(2.5) 2.3	(5.9) 5.7	(6.7) 7.6	(44.6)	44.5, 44.6, 44.6
2nd prep.	(40.3) 40.2	(2.5) 2.3	(5.9) 5.7	(6.7) 7.0	(44.6)	44.8, 44.6
3rd prep.	(40.3) 40.0	(2.5) 2.2	(5.9) 5.9	(6.7) 7.5	(44.6)	44.4, 44.4
$C_6H_2Cl_8O$ (s)	(19.3) 19.7	(0.6) 0.8	—	(4.3) 3.5	(75.9)	76.0, 76.1
C_3H_2ClN (l)	(41.2) 41.0	(2.3) 2.5	(16.0) 15.8	—	(40.5)	40.4
					S (%)	
$(C_2H_5)(CH_3)_2CO(CH_2)_2SH$ (l)	(56.7) 56.4	(10.9) 10.9	—	(10.8) 11.3	(21.6)	21.4

[a] s, solid; l, liquid.

[b] By difference.

equivalent of 8 μ of sulfur, which is the same as that produced by burning in a paper wrapper (*6*).

Table 3 shows results for more volatile compounds. Attempts were made to cover a range of volatilities beyond that which could be handled by simple filter paper absorption and tape encapsulation. Purities of test compounds, especially of the highly volatile materials in this list, were scrupulosuly considered. Of the more volatile compounds, only spectrophotometric grade solvents were used. Diphosgene was the only research sample. Analysis of this material showed 12.6% carbon compared with a calculated value of 12.1% and 16.1% oxygen, again by difference, compared with 16.2%.

Note the results of the analyses of methylene chloride. These, again were obtained using the same 3M No. 600 tape. Attempts to improve these results are discussed below.

DISCUSSION

Table 1 has illustrated the greater degree of accuracy available to the analyst in substituting a somewhat randomly selected pressure-sensitive tape as sample-wrapping material in the oxygen flask. Table 3 indicates that results using the techniques given, proved unreliable at a volatility somwhere above that of chloroform. Several avenues, e.g., a change in technique or in sample-wrapping material, could be followed in an effort to improve results on highly volatile materials. Since one change in sample-wrapping material, that from paper to tape, wrought such a drastic improvement, the election followed was to search for a tape with greater efficacy.

With this purpose in mind, publications of the Pressure Sensitive Tape Council were consulted (*16, 17*). Their "Test Methods" may be considered as related to the columns of Table 4, and their "Directory," to the rows. Their test methods were often either too exhaustive for our purposes (e.g., over a third of their procedures given concerned

TABLE 3

RESULTS ON VOLATILE LIQUIDS (arranged by volatility)

Material	bp (°C)	Cl (%)	
		Calc'd	Found
Diphosgene [$Cl_3COC(Cl)O$]	127	71.7	71.3
Ethylene dichloride	84	71.7	71.8, 72.0, 71.8
Carbon tetrachloride	77	92.2	92.3, 92.2, 92.2, 92.3
Chloroform	61	88.3	88.2, 88.3, 88.1
Methylene chloride	41	83.9	82.6, 82.8, 81.0

TABLE 4

DOES TAPE EXHIBIT HIGH DEGREE OF STUDIED QUALITY?

	Quality studied							
Tape identity	Availability	Pliability	Adhesiveness	Transparency	Wt	Combustion characteristics	Low chlorine	CH_2Cl_2 analysis
3M #600	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Permacel 404	Yes	No	Yes	Yes	Yes	Yes	No	Yes
Texcel	No	No	Yes	Yes	Yes	Yes	No	Yes
Electricians', Plymouth	No	Yes	Yes	No	No			
3M #800	No	Yes	No	Yes	Yes	Yes	Yes	
3M #853	No	Yes	No	Yes	Yes	Yes		
3M #810	Yes	Yes	No	No	Yes	Yes	Yes	No
3M #191-A	No	Yes	No	Yes	No	No		

adhesion); or they were misdirected, (e.g., their flammability test concerns a timed combustion in air with no flame quality noted). Pliability, plasticity, or conformability were not measured directly in their tests, but stiffness was measured by a determination of the bending moment of a free end of a specimen. With our very specific tape usage in mind, simpler methods directed toward this usage were desired. Rather than formally running many tests on many tapes, the choice was made to observe qualities in candidate tapes and to compare them with the 3M No. 600. Thus, for each quality a question could be posed: Does a tape under consideration equal or exceed 3M No. 600 in a quality important for this use? Yes or no? To include the cellophane tapes in Table 4, the question was restated as in the title of that table.

The specific tapes in Table 4 were selected by perusual of the Directory of the Pressure Sensitive Tape Council which lists over 1000 tapes from 17 manufacturers. Tapes 4 mils or thicker were eliminated, since their weight, estimated at approximately 90 g/inch, plus the weights of the sample, paper fuse and absorber would approach the limits of organic matter that the oxygen flask could completely oxidize. Adhesion was next consulted and tapes with adhesions of less than 35 oz/sq inch were eliminated. Tapes of unsuitable descriptions, as double coated, with polyvinyl chloride films or with opaque backings, were eliminated. The remaining tapes were typed and a sample of each suitable type was obtained and examined.

In Table 4, the quality of availability is first considered. Certainly for the limited quantity used for this purpose, a tape should be commercially available. The cellophane-backed tape is almost ubiquitous. All listed tapes are commercially available. The majority of the other qualities may be grouped for easy discussion: pliability, adhesiveness and transparency are most closely related to sample-wrapper fabrication; whereas weight, combustion characteristics, and low chlorine content (blank) are most closely related to use or combustion.

Pliability here is defined as the ability to conform to irregular geometries. Pliability is closely related to another quality, plasticity, defined here as the ability to maintain this conformation and which is considered in Table 4 as an integral part of pliability. These qualities are related to making a tight seal encaspsulating volatile materials, whereas adhesiveness is related to retaining this seal. When the final decision on best tape was reached, it developed that the deciding comparisons in favor of 3M No. 600 were made on the basis of these qualities.

As shown, the results using 3M No. 600 were excellent on materials boiling above 60°C. In themselves, these results are remarkable considering the oxidation resistance and the volatilities of these materials in

the context of the simplicity and rapidity of the combustion in the oxygen flask. However, for the nonvolatile materials, we have seen that the resistance to oxidation has been overcome using a simple tape-wrapper substitution. Thus, with very volatile materials, our main concern is overcoming the volatility obstacle, logically, by a superior volatility suppressor in the form of a tighter sample encapsulation. Again, observations indicate that the reason for the better results using 3M No. 600 tape over other tapes lie in the better and tighter wrapper-fabrication qualities of this tape. Analyzed deeper, it was shown that the pliability of cellophane film was adequate to allow it to be shaped to meet the moderate irregularities in wrapper fabrication; however, its plasticity failed in comparison with some of the other listed tapes. In overcoming its poor plasticity, No. 600 tape displayed an outstanding adhesive, which allowed it to be held fast to these irregularities. Secure seals were formed with more impervious sample wrappers, to the instant of combustion. The superior adhesion of No. 600 was noted by the fact that, invariably, combustion in this tape left the glass sample ampoule, heat distorted and fused to the platinum-wire sample carrier. Other tapes dropped their ampoules in the process of combustion over half of the time.

The pliability qualities may be readily observed by the feel of the tape and in making mock sample wrappers. Adhesiveness is specifically observed in noting the tenacity with which a tape holds an ampoule properly seated in its wrapper.

Transparency is a most necessary tape quality in wrapper use for a number of reasons: (1) to observe proper sealing of wrapper; (ii) to allow wrapper to be trimmed without loss of sample; and (iii) to observe placement of ampoule in the wrapper, both initially and at the moment before combustion. The transparency requirement eliminates the many opaque tapes from consideration. In Table 4, the electricians' tape represents this group. For the last of the three reasons given above, transparency may be considered as a quality necessary both to fabrication and in combustion.

In combustion, a useful tape must exhibit a reasonable weight so as not to overload the oxygen-flask system. Differing from sheer weight are other combustion characteristics based on chemical composition of the tape; for example, cellulose acetate (810 and 800) and polyester (853) all burn as well as cellophane film in the oxygen flask while polyethylene film (191-A) burns poorly. In the oxygen flask, burning polyethylene film forms dense soot, melts into globules, at times encapsulating unburned sample particles, and forms waxy slicks on interior flask surfaces and the interfaces of the absorption solution.

A low and reproducible chlorine content (blank) is essential. 3M No. 600 tape has a relatively high blank, a consequence of its caustic-process film manufacture. Experimentally, this blank is between 27 and 33 μ of chlorine/sq inch. Although the other listed office and household transparent tapes are lower in chlorine (for example, 18 μ of chlorine/sq inch for No. 800), the same precautions are required to correct for blank.

By closely observing the fabrication qualities of the various candidate tapes, their failure could be predicted. To verify this prediction, analyses on methylene chloride using other tapes were obtained. Typical with these were results with No. 810 which were 80.7 and 80.1% chlorine or several percent off values in Table 3.

In conclusion, 3M No. 600 tape is best for wrapping volatile and highly chlorinated substances for oxygen-flask combustion. This is not to say that other tapes will not perform better for related microanalytical purposes. From our experience, a good start in selecting a pressure-sensitive tape for sample wrappers in oxygen-flask combustion can be made by commencing with 3M No. 600.

SUMMARY

Following a rough comparison with other mineralization procedures, techniques are given for sampling and burning solids, and volatile and nonvolatile liquids in an oxygen flask, using sample wrappers fabricated from pressure-sensitive tape. Analytical results testing these techniques on chlorinated materials are given. Improvement in results is sought by selecting the best tape for a given analysis on the basis of its qualities.

REFERENCES

1. Arthur H. Thomas Company, Philadelphia, Pa 19105; Cat. 65, p. 362 (1965).
2. Awad, W. I., Gawargious, Y. A., Hassan, S. S., and Milad, N. E., Microdetermination of chlorine or bromine in highly halogenated organic compounds. *Anal. Chim. Acta* 36, 339–345 (1966).
3. Awad, W. I., Gawargious, Y. A., and Hassan, S. S., Further studies on the use of the oxygen flask for the microdetermination of chlorine or bromine in some highly halogenated organic compounds. *Mikrochim. Acta* 1967, 852–856.
4. Celon, E., and Bresadola, S., On the use of sodium borohydride in the microdetermination of chlorine or bromine in highly halogenated organic compounds. *Mikrochim. Acta* **1969**, 441–448.
5. Childs, C. E., Meyers, E. E., Cheng, J., Laframboise, E., and Balodis, R. B., A study of the oxygen flask combustion procedure. *Microchem. J.* **7**, 266–271 (1963).
6. Corliss, J. M., "Methods of Microanalysis. V. Oxygen Flask Combustion of Chemical Agents," 32 pp. AD 278 160. Armed Forces Techn. Inform. Agency, Arlington, Va, 1962.

7. Corliss, J. M., and Rhodes, E. J., Combustion separation of phosphorus in the microdetermination of sulfur. *Anal. Chem.* **36**, 394–397 (1964).
8. Corliss, J. M., and Scholtz, N. B., Perhalogenate microanalysis: oxygen flask combustion. Pap. No. 16, Anal. Chem., 6th Middle Atlantic Regional Meet., Baltimore, Md, 1971.
9. Corner, M., Rapid microdetermination of organically bound halogens, arsenic, phosphorus and boron. *Analyst (London)* **84**, 46–46 (1959).
10. Mazor, L., Papay, K., and Klatsmanyi, P., Application of the flask method to the analysis of highly halogenated organic materials. *Talanta* **10**, 557–560 (1963).
11. Rothstein, J. H., personal communication.
12. Schöniger, W., A rapid microanalytical method for halogen in organic materials. *Mikrochim. Acta* **1955**, 123–129.
13. Schöniger, W., The rapid microanalytical determination for halogen and sulfur in organic compounds. *Mikrochim. Acta* **1956**, 869–876.
14. Schwarzkopf, O., and Schwarzkopf, F., Chemical characterization of organometallic compounds. *In* "Characterization of Organometallic Compounds" (M. Tsutsui, ed.), Part I, pp. 35–72. Wiley (Interscience) New York, 1969.
15. Weast, R., (Ed.), "Handbook of Chemistry and Physics," 45th ed. Chem. Rubber Pub. Co., Cleveland, Ohio, 1964.
16. "Pressure Sensitive Tape Directory, 1971," prepared and published by Pressure Sensitive Tape Council, Glenview, Ill, 1971.
17. "Test Methods for Pressure Sensitive Tapes," 6th ed., developed and published by Specification and Tech. Comm., Pressure Sensitive Tape Council, Glenview, Ill, 1970.

MICROCHEMICAL JOURNAL 17, 148–150 (1972)

Some New Data Regarding the Formation of Alkylglycosides

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It has been shown first by Fischer (*5–9*) that the formation of methyl, ethyl, propyl, and isopropyl glucosides occurs even in aqueous solutions of the corresponding alcohols in the presence of an acid catalyst like HCl. The hydrolysis of alkylglycosides has been investigated in detail (*1, 2, 4, 10–18*).

The formation of alkyl glycosides in aqueous alcoholic solutions has great importance from the analytical point of view, because, on hydrolysis carried out in alcoholic aqueous solutions of water insoluble natural glycosides, less than the theoretical amount of glucose is formed due to the formation of alkylgylcoside (*5*).

For this reason, the formation of alkyl glucosides (at various concentrations) of methyl, ethyl, propyl, and isopropyl alcohol in aqueous solutions containing 5% HCl was investigated.

It was found that these reactions lead to true equilibria obeying the law of mass action; and the equilibrium constants (K) were measured as shown in Table 1.

D-Fructose forms glycosides under similar conditions to a much lesser extent. Under these conditions, it practically forms only methyl and ethyl glucosides as shown in Table 2.

Tertiary butanol does not form a glucoside with glucose in aqueous solution; for this reason, the degradation of water-insoluble natural glycosides to sugar and aglycon can be conveniently carried out in a mixture of *t*-BuOH and water.

EXPERIMENTAL METHODS

Glucose (0.120–0.600 g) was dissolved in a 30-ml mixture of alcohol and H_2O (containing 1.5 g of dissolved HCl gas), and was refluxed until the equilibrium was reached. The time required for this has been determined in several experiments. After cooling, the reaction mixture

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TABLE 1

Temp. (°C)	Alcohol (%)	K	$\frac{[\text{glucose}][\text{R–OH}]}{[\text{glucoside}][\text{HOH}]} = K$
Boiling	Methanol, 65	0.180	
	Methanol, 50	0.177	
	Methanol, 33	0.166	
	Methanol, 20	0.182	
26	Methanol, 33	0.721	
26	Methanol, 17	0.738	
Boiling	Ethanol, 50	0.243	
	Ethanol, 25	0.266	
	Propanol, 50	0.298	
	Propanol, 20	0.290	
	i-Propanol, 50	0.658	
	i-Propanol, 30	0.641	

TABLE 2

Temp. (°C)	Alcohol (%)	K	$\frac{[\text{fructose}][\text{R–OH}]}{[\text{fructoside}][\text{HOH}]} = K$
Boiling	Methanol, 76	2.47	
	Methanol, 64	2.36	
	Methanol, 56	2.39	
	Ethanol, 79	5.73	
	Ethanol, 72	5.09	

was diluted to 50 ml with H_2O and the amount of free glucose was measured in an aliquot according to Bertrand's method (3).

REFERENCES

1. Armstrong, E. F., Hydrolyse isomerer Glucoside und Galactoside durch Sauren und Enzyme. *Proc. Roy. Soc., London* **74**, 188–194 (1904).
2. Bamford, C., Capon, B., and Overend, W. C., Kinetics and mechanism of the acid catalyzed hydrolyzis of ethyl and phenyl, 1-thio-β-D-glucopyranoside. *J. Chem. Soc.* **1962**, 5138–5141.
3. Bertrand, A., Determination of the reducing sugars. *Bulletìne de la Societé Chimìe de France* [3] **35**, 1287 (1906). *Bertrand, A.*, Determination of the reducing sugars. *Bulletìne de la Societé Chimie de France* [3] **35**, 1292 (1906).
4. Bolin, I., Stability maxima of some organic compounds. *Z. Anorg. Allg. Chem.* **143**, 201–230 (1925).
5. Fischer, E., Ueber die Glucoside der Alkohole, *Chem. Ber.* **26**, 2400–2412 (1893).
6. Fischer, E., Ueber einige synthetischen Glucoside. *Chem. Ber.* **27**, 2478–2486 (1894).
7. Fischer, E., Einfluss der Configuration die Wirkung der Enzyme. *Chem. Ber.* **27**, 2985–2993 (1894).

8. Fischer, E., Ueber die Verbindungen der Zucker mit den Alkoholen und Ketonen. *Chem. Ber.* **28**, 1145–1167 (1895).
9. Fischer, E., Struktur d. beid. Methyl-glycoside und ein dritt. Methyl-glycosid. *Chem. Ber.* **47**, 1980–1991 (1914).
10. Freudenberg, K., Dürr, W., and Hochstetter, H., Hydrolysis of some disaccharides, glucosides and acetone sugars. *Chem. Ber.* **61 B**, 1735–1743 (1928).
11. Hartley, F., and Linnel, W. H., Structure of -γ-sugars. *Quart. J. Pharm. Pharmacol.* **13**, 332–343 (1940).
12. Haworth, W. N., and Porter, C. R., Isolation of crystalline α- and β-ethyl-glucofuranosides (:γ-ethyl glucosides:) and other crystalline derivatives of glucofuranose. *J. Chem. Soc., London* **1929**, 2796–2806.
13. Haworth, W. N., Porter, C. R., and Waine, A. C., Crystalline α-methyl glucofuranoside (:γ-methyl glucoside:) and its derivatives. *J. Chem. Soc., London* **1932**, 2254–2258.
14. Heidt, L. J., and Purves, C. B., Termal rates and activation energies for the aqueous acid hydrolysis of α- and β-methyl-, phenyl- and benzyl-D-glucopyranosides. *J. Amer. Chem. Soc.* **66**, 1385–1389 (1944).
15. Moelwyn-Hughes, E. A., The kinetics of the hydrolysis of certain glucosides. Trehalose, α-methylglucoside and tetramethyl-α-methylglucoside. *Trans. Faraday Soc.* **25**, 81–92 (1929).
16. Moelwyn-Hughes, E. A., Kinetics of the hydrolysis of certain glucosides. β-Methylglucoside, cellobiose, melibiose and furanose. *Trans. Faraday Soc.* **25**, 503–520 (1929).
17. Overend, W. G., Rees, C. W., and Sequeira, J. S., The acid catalyzed hydrolysis of glycosides. *J. Chem. Soc.* **1962**. 3429–3440.
18. Timel, T. E., Effects of polar groups in the aglucon on the acid hydrolysis of glucosides. *Chem. Ind. London* **29**, 1208–1209 (1963).

MICROCHEMICAL JOURNAL 17, 151–159 (1972)

Charge-Transfer Complexes and Their Applications; Distinction and Determination of Some Aromatic Amines

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INTRODUCTION

Analytical applications of the charge-transfer complexes are often based on their spectrophotometric studies which enable one to distinguish and determine the various donor and acceptor components. Some mono-, di-, tri-, and tetrasubstituted ethylenes have been distinguished by the differences in the positions of the maxima of the ultraviolet charge-transfer bands of their iodine and tetracyanoethylene complexes (*1*). These band maxima show a red shift with increasing double bond substitution. A study of pi-complexes of 2,4,7-trinitrofluorenone with phenols, aromatic amines, and hydrocarbons has resulted in the distinction and determination of some of these compounds (*2*).

The π–π complexes of aromatic amines and nitro compounds have long been known and their spectral and thermodynamic constants have been reported (*3*). The present paper deals with the distinction and determination of some aromatic amines utilizing their charge-transfer interactions with *s*-trinitrobenzene.

EXPERIMENTAL METHODS

Materials. A.R. (B.D.H.) samples of aniline, *o*-toluidine, *m*-toluidine, *N*-methylaniline, *N,N*-dimethylaniline and *N,N*-diethylaniline were dried for several hours over sodium hydroxide pellets and distilled under reduced pressure over zinc dust. The middle fractions boiling at constant temperature were collected and used in the present study. The samples should be protected from atmospheric oxygen as far as possible. After a few days the samples get discolored and fresh distillation is required. *p*-Toluidine (A.R., B.D.H.) was used from a freshly opened bottle without any further purification. *s*-Trinitrobenzene was of "organic reagents for organic analysis" grade sample (Hopkins and Wil-

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liams Ltd., London) and was dried in a vacuum desiccator before use. A.R. (B.D.H.) chloroform (sp. d. 1.474, refractive index N_D^{20} 1.445) was used and found to be very satisfactory for the purpose.

Concentration of *s*-trinitrobenzene for its use as the reagent for the determination of amines was about 1.5 g/250 ml in chloroform. This is almost a saturated solution and hence a higher concentration could not be used.

Method

Spectra. Absorbance measurements were done with a Beckman model DU spectrophotometer at room temperature (20 $\pm$ 0.5°). Matched silica cells of 1-cm pathlength were used and care was taken always to use the same cell as the reference cell. The spectra of the mixtures of amines (the donors) with the acceptor (*s*-trinitrobenzene) in chloroform were scanned in the region of 300–700 mμ, using pure solvent in the reference cell. The free donor and the acceptor absorption spectra were also recorded in the said region. Trinitrobenzene and all the donors do not possess any appreciable amount of absorption beyond 390 mμ. The appearance of a new band (charge-transfer bands) in the spectra of the mixtures in the region of 400 to 600 mμ indicates the complex formation. Three representative absorption spectra of the complexes in chloroform are shown in Fig. 1 to 3. The absorption spectra of *s*-trinitrobenzene (the acceptor component) is shown by the curve 1 in Fig. 1. The free donor component absorptions are shown in the corresponding figures.

Procedure

Suitable aliquots of amines from the stock solutions, prepared by dissolving accurately weighed samples in chloroform, were transferred to 25-ml volumetric flasks and 5 ml of the reagent were added to each flask. The contents were made up to the mark with chlororoform and shaken to mix well after properly stoppering the flask. The absorbance of the solutions were measured at the appropriate wavelength (Table 1) against the reagent blank. The concentrations of the unknown solutions were calculated by referring the absorbance data to the calibration curves which may be easily prepared using the known concentrations.

Various sets of mixtures were prepared by transferring suitable aliquots of the component solutions to 25-ml flasks. Five ml of the reagent were added and the contents were made up to the mark with the solvent. The absorbance of the mixed solutions were measured at two, three, or four appropriate wavelengths (depending upon the

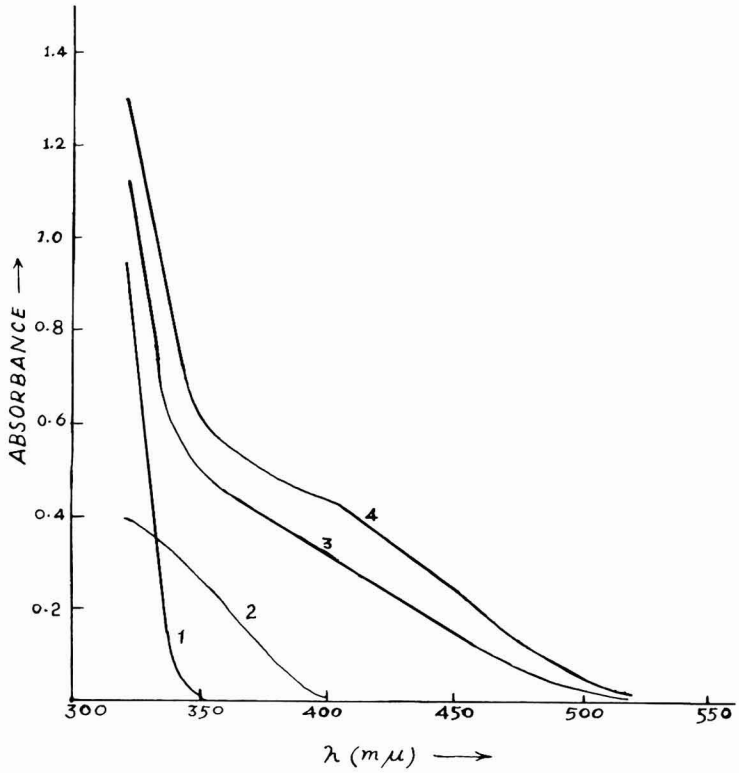

FIG. 1. Visible absorption spectra of solutions of *s*-trinitrobenzene–aniline mixtures in chloroform; pathlength, 1 cm, temp., 20°C: (1) aniline, 63.03 × 10^{-2} *M*; (2) *s*-trinitrobenzene, 15.23 × 10^{-4} *M*; (3) aniline, 46.59 × 10^{-2} *M*; *s*-trinitrobenzene, 15.23 × 10^{-4} *M*; and (4). aniline, 63.03 × 10^{-2} *M*; *s*-trinitrobenzene, 15.23 × 10^{-4} *M*.

number of components in the mixture). The concentrations of the components were calculated by solving the simultaneous equations.

RESULTS AND DISCUSSION

The charge-transfer band maxima of all the complexes studied lie beyond 390 mμ. The resolved charge-transfer bands by the procedure of Reid and Mulliken and others (*4*) appear like those given in Fig. 4. For the distinction and the determination of the amines one does not need to resolve the CT (charge-transfer) bands and the determination can be done directly utilizing the CT band maxima listed in Table 1. It is clear from the λ_{max} values that gradual red shifts are obtained in passing from primary to tertiary amines. Thus aniline and isomeric

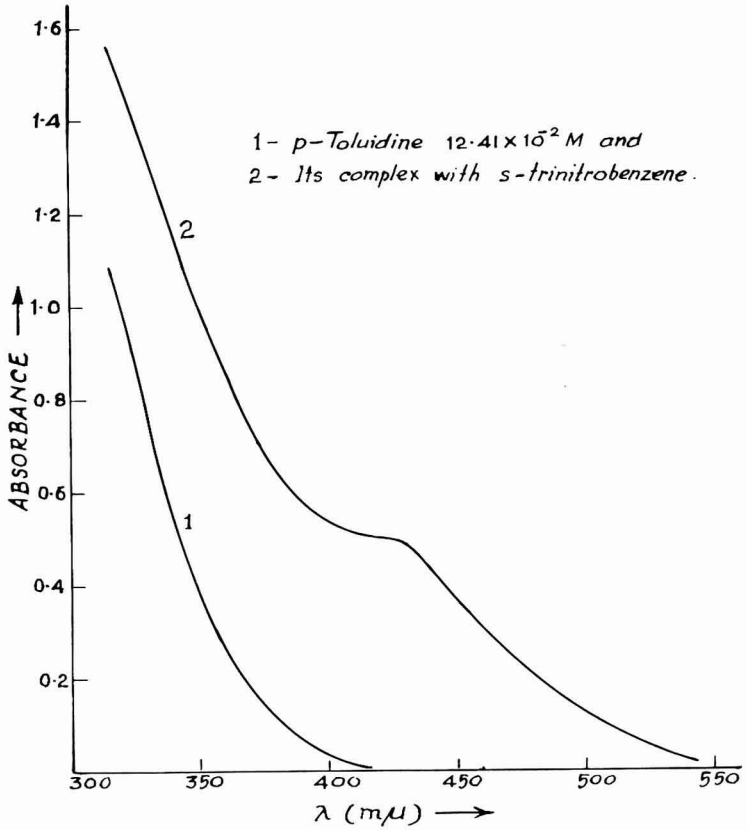

FIG. 2. Absorption spectrum of *p*-toluidine complex with *s*-trinitrobenzene in chloroform at 20°C, pathlength 1 cm: (1) *p*-toluidine, 42.41 × 10^{-2} *M*; (2) *p*-toluidine, 42.41 × 10^{-2} *M*, *s*-trinitrobenzene (TNB), 23.45 × 10^{-4} *M*.

toluidines (primary amines) may easily be distinguished from *N*-methylaniline (secondary) and *N,N*-dimethyl- and *N,N*-diethylanilines (tertiary). In general, a primary amine will have a lowest λ_{max} in its CT complex and a tertiary the highest; this is because the energies of the CT band maxima are directly proportional to the first ionization potential of the donors (*5*) with a given acceptor, and the ionization potentials of amines are expected to decrease progressively on passing from primary → secondary → tertiary amine due to the gradual increase in the inductive effect of alkyl groups substituted at the nitrogen atom.

Since the band maxima of *o*- and *m*-toluidine complexes fall very close to each other, it is not possible to distinguish between the two. However, *p*-toluidine can easily be distinguished from its *o*- and *m*-isomers as well as from aniline. Even the two tertiary amines in question

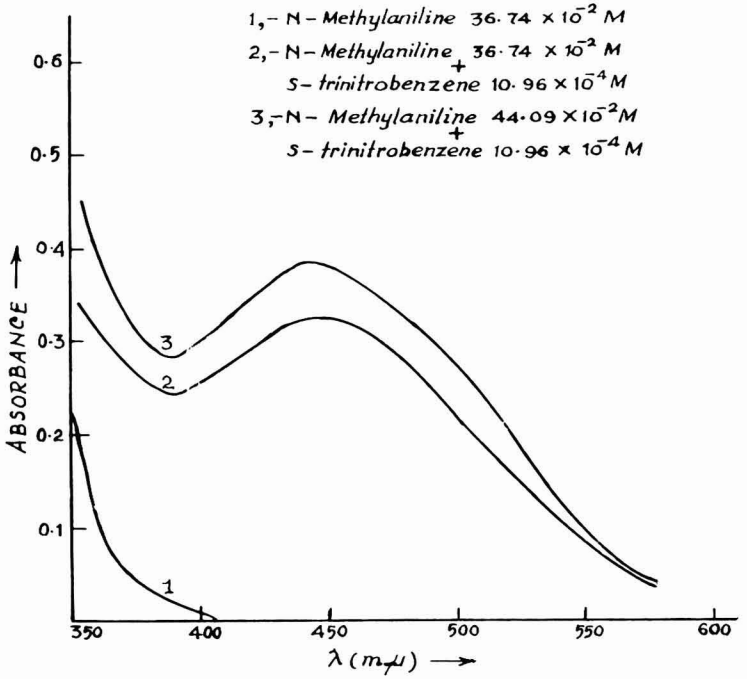

FIG. 3. Absorption spectra of *N*-methylaniline–*s*-trinitrobenzene complex in chloroform solution at 20°C; pathlength, 1 cm.

may be easily distinguished as the λ_{max} of the CT bands of their complexes are wide apart.

The Beer-Lambert law was applicable for all the amines studied at the respective CT band maxima of their complexes. The range of concentrations in which the Beer's law was obeyed and the results of determinations are given in Table 2. The lower limits of concentrations of amines (obeying Beer's law) are expected to zero but the optimum absorbance readings could only be obained with the concentrations indicated in Table 2. Though the molar extinction coefficient of aniline

TABLE 1

λ_{max} VALUES OF THE CT BANDS OF THE COMPLEXES IN CHLOROFORM AT 20°C (±0.5°)

Donor	λ_{max} (mμ)	Donor	λ_{max} (mμ)
Aniline	400 ± 3	*N*-Methylaniline	446 ± 1
o-Toluidine	415 ± 2	*N*,*N*-Dimethylaniline	485 ± 1
m-Toluidine	412 ± 2	*N*,*N*-Diethylaniline	501 ± 1
p-Toluidine	425 ± 2		

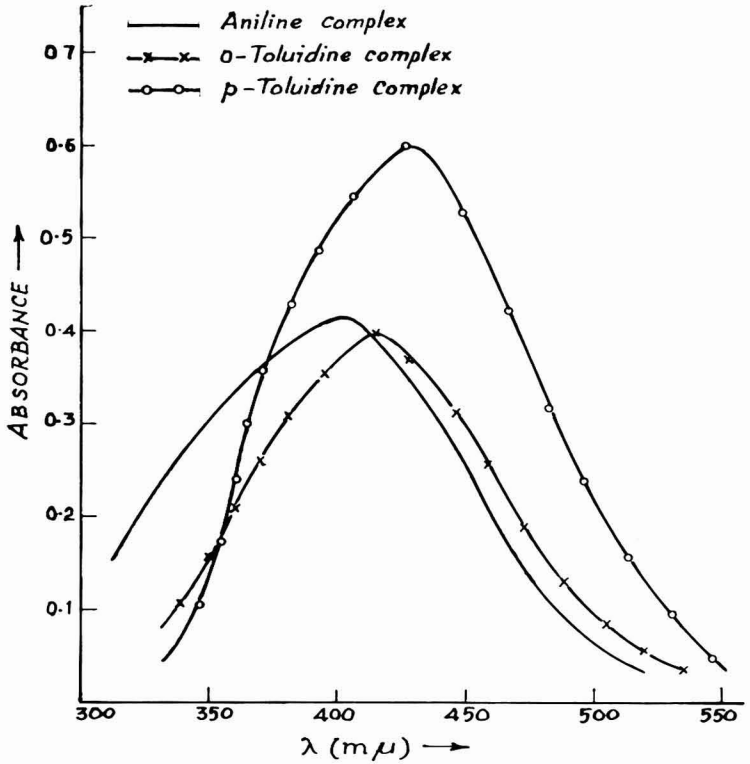

FIG. 4. The charge-transfer bands of aniline, *o*-toluidine, and *p*-toluidine complexes with *s*-trinitrobenzene in chloroform at 20°C.

complex is maximum, the senstivity of the method is the least for this compound because of the lowest K value (*3*) for the system.

For a binary mixture two wavelengths are required for the determination of the components. Suppose the absorptions due to the unit concentrations of the two components (M and N) at the two wavelengths λ_1 and λ_2 are A_m^1, A_m^2 and A_n^1, A_n^2, respectively (determined from the calibration curves), and the total absorption due to the mixture at the two wavelengths are A^1 and ^{2}. If the unknown concentrations of the components be x and y, it follows that:

$$A^1 = x \cdot A^1_M + y \cdot A^1_N, \qquad (1)$$

$$A^2 = x \cdot A^2_M + y \cdot A^2_N. \qquad (2)$$

A^1 and A^2 are experimentally determined quantities and thus solving Eqs. (1) and (2), x and y may be determined. Mixtures containing

TABLE 2

DETERMINATION OF AROMATIC AMINES (temp, 20 ± 0.5°)

Amine	Conc (mg/ml of final solution)		Approximate range for obeying Beer's law (mg/ml)
	Added	Found	
Aniline	2.05	2.00	
	3.07	3.10	2.00
	4.11	4.12	to
	8.60	8.41	35.00
	12.35	12.24	
o-Toluidine	2.45	2.43	
	5.34	5.29	2.00
	7.10	7.17	to
	13.05	13.00	35.00
	25.06	24.71	
m-Toluidine	2.48	2.46	2.00
	6.67	6.45	to
	20.19	20.00	35.00
p-Toluidine	1.85	1.83	1.50
	3.32	3.30	to
	14.12	14.01	35.00
N-Methylaniline	0.66	0.65	0.50
	3.94	3.92	to
	19.02	18.90	38.00
N,*N*-Dimethylaniline	0.62	0.61	
	3.82	3.80	0.40
	20.15	20.00	to
	31.26	30.95	40.00
N,*N*-Diethylaniline	1.80	1.74	1.5
	4.37	4.33	to
	26.50	26.32	35.00

more than two species may similarly be analyzed provided an additional absorbance measurement is done for each added component at a suitable wavelength. However, the accuracy of the method goes on decreasing with increasing components. Results of determinations with binary, ternary, and quaternary mixtures of primary, secondary, and tertiary amines (utilizing the CT band maxima of their complexes for absorbance measurements) are given in Tables 3 and 4.

TABLE 3

DETERMINATION OF MIXTURES OF PRIMARY, SECONDARY AND TERTIARY AMINES (Binary mixtures) (temp, 20 ± 0.5°)

Systems	Wavelengths selected for the detn (mμ)	Amount of the components (mg/ml) Added	Found
1. Aniline (*x*)	400 and	*x* = 3.98	3.97
N-Methylaniline (*y*)	446	*y* = 3.56	3.52
		x = 7.89	7.85
		y = 3.56	3.51
2. Aniline (*x*)	400 and	*x* = 3.98	3.98
N,*N*-dimethylaniline (*y*)	485	*y* = 3.82	3.80
		x = 7.89	7.86
		y = 3.82	3.81
3. *N*-Methylaniline (*x*)	446 and	*x* = 3.56	3.54
	485	*y* = 3.82	3.81
N,*N*-dimethylaniline (*y*)		*x* = 7.89	7.86
		y = 3.82	3.82
4. *N*-Methylaniline (*x*)	446 and	*x* = 3.56	3.54
	510	*y* = 3.74	3.73
N,*N*-diethylaniline (*y*)		*x* = 3.56	3.55
		y = 7.48	7.48
5. *N*,*N*-dimethylaniline (*x*)	485 and	*x* = 3.82	3.80
	510	*y* = 3.74	3.73
N,*N*-diethylaniline (*y*)		*x* = 7.64	7.64
		y = 3.74	3.72
		x = 3.82	3.81
		y = 7.48	7.48

SUMMARY

Charge-transfer interactions between aromatic amines and *s*-trinitrobenzene have been utilized for the distinction and determination of some primary, secondary, and tertiary aromatic amines. Charge-transfer (CT) band maxima of the complexes show red shifts in passing from primary → secondary → tertiary amines. Beer's law was obeyed at the respective CT band maximum and the results of determinations of pure amine samples as well as of their binary, ternary, and

TABLE 4

DETERMINATION OF TERNARY AND QUATERNARY MIXTURES OF PRIMARY SECONDARY, AND TERTIARY AMINES (temp 20 ± 0.5°)

Systems	Wavelengths of detns (mμ)	Amount of component (mg/ml) Added	Found
1. Aniline	400, 446,	3.98	3.97
N-methylaniline	and 485	3.56	3.53
N,N-dimethylaniline		3.82	3.80
2. Aniline	400, 446,	3.98	3.96
N-methylaniline	and 510	3.56	3.52
N,N-diethylaniline		3.74	3.70
3. *N*-methylaniline	446, 485,	3.56	3.52
N,N-dimethylaniline	and 510	3.82	3.78
N,N-diethylaniline		3.74	3.70
4. Aniline	400, 446, 485,	3.98	3.93
N-methylaniline	and 510	3.56	3.50
N,N-dimethylaniline		3.82	3.76
N,N-diethylaniline		3.74	3.65

quaternary mixtures are described. Compounds containing both donor and acceptor groups cannot be determined by the recommended procedure.

REFERENCES

1. Long, D. R., and Neuzil, R. W., Determination of olefins by means of iodine complexes. Ultraviolet absorption method. *Anal. Chem.* **27,** 1110–1114 (1955); Bauer, R. H., Determination of substitution of unconjugated olefins from pi-complexes with tetracyanoethylene. *Anal. Chem.* **35,** 107–110 (1963).
2. Schenk, G. H., Vance, P. W., Pietrandrea, J., and Mojzis, C., Study of the pi-complexes with 2, 4, 7-trinitrofluorenone with phenols, aromatic hydrocarbons, and aromatic amines. *Anal. Chem.* **37,** 372–378 (1965).
3. Foster, R., and Hammick, D. L., Interaction of polynitro compounds with aromatic hydrocarbons and bases. XII. Complexes of *N*-alkylated anilines with *s*-trinitrobenzene and *s*-trinitrotoluene. *J. Chem. Soc.* 2685–2692. **1954;** Bier, A., Complexes of aromatic nitro-compounds. *Rec. Trav. Chim.* **75,** 866–870 (1956).
4. Reid, C., and Mulliken, R. S., Molecular compounds and their spectra. IV. The pyridine-iodine system. *J. Amer. Chem. Soc.* **76,** 3869–3874 (1954). Tsubomura, H., and Lang, R. P., Molecular complexes and their spectra. XIII. Complexes of iodine with amides, diethyl sulphide and diethyl disulphide. *J. Amer. Chem. Soc.* **83,** 2085–2092 (1961).
5. McConnel, H., Ham, J. S., and Platt, J. R., Regularities in the spectra of molecular complexes. *J. Chem. Phys.* **21,** 66–70 (1953).

MICROCHEMICAL JOURNAL 17, 160–166 (1972)

A Micromethod for the Determination of Blood Urea Nitrogen in Serum

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The objective of this paper is to describe a micromethod (BUN-Strate™) for the determination of blood urea nitrogen (BUN), and compare it with a stat procedure (Urograph®) and two routine automated laboratory procedures (the Technicon single channel AA and SMA-12/60 procedures). This microprocedure has compared favorably in parallel studies with the routine laboratory procedures over a period of several months, and appears to bring another microprocedure within the realm of the routine clinical laboratory.

INTRODUCTION

Due to increased automation in our laboratories, we found ourselves using more than one method for a simple routine procedure such as blood urea nitrogen. Following the acquisition of the SMA-12/60, our hospital established a policy whereby all patients over the age of 10 years have a 12/60 survey done on admission. When an elevated BUN is reported on a patient, a repeat analysis is completed using a single channel basic AA procedure, N-1c. However, during the hours from 11:00 p.m. to 7:00 a.m., a stat procedure such as Urograph has been used. Consequently, it would be desirable to introduce a good microchemical method for these repeat BUN analyses, that would meet several criteria:

1. A microsample size is highly desirable for pediatric and geriatric patients.
2. A direct and reliable chemical reaction is required.
3. Results must be expressed in units which are directly related to the routine method employed in the laboratory.
4. The method must be simple, fast, precise, and accurate.

The assay system which we have evaluated is the general Diagnostics BUN-Strate, which utilizes a modified Berthelot procedure (*2*). This

system employs a 10 μl serum sample (easily obtained from a finger prick or heel puncture).

The reaction is as follows:

$$\text{Urea} \xrightarrow[37°C]{\text{Urease}} 2NH_4^+ + CO_2, \quad (1)$$

$$NH_4^+ + OCl^- + \text{phenol} \xrightarrow[\text{prusside}]{\text{Nitro-}} \underset{\text{(blue)}}{\text{Indophenol.}} \quad (2)$$

The directions of the manufacturer were followed exactly as described for this procedure.

MATERIALS AND METHODS

Materials

Caraway micro blood collecting tubes; (nonheparinized)[1] (74–76 mm; 1.8–2.2 mm i.d.; 3.9–4.1 mm o.d.)

Urograph®, General Diagnostics [2]

Centrifuge

BUN-Strate™, General Diagnostics [2]

Eppendorf 10 μl pipette [3]

12/60 SMA Technicon [4]

Single channel AA Technicon [4]

Collection of Sample

1. Make two good clean skin punctures side-by-side using B-D long point Microlance or a small incision with a Bard Parker No. 11 scalpel blade. A sufficient flow of blood is essential for satisfactory collection.
2. Remove first drop of blood with gauze; fill 4–6 Caraway tubes.
3. Seal Caraway tubes with Critocaps (Clay Adams No. A-2942).
4. Insert the Caraway tubes into a test tube and centrifuge at 10,000 rpm for approximately 3 minutes.
5. Cut tubes with a sharp ampul file 0.5 mm above the buffy coat.
6. Allow serum to drain into a 0.5 ml specimen cup. The serum sample is collected from this pooled specimen using Eppendorf 10 μl pipette.

STUDY PROTOCOL

Our clinical study consisted of parallel evaluations of 100 normal patient sera (having SMA-12/60 survey values of less than 20 mg/100 ml) and 50 abnormal patient sera (with SMA-12/60 values greater

[1] Clay-Adams Company, New York, NY.

[2] General Diagnostics Division of Warner-Lambert Company, Morris Plains, NJ.

[3] Eppendorf-Brinkmann Instruments, Inc., Westbury, NY.

[4] Technicon Instrument Corporation, Tarrytown, NY.

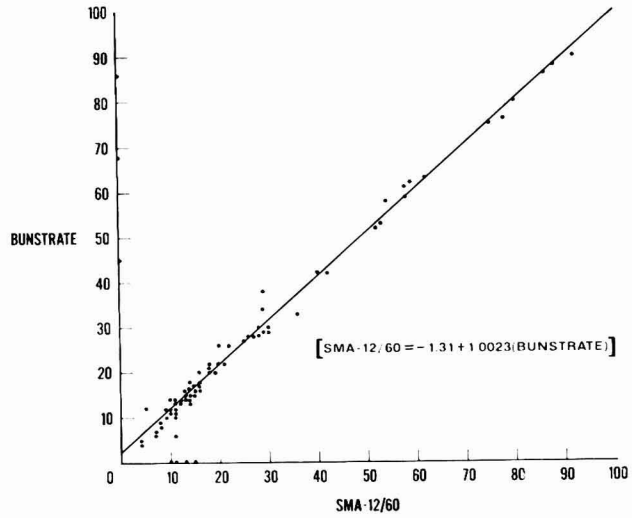

FIG. 1. Plot of blood urea nitrogen (mg/100 ml) found by BUN-Strate versus that found by SMA-12/60.

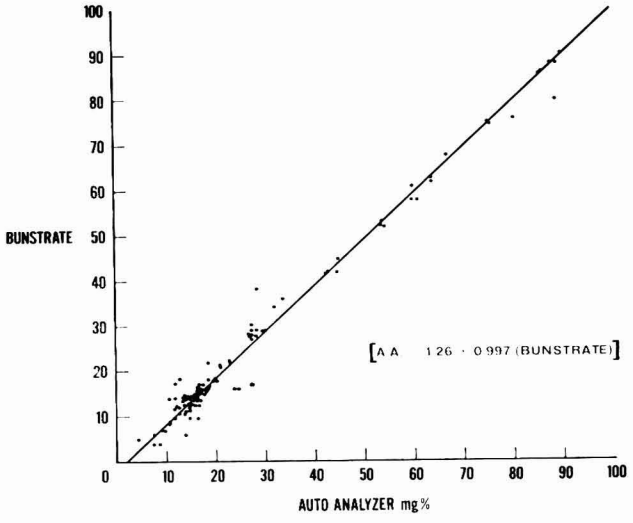

FIG. 2. Plot of blood urea nitrogen (mg/100 ml) found by BUN-Strate versus that found by AutoAnalyzer.

than 29 mg/100 ml) by three BUN methods (*1–3*): routine AA, Urograph, and BUN-Strate. All three methods were standardized and controlled with the same five reference and control sera listed below:

Serum	Lot no.	Urea nitrogen (mg/100 ml)
Versatol	2276049	12.1
Versatol A	220039	30.4
A Alternate	2204049	61.0
Local pool normal		26.0
abnormal		57.0

The resulting data was then submitted for regression analysis to establish the degree of correlation among the various methods studied.

RESULTS AND DISCUSSION

Excellent correlations were achieved among the various procedures, as illustrated by Figs. 1–6. The correlation coefficients for all comparisons were $R = 0.98 - 0.99$ (Table 1), indicating excellent agreement among the automated, Urograph, and BUN-Strate procedures.

The normal range values obtained under the conditions of this study are summarized below:

	Normal range (mg/100 ml; $\bar{X} \pm 2$ SD)
BUN-Strate	3–25
Urograph	8–23
SMA-12/60	3–22
AutoAnalyzer	6–25

CONCLUSIONS

The BUN-Strate microassay system represents a highly useful, precise and accurate microchemical procedure. BUN-Strate shows an excellent correlation with the SMA-12/60 and single channel AA automated procedures, as well as with the Urograph assay system. Thus, BUN-Strate meets all of the criteria outlined above for a microchemical method.

The Urograph procedure has also exhibited excellent correlations with the automated procedures, and represents an effective and meaningful BUN procedure for routine laboratory use.

SUMMARY

A microprocedure for determination of blood urea nitrogen, using a commercial product (BUN-Strate), is described. This method has the advantage of: use

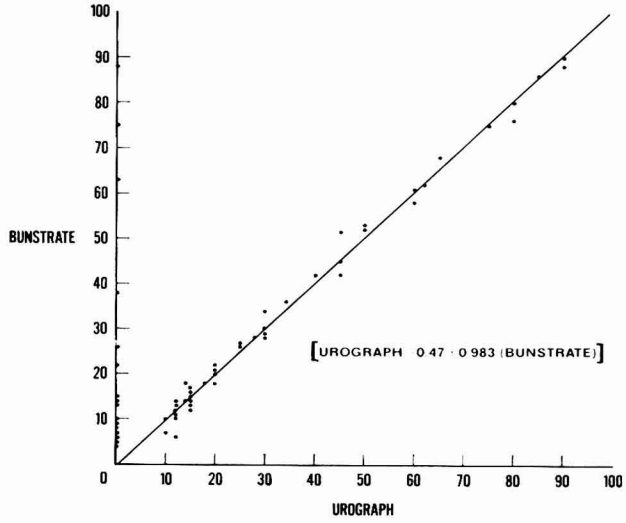

FIG. 3. Plot of blood urea nitrogen (mg/100 ml) found by BUN-Strate versus that found by Urograph.

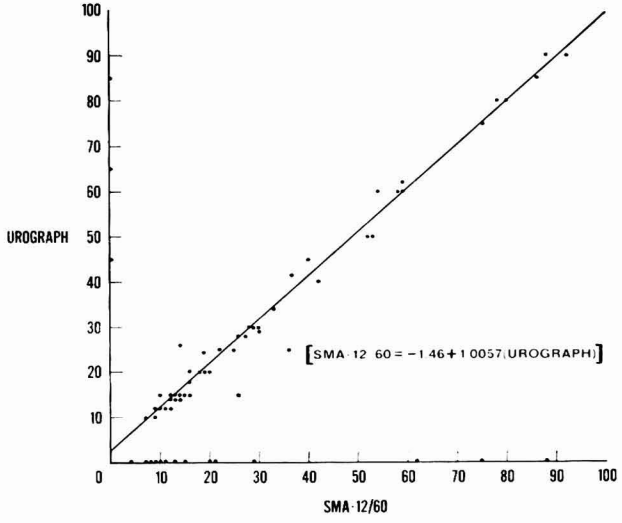

FIG. 4. Plot of blood urea nitrogen (mg/100 ml) found by Urograph versus that found by SMA-12/60.

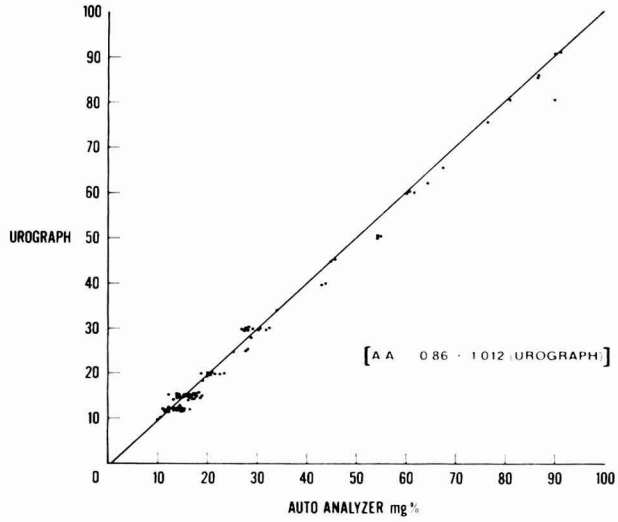

FIG. 5. Plot of blood urea nitrogen (mg/100 ml) found by Urograph versus that found by AutoAnalyzer.

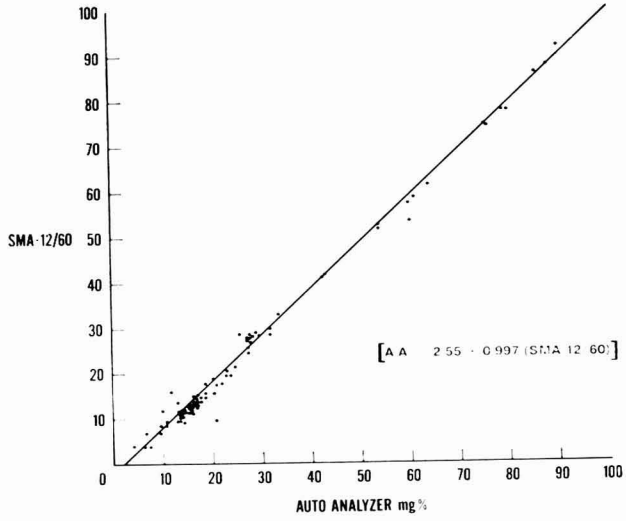

FIG. 6. Plot of blood urea nitrogen (mg/100 ml) found by SMA-12/60 versus that found by AutoAnalyzer.

TABLE 1
REGRESSION EQUATIONS RELATING FOUR BUN PROCEDURES

	Regression equation	Correlation coefficient r
BUN-Strate relationships		
—vs SMA-12/60	BUN-Strate = 1.74 + 0.9800 (SMA-12/60)	0.991
(see Fig. 1)	SMA-12/60 = −1.31 + 1.0023 (BUN-Strate)	
—vs AutoAnalyzer	BUN-Strate = −0.31 + 0.969 (AA)	0.983
(see Fig. 2)	AA = 1.26 + 0.997 (BUN-Strate)	
—vs Urograph	BUN-Strate = 0.03 + 0.999 (Urograph)	0.992
(see Fig. 3)	Urograph = 0.47 + 0.983 (BUN-Strate)	
Urograph relationships		
—vs SMA-12/60	Urograph = 1.82 + 0.9798 (SMA-12/60)	0.993
(see Fig. 4)	SMA-12/60 = −1.46 + 1.0057 (Urograph)	
—vs AutoAnalyzer	Urograph = −0.15 + 0.964 (AA)	0.988
(see Fig. 5)	AA = 0.86 + 1.012 (Urograph)	
Automated relationship		
SMA-12/60 vs AutoAnalyzer	SMA-12/60 = −1.98 + 0.9816 (AA)	0.989
(see Fig. 6)	AA = 2.55 + 0.9967 (SMA-12/60)	

of disposable pipettes and the elimination of obtaining specimen by venipuncture. Data presented shows that BUN-Strate is as accurate and precise as three widely used methods for BUN determinations.

REFERENCES

1. Conway, E. J., "Microdiffusion Analysis and Volumetric Error," 4th ed., MacMillan, New York, 1963.
2. Henry, R. J., "Clinical Chemistry, Principles and Technics," p. 264. Harper & Row (Hoeber), New York, 1964.
3. Technicon Corporation, "N-Methodology: Systems Instruction Manual T-67-109-A," Tarrytown, New York, 1968.

MICROCHEMICAL JOURNAL 17, 167–172 (1972)

Spectrophotometric Determination of Titanium with Tiron

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INTRODUCTION

Tiron (disodium 1,2-dihydroxybenzene-3,5-disulfonate), which forms a soluble yellow complex with titanium, was first suggested as a reagent for the colorimetric determination of titanium by Yoe and Jones (*6*). Further work on the reagent (*4,5*) has confirmed its usefulness in the determination of small quantities of titanium.

The soluble yellow complex of Tiron with titanium is stable in the pH range 4.3 to 9.6 and shows an absorption maximum at 380 mμ (*5*). However ferric iron also forms a colored complex with Tiron, the color varying from blue below pH 5.7 to intense red above pH 7.0 (*6*). The dark color of this complex interferes with the determination of titanium.

Since the Fe(III)–Tiron complex can be reduced by sodium dithionite to a colorless compound, Tiron can be used as reagent for iron and titanium in one solution, by first determining iron and then titanium after bleaching the Fe(III)–Tiron complex (*5*). The procedure given by Yoe and Armstrong (*5*) consisted of adding Tiron to the test solution, followed by ammonia until the solution was neutral to Congo red, and then pH 4.7 buffer. The absorbance of the Fe(III)–Tiron complex was measured at 560 mμ, 25 mg of sodium dithionite were added, and the absorbance of the yellow Ti–Tiron complex was measured at 410 mμ.

In an adaptation of this procedure to silicate analysis, Corey and Jackson (*1*) added the test aliquot to a volumetric flask containing the Tiron reagent and pH 4.7 buffer, diluted the solution to volume, and determined the iron at 560 mμ. Titanium was determined at 410 mμ after reducing the Fe(III)–Tiron complex.

The difficulties that may be experienced with this procedure are:

1. Test solutions containing titanium are generally very acidic (about 0.4 *N* HCl), so that the pH of the solution even after addition of pH 4.7 buffer and Tiron may be lower than 4.3.

2. Sodium dithionite in solution absorbs strongly at wavelengths shorter than 400 mμ. Therefore the absorbance of the Ti–Tiron com-

plex cannot be measured at 380 mμ, but must be done at 400–410 mμ, reducing the sensitivity of the method.

3. Use of dithionite as reductant often results in precipitation of sulfur if the iron content of the solution is high.

4. The reduction by dithionite is temporary and the blue color of the Fe(III)–Tiron complex reappears in a few minutes.

Further, if iron and titanium contents differ in order of magnitude, as is usually the case, separate aliquots have to be used to determine both elements. Since iron can be very conveniently determined by the orthophenanthroline method (*2,3*), Tiron is best used specifically for titanium.

Several reductants were tried for bleaching the Fe(III)–Tiron complex, and thioglycolic acid was found to be the most suitable. A procedure using this reductant is discussed below.

MATERIALS AND METHODS

Standard 100 ppm titanium solution was prepared by fusing 0.1668 g of spectroscopically pure titanium dioxide with potassium pyrosulfate, taking up the melt in 60 ml of 6 *N* HCl and diluting with water to 1000 ml. This stock solution was diluted with 0.4 *N* HCl to give a 10 ppm working solution.

Standard 500 ppm iron solution was prepared by dissolving 0.7147 g of spectroscopically pure ferric oxide in 6 *N* HCl with concentrated nitric acid and diluting to 1000 ml.

Reagent grade chemicals were used to prepare standard solutions of aluminum, manganese, phosphorus, zirconium, copper, nickel, citrate, and fluoride.

N Sodium acetate–acetic acid buffer of pH 5.0 was prepared by dissolving 82 g of anhydrous sodium acetate and 27 ml of glacial acetic acid in 1000 ml of water and adjusting the pH to 5.0 with the help of a pH meter.

Thioglycolic acid (80%) was diluted with water to give a 40% solution.

Tiron reagent was prepared by dissolving 4.0 g of laboratory grade Tiron in about 80 ml of water and diluting to exactly 100 ml. Fresh reagent was prepared daily, though the solution will keep for a week.

Transmittancies of the solutions were measured on a Bausch and Lomb Spectronic 20 spectrophotometer using ½-inch test tubes.

Proposed method. Five ml of 4% Tiron solution is taken in a 50 ml volumetric flask, and 20 ml of pH 5.0 buffer are added. The test solution is added and the contents are mixed. One ml of 40% thioglycolic acid is then added, the solution is diluted to volume and mixed. The transmittancy is measured at 380 mμ, and the titanium content is determined from a standard curve prepared in a similar manner using differ-

ent amounts of standard titanium solution. Beer's law is obeyed in the range 0.4–2.0 ppm Ti.

EXPERIMENTAL RESULTS

The spectral transmittancy values for 40% thioglycolic acid over the wavelength range 330–600 mμ are given in Table 1. The reagent shows no absorption in the entire range.

The absorbance curve for the Ti–Tiron complex given in Fig. 1 confirms that maximum absorption occurs at 380 mμ. The absorbance at 410 mμ is about 12% less. Since the reductant does not absorb at 380 mμ, the highest sensitivity of the method can be attained by using this wavelength.

The standard curve for titanium was obtained both with and without iron (Table 2). Up to 50 ppm of iron does not interfere. The final pH obtained was 4.6–5.0, showing that use of pH 5.0 buffer in the amount mentioned was advantageous. A straight-line curve was obtained in the range 0.4–2.0 ppm Ti.

The limiting concentrations of various ions permissible in the procedure were determined by using varying concentrations of the ions. The values given in Table 3 show that only copper interferes at low concentrations (greater than 0.2 ppm). Up to 10 ppm Ni is tolerated, and much larger concentrations of Mn, Zr, Al, P, F, and citrate do not interfere.

Interference by Al, Fe, Mn, and Zr in presence of P was also studied (Table 4). In presence of 200–400 ppm P, Al does not interfere up to

TABLE 1

SPECTRAL TRANSMITTANCE VALUES FOR 40% THIOGLYCOLIC ACID

Wavelength (mμ)	Transmittance (%)	Wavelength (mμ)	Transmittance (%)
330	100.0	470	100.0
340	100.0	480	100.0
350	100.0	490	100.0
360	100.0	500	100.0
370	99.0	510	100.0
380	100.0	520	100.0
390	100.0	530	100.0
400	100.0	540	100.0
410	99.0	550	100.0
420	100.0	560	100.0
430	99.0	570	100.0
440	100.0	580	100.0
450	99.0	590	100.0
460	100.0	600	100.0

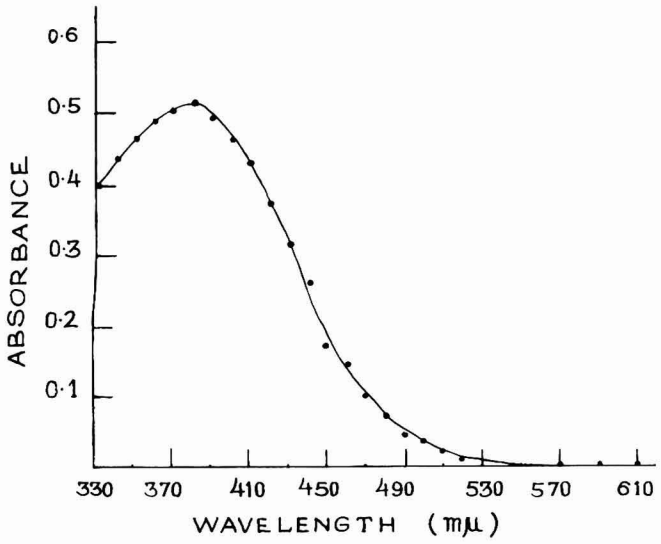

FIG. 1. Absorbance curve of the titanium–Tiron complex.

a level of 400 ppm, Fe to 20 ppm, and Mn to 200 ppm. These were the highest concentrations of the cations studied. However 1 ppm Zr interferes. Therefore the test solutions should not contain P and Zr together.

DISCUSSION

Use of a pH 5.0 buffer in the system makes it possible to maintain the final pH of the solution around 4.6 even with the highly acidic Ti

TABLE 2

ABSORBANCIES AT 380 mμ OF THE Ti–TIRON SOLUTIONS WITH VARYING AMOUNTS OF TITANIUM

Content (ppm)			
Titanium	Iron	Absorbance	pH
0.4	—	0.137	5.0
0.8	—	0.276	5.0
1.2	—	0.420	5.0
1.6	—	0.553	5.0
2.0	—	0.699	5.0
0.4	50.0	0.137	4.6
0.8	50.0	0.276	4.6
1.2	50.0	0.420	4.6
1.6	50.0	0.545	4.6
2.0	50.0	0.699	4.6

TABLE 3

INFLUENCE OF OTHER IONS ON THE DETERMINATION OF TITANIUM WITH TIRON

Titanium taken (ppm)	Other ion		Titanium found (ppm)	Interference
	Species	Conc. (ppm)		
0.80	Cu	0.2	0.80	No
0.80	Cu	0.5	0.83	Yes
0.80	Ni	10	0.80	No
0.80	Ni	15	0.85	Yes
0.80	Mn	400	0.80	No
0.80	Zr	800	0.80	No
0.80	Al	600	0.80	No
0.80	PO_4	400 [a]	0.80	No
0.80	Fe	50	0.80	No
0.80	F	40	0.80	No
0.80	Citrate	2500	0.80	No

[a] Expressed as P.

solutions. This pH lies within the stability range of the Ti–Tiron complex (pH 4.3–9.6). Since iron is not proposed to be determined by this method, maintenance of pH at exactly 4.7 as recommended by earlier workers (*1,5*) is not necessary.

Thioglycolic acid reduces the interfering Fe(III)–Tiron complex almost instantly, and prevents subsequent oxidation. This permits preparation of a large number of samples prior to absorptiometry. Since thioglycolic acid does not absorb in the range 330–660 mμ, the color of the Ti–Tiron complex can be measured at 380 mμ, the wavelength of maximum absorption. Absorbance at this wavelength is about 12% higher than at 410 mμ, the wavelength hitherto used.

TABLE 4

INFLUENCE OF OTHER IONS IN PRESENCE OF PO_4 ON THE DETERMINATION OF TITANIUM BY TIRON

Concentration of titanium taken = 0.8 ppm.

Final concn. of other ions (ppm)					Titanium found (ppm)	Interference
PO_4[a]	Al	Fe	Mn	Zr		
200	400	0	0	0	0.8	No
400	0	20	0	0	0.8	No
200	0	0	200	0	0.8	No
400	0	0	0	1	0.75	Yes

[a] Expressed as P.

SUMMARY

The procedure for spectrophotometric determination of titanium with Tiron (disodium 1,2-dihydroxybenzene-3,5-disulfonate) has been modified by using thioglycolic acid to reduce the Fe(III)-Tiron complex which otherwise interferes with transmittance measurement. Reducation by thioglycolic acid is instantaneous and reoxidation does not occur. Addition of a pH 5.0 buffer to the system maintains the pH within the stability range of the Tiron complex. Large amourts of Mn, Zr, Al, P, F, and citrate do not interfere, but more than 0.2 ppm Cu and 10 ppm Ni are not permissible. P and Zr should not be present together.

ACKNOWLEDGMENT

The authors are grateful to Dr. C. Dakshinamurti, Head, Division of Agricultural Physics, Indian Agricultural Research Institute for providing the necessary research facilities.

REFERENCES

1. Corey, R. B., and Jackson, M. L., Silicate analysis by a rapid semimicrochemical system. *Anal. Chem.* **25,** 624–628 (1953).
2. Krishna Murti, G. S. R., Moharir, A. V., and Sarma, V. A. K., Spectrophotometric determination of iron with orthophenanthroline. *Microchem. J.* **15,** 585 (1970).
3. Sandell, E. B., "Colorimetric Determination of Traces of Metals," 487 pp. Wiley (Interscience), New York, 1944.
4. Yoe, J. H., and Armstrong, A. R., A new colorimetric reagent for titanium. *Science* **102,** 207 (1945).
5. Yoe, J. H., and Armstrong, A. R., Colorimetric determination of titanium with disodium-1,2-dihydroxybenzene-3,5-disulfonate. *Ind. Eng. Chem., Anal. Ed.* **19,** 100–102 (1947).
6. Yoe, J. H., and Jones, A. L., Colorimetric determination of iron with disodium-1,2-dihydroxybenzene-3,5-disulfonate. *Ind. Eng. Chem., Anal. Ed.* **16,** 111–115 (1944).

MICROCHEMICAL JOURNAL 17, 173–185 (1972)

Identification of the Source of the Crude Drug Tu-zhu-ye Using Low-temperature Plasma Ashing

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INTRODUCTION

Low-temperature oxygen plasma excited in a high-frequency electromagnetic field under low pressure gently removes organic substrates from specimens at an ordinary temperature. Mineral constituents remaining as an ash are partly volatilized during heating in a platinum or porcelain crucible by the conventional high-temperature ignition method. The plasma ashing can therefore be successfully used for mineralization of organic substances for atomic absorption spectrometry and other analytical techniques *(1–6)*.

Usage of the plasma ashing technique has been recently extended by the present authors to the ashing of plant tissues. The resultant ashed tissues are microscopically examined to determine the characteristic presence of inorganic crystals such as calcium oxalate, calcium carbonate and silicon bodies in the original tissue matrix *(7–14)*. Since the ashed tissues completely preserve the structural integrity of the mineral constituents in the specimens, and distribution patterns of the above-mentioned materials are highly specific for the individual plant tissue it has been possible to use the plasma ashing technique for identification and taxonomical studies of plants.

The present authors have reported an application of the plasma ashing method to an identification of the plant source of the Chinese crude drug "Dan-zhu-ye" which had been traditionally used as a diuretic and had not been identified by the conventional histological method *(7, 14)*. This paper reports more recent work carried out for identification of the Korean crude drug "Tu-zhu-ye" which has uses similar to "Dan-zhu-ye" and has been thought to be made from the leaves of one of the *Phyllostachys* bamboo species.

MATERIALS AND METHODS

Plasma Asher

Although any type of plasma asher which is commercially available may be used, a relatively small power apparatus is suggested for ashing specimens such as plant leaves on microscope glass slides *(5, 8)*. The apparatus used in this work had a maximum output power of 50 W at 14 MHz which could be smoothly reduced down to the minimum of 10 W. The electrical set-up is easily done in a laboratory workshop.

The flow sheet and the power unit are schematically illustrated in Fig. 1. Oxygen is supplied from a tank A at a flow rate of 30 ml/min towards a needle valve B, a flow meter C and a three-way juncture D. A 10 ml/min portion of the oxygen is introduced to a plasma tube G via a flow meter E and needle valve F by evacuating the plasma tube G with a rotary pump J via a MacLeod vacuum gauge H and a three-way stopcock I. The plasma tube is made of Pyrex with an outside diameter of 35 mm and a length of 40 cm. A pumping speed of more than 50 l/min is necessary for evacuating the plasma tube at around 1 mm Hg. A specimen on a glass slide M is located at 5–10 cm downstream from a high-frequency coil K which has been tuned at 14 MHz by adjusting a variable air condenser VC tapped by a coaxial cable L.

Preparation and Ashing of Specimens

A plant leaf is cut into small pieces about 30×40 mm and fixed on a glass slide using cellophane tape as illustrated in Fig. 2. Fresh leaves

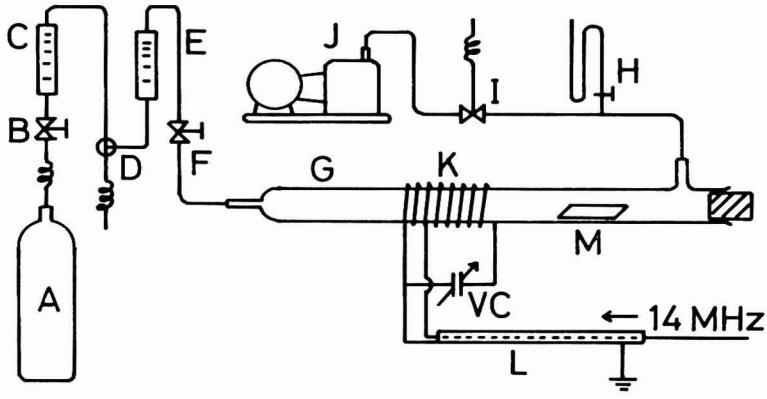

FIG. 1. Schematic diagram of low-temperature plasma asher: (A) oxygen tank; (B), (F) needle valve; (C), (E) flow meter; (D) three-way juncture; (G) Pyrex tube, o.d. 35 mm; (H) MacLeod guage; (I) three-way stopcock; (J) rotary pump; (K) HF coil, 10 t/7 cm; (L) coaxial cable, 75Ω impedance; (M) specimen on glass slide; (VC) variable air condenser, 150 pF.

are generally more smoothly ashed than dried materials because of closer fit of the fresh specimens to the surface of glass slides and a concomitant rate controlling effect during the plasma oxidation probably due to the presence of moisture in the specimens *(8)*. It is therefore advisable to soak dried leaves in water for a few hours prior to cutting.

A specimen thus prepared is inserted into the plasma tube and is placed as described above. Plasma ashing is normally carried out for a few hours but it sometimes requires several hours for specimens involving hard tissues, such as costae in bamboo leaves.

The glass slide with the ashed tissue is then carefully taken out of the plasma tube and a low-viscosity Canada balsam-xylene mixture (2:3) is slowly impregnated into the ashed tissue. The glass slide is left to dry overnight, protected from possible contamination. A few drops of high-viscosity Canada balsam–xylene mixture (6:1) are then added onto the specimen and a cover glass is mounted to make a permanent preparation *(8, 11)*.

EXPERIMENTAL METHODS

Specimens for comparative study

Tu-zhu-ye is marketed at Seoul and Taegu, Korea, both products having been thought to be leaves of one of *Phyllostachys* by conventional histological study. The present work was carried out using specimens of *Phyllostachys heterocycla* MITF. var. *pubescens* OHWI, *Phyllostachys nigra* MUNRO var. *henonis* STAPF, *Phyllostachys bambusoides* SIEB. et ZUCC. and *Bambusa multiplex* RAEUSCHEL collected during the summer season of 1969 or 1970 for a comparative study with the two specimens of "Tu-zhu-ye." Microscopical observations of the individual ashed tissues are described below.

Phyllostachys heterocycla MITF. var. *pubescens* OHWI. Growing districts: Takatsuki (Osaka pref.), Yamasaki (Kyoto pref.) and Mt. Ibuki (Shiga pref.), 1970.

The costa running along the central axis of the ashed leaf is densely

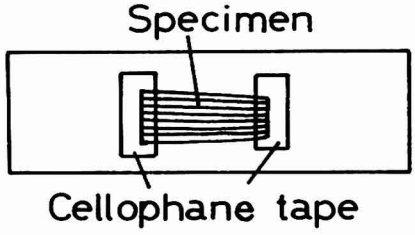

FIG. 2. Preparated specimen on glass slide.

lined by silicon bodies (Fig 3-A) of rounded square shape with some deformations (Fig. 3-B). Arrangement of the crystals in double lines at both surfaces of the leaf is also noted. The tracheid, which had been the structural material of the costa, has completely disappeared in the

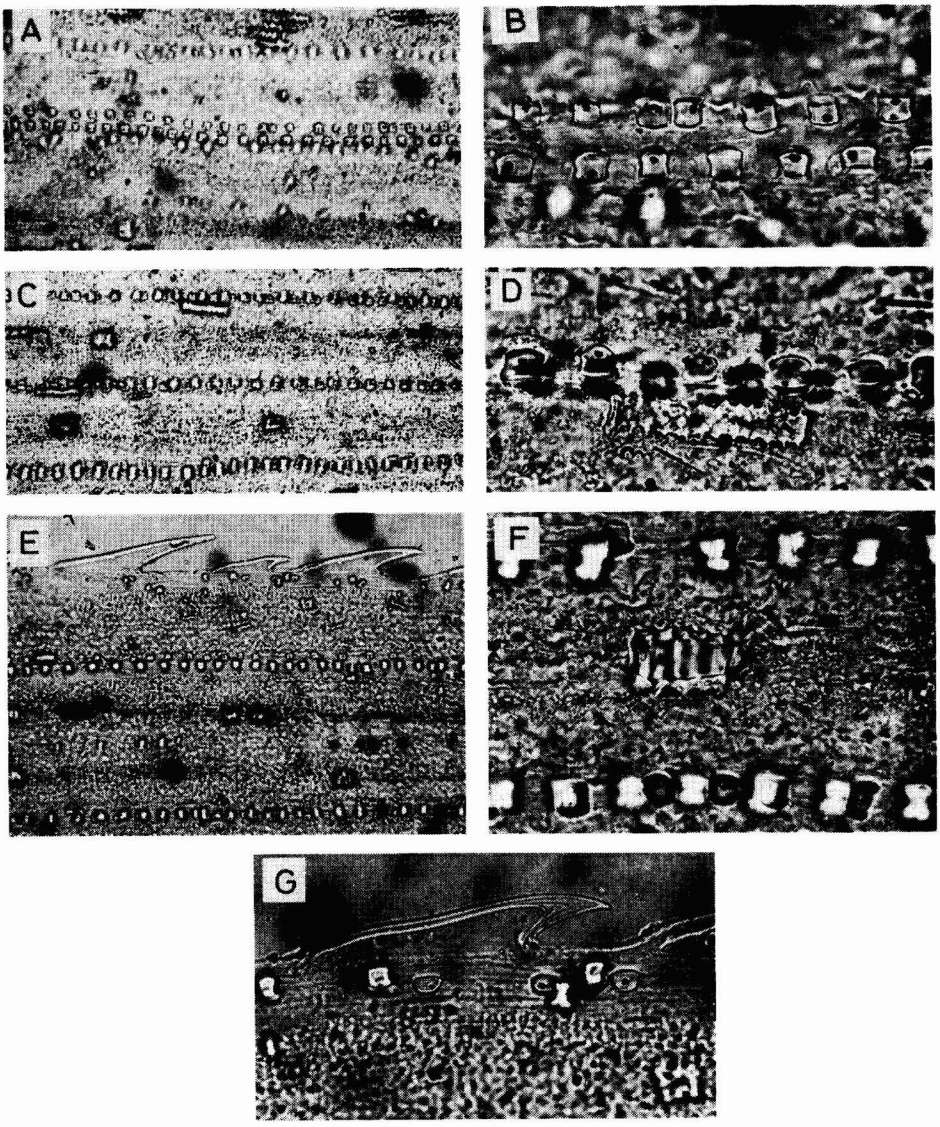

FIG. 3. Ashed leaf of *Phyllostachys heterocycla* MITF. var. *pubescens* OHWI: Costa, (A) 100×, (B) 400×; Mesophyllum, (C) 100×, (D), (F) 400×; Margin, (E) 100×, (G) 400×.

ashed tissue while some veinlets crossing between the costa and the neighboring veins remain in the original form although these materials have not been well focused in the photographs. The veinlets are not lined by silicon bodies but smaller grains are found along the network.

Along the veins at the mesophyllum, single lines of silicon bodies having the same shapes as those in the costa are deposited at both surfaces of the leaf (Fig. 3-C, D). Smaller grains are found at locations where cell membranes and their contents might have been in the original tissue. Idioblasts with rugged surfaces (Fig. 3-F) or of slender rectangle shape (Fig. 3-D) are also found in the mesophyllum along the veins. No hard hair is observed on the surface of the mesophyllum but some stomata are found at interveins near the costa.

The arrangement of the silicon bodies tends to be irregular at the margin (Fig. 3-E) and the crystals become smaller and more rounded (Fig. 3-G). Hard hairs at the margin are unicellular and their membranes are relatively thickened exhibiting multilayer system (Fig. 3-G).

Phyllostachys nigra MUNRO var. *henonis* STAPF. Growing district: Kanzaki-gun (Hyogo pref.), 1969.

Several lines of rectangular, rounded rectangular or biconcave form silicon bodies are deposited along the costa. It is quite characteristic of this species that most of the silicon bodies are longitudinal along the costa (Fig. 4-A, B). The nuclear points are mainly found at the central positions of the silicon bodies. Parallel stripes are observed in the crystals rather than concentric layers encircling the nuclear points as in other species. The arrangement of the crystals is not dense and the tracheid at the same place has disappeared in the ashed tissue.

The veins at the mesophyllum of this specimen have more sparsely distributed silicon bodies than *P. heterocycla* var. *pubescens,* but the arrangement of the crystals is relatively regular (Fig. 4-C). The crystals on the veins differ greatly from those on the costa and are longitudinal across the vein (Fig. 4-D). The nuclear points in the silicon bodies are located at either the central or eccentric position. The tracheid and the sieve tubes are not preserved in the ashed tissue. Some idioblasts are found in the mesophyllum mostly lying longitudinally along the vein and having irregular prominences at the profiles (Fig. 4-F). Another type of idioblast is also observed (Fig. 4-E). No hard hair exists in this area.

At the margin, fewer silicon bodies are observed and the crystals become smaller with irregular arrangement (Fig. 4-G). The round rectangular crystals are generally longitudinal across the vein (Fig. 4-H) and the nuclear points are located either at the central or eccentric position. Slender idioblasts having serrated profiles are found lying

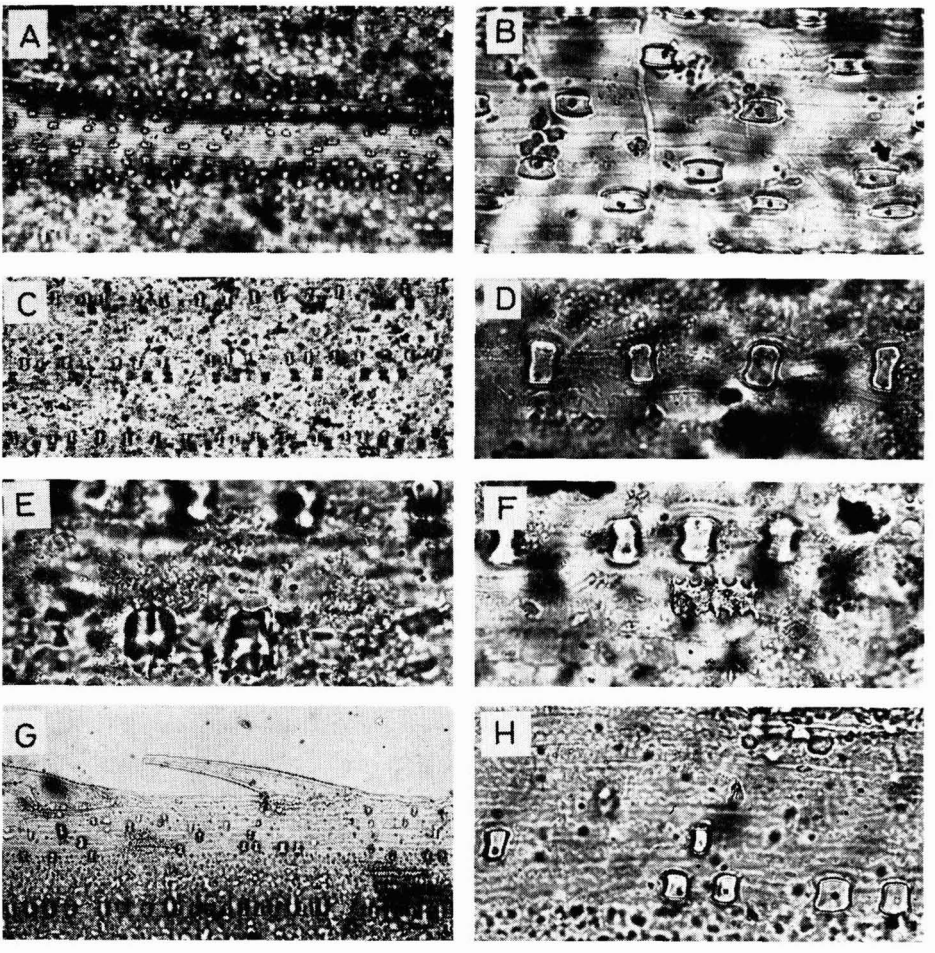

FIG. 4. Ashed leaf of *Phyllostachys nigra* MUNRO var. *henonis* STAPF: Costa. (A) 100×, (B) 400×; Mesophyllum, (C) 100×, (D), (E), (F) 400×; Margin. (G) 100×, (H) 400×.

parallel to the vein. Hard hairs at the margin are much larger than those of the *P. heterocycla* var. *pubescens*. They have thicker membranes (Fig. 4-G) and some of them consist of a double-cellular system.

Phyllostachys bambusoides SIEB. et ZUCC. Growing district: Mt. Ibuki (Shiga pref.), 1970.

The silicon bodies along the costa are somewhat more irregularly arranged compared with those of *P. heterocycla* var. *pubescens* and *P. nigra* var. *henonis* in terms of both the linearity of the linings and the separation of the crystals (Fig. 5-A). The shapes of the crystals differ from the above-mentioned species but are similarly biconcave

lying longitudinal across the costa (Fig. 5-B, D). Nuclear points in the crystals are generally located at the eccentric position with one point for one crystal but occasionally two or more nuclear points are found

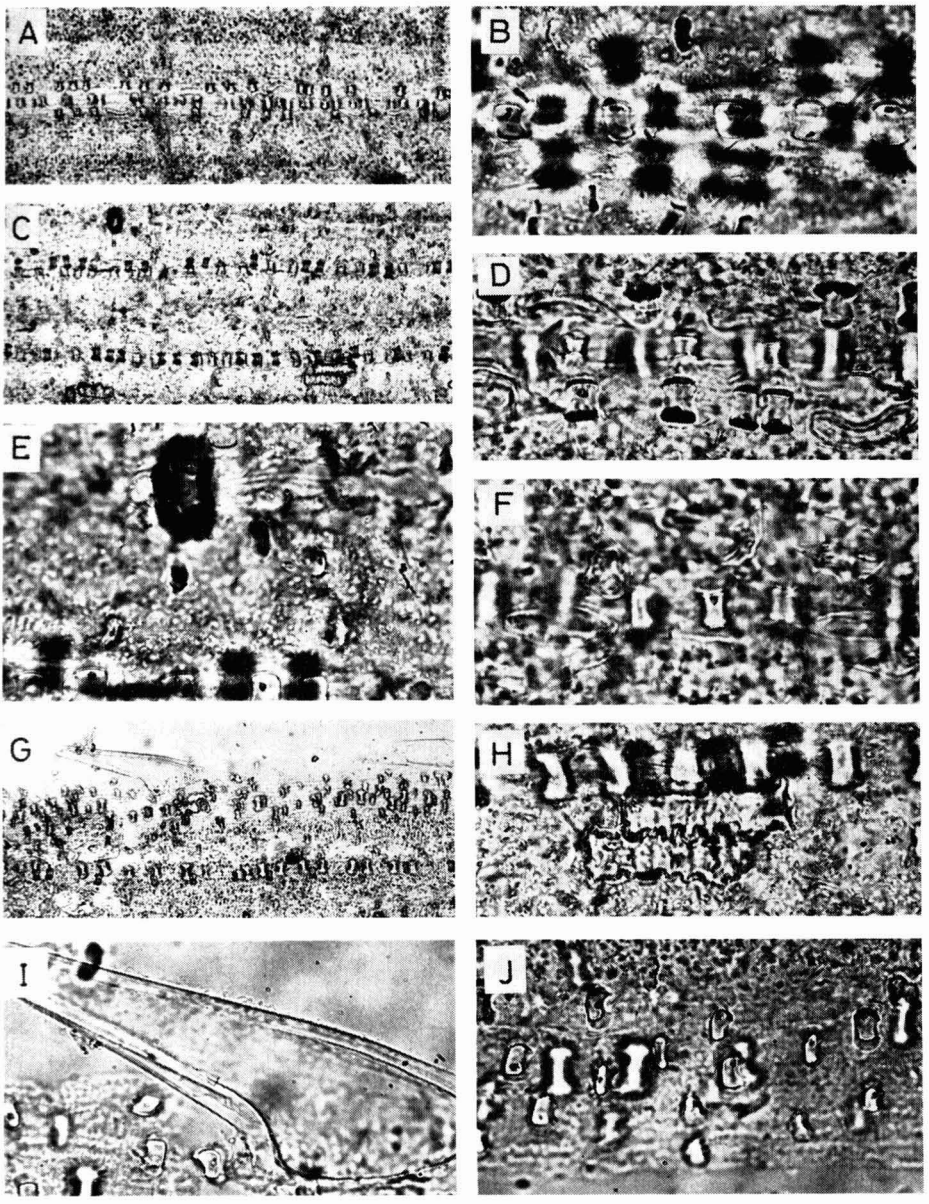

FIG. 5. Ashed leaf of *Phyllostachys bambusoides* SIEB. et ZUCC.: Costa, (A) 100×, (B), (D) 400×; Mesophyllum, (C) 100×, (E), (F), (H) 400×; Margin, (G) 100×, (I), (J) 400×.

in one crystal. A multilayer system is observed encircling the nuclear point. The tracheid has disappeared in the ashed tissue.

The veins at the mesophyllum are lined by silicon bodies that are more irregularly arranged than the above two species but the spacings are almost identical to *P. heterocycla* var. *pubescens*. The shape of the crystals is biconcave, longitudinal across the vein, and the nuclear points encircled by the multilayer system are found to be identical to those on the costa (Fig. 5-E).

Smaller silicon bodies than those on the veins are spread out at the intervein (Fig. 5-B, C, E). Idioblasts are also found at the intervein having varied shapes with or without prominences on their surfaces (Fig. 5-E, H). The stomata are not observed in the ashed tissue. Very short and unicellular hard hairs are sometimes found, having thickened membranes (Fig. 5-F). Some other crystalline materials exhibiting long prismatic or needle shape are also observed at the mesophyllum. Occasionally, relatively large clustered crystals which have not been chemically identified are found at the same time as are small grains of irregular sizes which probably functioned as cell constituents.

The arrangement of the silicon bodies at the margin tend to be very irregular but relatively dense (Fig. 5-G). This phenomenon is significantly different from other species studied in the present work. It is also seen that the silicon bodies deposited longitudinally across the vein have irregular sizes and are much thinner than those located at the costa or mesophyllum (Fig. 5-I, J).

The nuclear points are often located at the eccentric position encircled by a concentric multilayer system. Roundish or rectangular idioblasts are sometimes found and their cell membranes have serrate prominences. The hard hairs at the margin are slightly larger than *P. heterocycla* var. *pubescens* and *Bambusa multiplex*, but are still smaller than *P. nigra* var. *henonis* (Fig. 5-G). Their membranes are relatively thickened (Fig. 5-I) having a unicellular system, but no double-cellular system has been observed.

Bambusa multiplex RAEUSCHEL. Growing district: Yamashina (Kyoto pref.), 1970.

The arrangement of the silicon bodies along the costa exhibits more irregularity than the three species described above (Fig. 6-A). A variety of the crystal sizes should also be noted. All the crystals are longitudinal across the costa and their biconcave shapes are much more constricted than for any other species studied in the present work (Fig. 6-B). Relatively large nuclear points exist in the crystals mostly located at the eccentric positions. Parallel stripes are generally observed in the crystals rather than concentric layers.

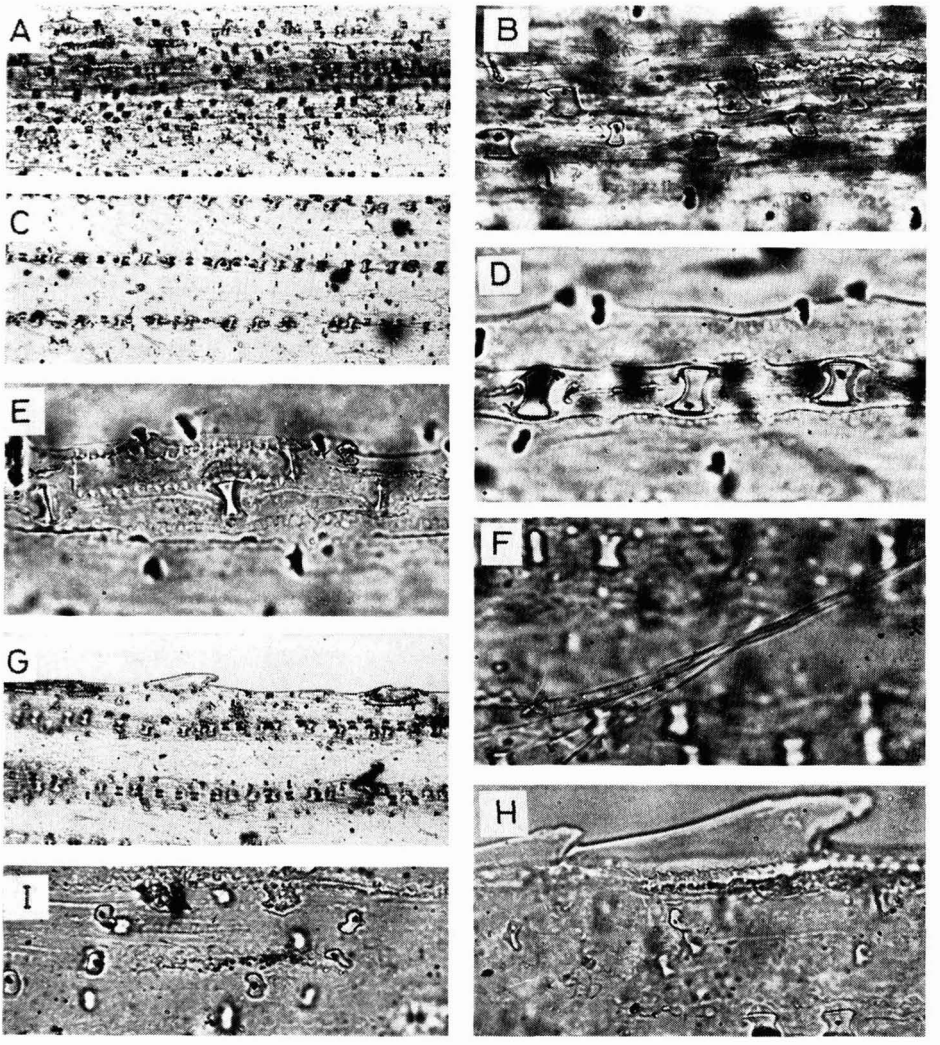

FIG. 6. Ashed leaf of *Bambusa multiplex* RAEUSCHEL: Costa, (A) 100×, (B) 400×; Mesophyllum, (C) 100×, (D), (E), (F) 400×; Margin, (G) 100×, (H), (I) 400×.

Exceptionally small silicon bodies are spread in very low density having practically no regular order. Their shapes are ellipsoidal or cocoon-like and longitudinal and lie across the costa. The presence of nuclear points in these small crystals is inconclusive but still a concentric multilayer system is observed.

Neither parenchyma nor vascular bundle systems from the original specimen remain in the ashed tissue but membranes of the epidermis

have been preserved to some extent. Idioblasts exhibited a variety of shapes such as long, short or bent bars and rectangular. Serrations are generally observed at the profiles.

The silicon bodies along the veins at the mesophyllum are arranged in single or partly double lines (Fig. 6-C). The sizes of the crystals are as large as those on the costa and have practically the same shape (Fig. 6-D). Some smaller crystals are found at the intervein as in *P. bambusoides* (Fig. 6-A, E). Extremely long idioblasts having serrate profiles lie along the vein (Fig. 6-E). The veinlets crossing between the veins have disappeared in the ashed tissue.

A very irregular arrangement of the silicon bodies is observed at the margin (Fig. 6-G). The crystals are relatively small and are spread in lower density than *P. bambusoides,* but still in higher density than *P. heterocycla* var. *pubescens* or *P. nigra henonis.* The shapes are cocoon-like being longitudinal across the vein (Fig. 6-H, I). The nuclear points in the crystals are located at the eccentric positions encircled by concentric multilayer system. Prismatic or twin crystals irregularly located are often found. Serrate hard hairs at the margin are unicellular (Fig. 6-G) smaller sizes than *P. nigra* var. *henonis* or *P. bambusoides* but nearly the same as *P. heterocycla* var. *pubescens.* Their membranes are considerably thickened (Fig. 6-G, H).

Relatively slender unicellular hairs are observed throughout the surface of the specimen and their membranes look thinner than those at the margin (Fig. 6-F).

Tu-zhu-ye (Seoul). The specimen had been marketed at Seoul, Korea, in 1940 and has been conserved at the Faculty of Pharmaceutical Sciences, Kyoto University.

The ashed tissue is identical with all the remarks described for *P. nigra* var. *henonis.* An example of the crystal arrangement on the costa is illustrated in Fig. 7 for comparison with Fig. 4-B.

Tu-zhu-ye (Taegu). The specimen had been marketed at Taegu,

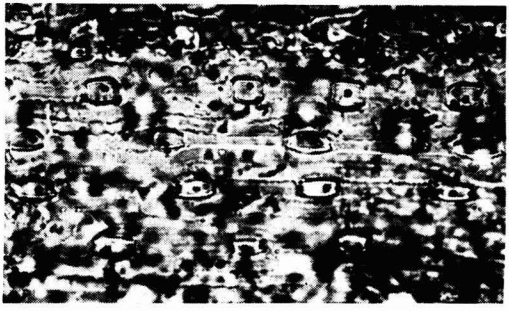

FIG. 7. Ashed tissue of "Tu-zhu-ye" at Seoul, Korea: Costa, 100×.

Korea, in 1940 and has been conserved at the Faculty of Pharmaceutical Sciences, Kyoto University.

The ashed tissue of this specimen differs greatly from all the specimens examined in this work. The silicon bodies are arranged on the veins with relatively straight lines having irregular spacing (Fig. 8-A). The shape of the crystals is generally rectangular with extraordinarily sharp corners (Fig. 8-C) and the nuclear points locate either at the central or eccentric position. Smaller silicon bodies are sometimes found at the intervein, these have a single nuclear point.

The decisive evidence isolating this specimen from the other bamboo species studied in this work is the relatively straight line of irregularly spaced idioblasts running parallel to the veins (Fig. 8-B). Some idioblasts attached to each other while solitary bodies are diffuse. Another type of slender idioblasts is found lying longitudinally across the vein. It appears therefore that the plant of origin of this specimen will differ considerably from the *Phyllostachys*.

CONCLUSION

Microscopical examinations of the ashed tissues preserving mineral microstructures of several bamboo specimens, namely, *Phyllostachys heterocycla* MITF. var. *pubescens* OHWI, *Phyllostachys nigra* MUNRO

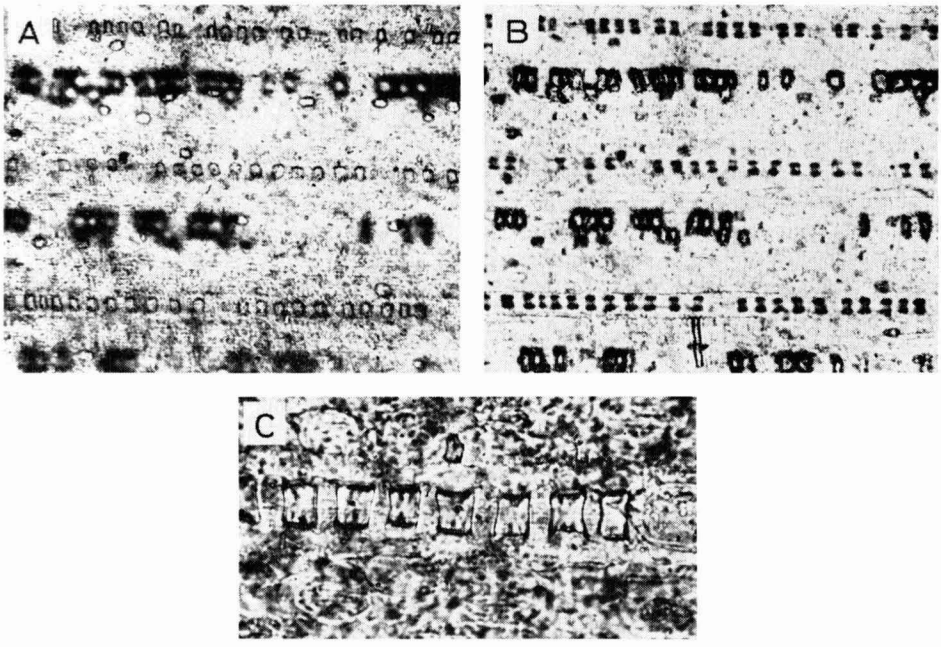

FIG. 8. Ashed tissue of "Tu-zhu-ye" at Taegu, Korea: Mesophyllum, (A), (B) 100×; (C) 400×.

var. *henonis* STAPF, *Phyllostachys bambusoides* SIEB. et ZUCC. and *Bambusa multiplex* RAEUSCHEL have revealed the characteristic presence of silicon bodies deposited along the veins and other inorganic materials which are highly specific for the individual species. Major identifying features are summarized as follows:

1) *P. heterocycla* var. *pubescens:* The shape of silicon bodies is generally roundish-square.

2) *P. nigra* var. *henonis:* The silicon bodies at the costa lie mostly longitudinally along the vein and the hard hairs at the margin are exceptionally large.

3) *P. bambusoides:* Silicon bodies at the margin are very irregularly arranged in high density. At the intervein, silicon bodies smaller than those along the veins are observed.

4) *Bambusa multiplex:* All the silicon bodies lie longitudinally across the vein. They have an extremely constructed hour-glass shape. Nuclear points in the crystals are relatively large. Smaller silicon bodies are found in the intervein.

A comparative study for identification of the plant of origin of the Korean crude drug "Tu-zhu-ye" using the ashing method has led to the conclusion that the specimen marketed at Seoul is identical to the *Phyllostachys nigra* MUNRO var. *henonis* STAPF while the specimen marketed at Taegu completely differs from any other specimens employed in the present work. Extensive study using a wider variety of bamboo species should therefore be requested.

SUMMARY

Tu-zhu-ye is a Korean crude drug for use as a diuretic but its plant source has not been identified. The present work has been attempted for identification of the original plant using specimens collected both at Seoul and Taegu, Korea, their mineral microstructures being examined after ashing the specimens by a low-temperature method with high-frequency oxygen plasma. A preliminary survey of the specimens with the naked eye suggested that the Tu-zhu-ye was near to *Phyllostachys* or its closely related bamboo species. Several specimens, namely, *Phyllostachys heterocycla* MITF. var. *pubescens* OHWI, *Phyllostachys nigra* MUNRO var. *henonis* STAPF, *Phyllostachys bambusoides* SIEB. et ZUCC. and *Bambusa multiplex* RAEUSCHEL were therefore ashed by the low-temperature method and the comparative microscopic studies with the resultant ashed tissues concluded that the "Tu-zhu-ye" from Seoul was perfectly identical with *Phyllostachys nigra* MUNRO var. *henonis* STAPF. A further investigation is continued for the specimen from Taegu since it is different from any other specimens provided for the present work.

REFERENCES

1. Berkley, C., Churg, J., Selikoff, I. J., and Smith, W. E., The detection and localization of mineral fibers in tissue. *Ann. N. Y. Acad. Sci.* **132**, 48–63 (1965).

2. Gleit, C. E., Electronic apparatus for ashing biologic specimens. *Amer. J. Med. Electron.* **2**, 112–118 (1963).
3. Gleit, C. E., and Holland, W. D., Use of electrically excited oxygen for the low-temperature decomposition of organic substances. *Anal. Chem.* **34**, 1454–1457 (1962).
4. Hollahan, J., Analytical applications of electrodelessly discharged gases. *J. Chem. Educ.* **43**, A401–416 (1966).
5. Hozumi, K., Chemistry of low-temperature oxygen plasma and its applications. *Kagaku-no-Ryoiki (J. Japanese Chem.)* **25**, 713–723 (1971).
6. Hozumi, K., and Matsumoto, M., Parameters for oxidation rate of organic substances in low-temperature oxygen plasma. *Japan Analyst* **21**, 206–214 (1972).
7. Umemoto, K., and Hozumi, K., A pharmacognostical application of low-temperature ashing: Microscopic observations on the mineral structure of "Dan-zhu-ye" and its related species of graminae. *Chem. Pharm. Bull. (Tokyo)* **19**, 217–219 (1971).
8. Umemoto, K., and Hozumi, K., Observations of calcium oxalate crystals in ashed tissues of leaves of *Cassia angustifolia* VAHL and *Camellia japonica* L. *Yakugaku Zasshi (J. Pharm. Soc. Japan)* **91**, 828–833 (1971).
9. Umemoto, K., and Hozumi, K., Observations of calcium carbonate crystals in ashed tissues of leaves of *Ficus elastica* ROXB., *Himulus japonicus* SIEB. et ZUCC. and *Justica procumbens* LINN., *Yakugaku Zasshi (J. Pharm. Soc. Japan)* **91**, 845–847 (1971).
10. Umemoto, K., and Hozumi, K., Observations of silicon bodies in ashed tissues of leaves of *Bambusa multiplex* RAEUSCHEL and Stems of *Equisetum hyemale* LINN. and *Equisetum ramosissimum* DESF. var. *japonicum* MILDE. *Yakugaku Zasshi (J. Pharm. Soc. Japan)* **91**, 850–854 (1971).
11. Umemoto, K., and Hozumi, K., Method for observation of mineral microstructure. *Yakugaku Zasshi (J. Pharm. Soc. Japan)* **91**, 890–895 (1971).
12. Umemoto, K., and Hozumi, K., Identifications of crude drugs Uvae Ursi folium and Vitis-Idaeae folium and their related plants by calcium oxalate crystals. *Yakugaku Zasshi (J. Pharm. Soc. Japan)* **91**, 1047–1058 (1971).
13. Umemoto, K., and Hozumi, K., Interrelation between grade of air pollution and distribution patterns of calcium oxalate crystals in leaves of *Gingko biloba* LINN. *Anal. Chem.*, in press.
14. Umemoto, K., Hutoh, M., and Hozumi, K., Identification of the plant source of the crude drug "Dan-zhu-ye" using low-temperature plasma ashing. *Mikrochim. Acta*, in press.

MICROCHEMICAL JOURNAL 17, 186–192 (1972)

Spectrophotometric Studies of the Complexes of Quadrivalent Titanium, Zirconium, and Hafnium with Dibromopyrogallol Sulfonphthalein

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INTRODUCTION

Dibromopyrogallol sulfonphthalein, which has a trivial name, bromopyrogallol red (BPGR) has been used as a sensitive metallochromic indicator in the titration of Bi^{3+}, Ni^{2+}, Co^{+2}, Cd^{+2}, Mg^{2}, and rare earths (*1*). The use of 1/10 Phenonthralin/BPGR indicator in the visual and photometric titrations of CN^- with $AgNO_3$ by Dagnall and Elghamry (*1*) and BPGR is reported to have been used in rare earth determinations (*3*). A detailed study of pyrogallol red and bromopyrogallol red as interesting chromogenic reagents has been reported by Pande and Sangal (*6*). The color of the dye in the acid solution was found to change from yellowish-orange to reddish violet by addition of titanium, zirconium, and hafnium ions. In the present communication, detailed investigation of metal chelates of titanium(IV), zirconium(IV), and hafnium(IV) with bromopyrogallol red is reported.

EXPERIMENTAL METHODS

Reagents

BPGR Solution. Dibromopyrogallol sulfonphthalein (B.D.H.) was dissolved in 40% alcohol. Carbon dioxide-free distilled water was used for making up the solutions to a required volume.

Titanium sulfate solution. An anhydrous titanium dioxide is fused with Na_2CO_3 and the fused mass is placed into acidified water, dissolved, and made up to desired volume. An aliquot is taken from the prepared solution and titanium hydroxide is precipated with ammonia and the residue is ignited to TiO_2.

Zirconium and hafnium–oxychloride solution. A known quantity of A.R. zirconium and hafnium oxychlorides are dissolved and the stock solution is kept strongly acidic.

Ion-exchange resins. Cationic and anionic resin beds are prepared by using Amberlite I R-45 and Amberlite IR-120.

Instruments

pH meter. Hydrogen ion concentration of the solution was measured with a Leeds and Northrup direct reading pH indicator with a glass calomel electrode system, operated on 220/V/50 cycles AC mains. The instrument was standardized from time to time with a standard buffer of pH 6.88 or 4.0 supplied along with the instrument.

Spectrophotometer. All spectrophotometric studies were made on a Beckman spectrophotometer model-B, using matched glass cells of 1 cm. thickness.

Condition of study. All experiments were performed at 25° ± 2°C. The total volume of the mixture prepared for the measurements was kept at 25 ml in each case. The pH of all solutions and mixtures was adjusted by the addition of sodium hydroxide and hydrochloric acid solutions.

Absorption spectra of BPGR Solution and BPGR–Metal Chelates

Bromopyrogallol red solution of the order 10^{-5} *M* was prepared at different pH values and their absorbances were recorded at different pH values and the region of maximum absorption were found to shift as shown in Table 1 (Fig. 1).

The method of Vosburg and Cooper (*7*) was employed to determine the nature of the complexes formed. Several solutions containing different ratios (0 : 1, 1 : 0.5, 1 : 1, 1 : 2, 1 : 3, 1 : 4) of metal and their absorption spectra was recorded from 400 to 675 mμ. Due to shortage of space only one figure is shown with Ti(IV) and BPGR (Fig. 2).

Variation of λ_{max} of the chelates with pH. The λ_{max} of the chelate is found at different pH values and the readings are given in Table 2 and Fig. 3. Here also due to shortage of space one figure with Ti(IV) and BPGR is shown.

Determination of Composition and Stability of Constants

Composition of the chelates. Composition of the chelates has been determined by absorptiometric measurements in which two different methods were used, the method of continuous variations (*6*) and the mole ratio method (*7*). The results obtained are in agreement with each other and show that each of the metal forms 1 : 1 chelate with BPGR Figs. 3 and 4.

Evaluation of conditional stability constants. The stability constant is useful for the understanding of the characteristic of a chelate (or a complex) but the determination of thermodynamic constants is beset

TABLE 1

VARIATION OF λ_{max} OF BPGR WITH pH

Reagent	pH	λ_{max} (mμ)	Remarks
BPGR	0.0–0.6	480	The dye is unstable above pH 8.0.
	0.5–3.2	430	
	3.2–4.5	570	
	4.5–8.0	580	

with difficulties and it is often convenient and yet valuable to determine the stoichiometric constants, which describe the stability of species under a given set of experimental conditions. In the present study the constants determined are those obtained at fixed temperature and pH as mentioned. This constant has been termed a conditional stability constant in the present work. The values are determined by two different methods, i.e., the method of Mukherj and Dey (*5*), and the mole ratio method, (Fig. 5). The results of the stability constant are shown in Table 3.

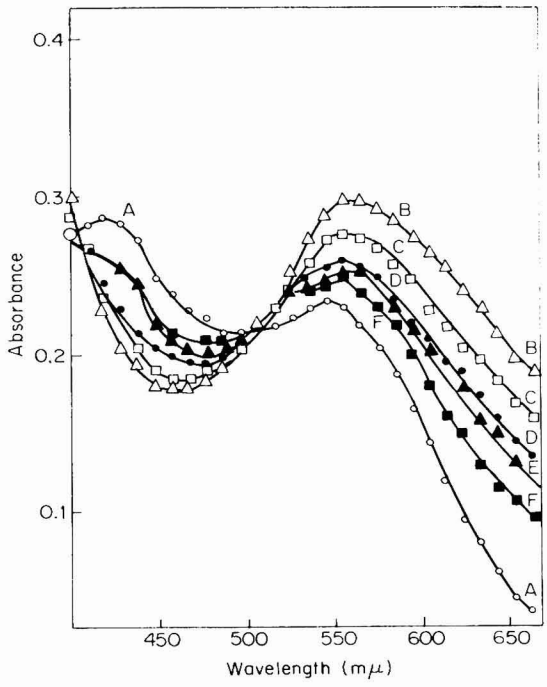


FIG. 1. Absorption spectra of the titanium(IV)–BPGR chelate at pH 1.5. Molar ratio of titanium(IV) to BPGR: (A) 0 : 1.0; (B) 1 : 0.5; (C) 1 : 1; (D) 1:2.0; (E) 1:3.0.

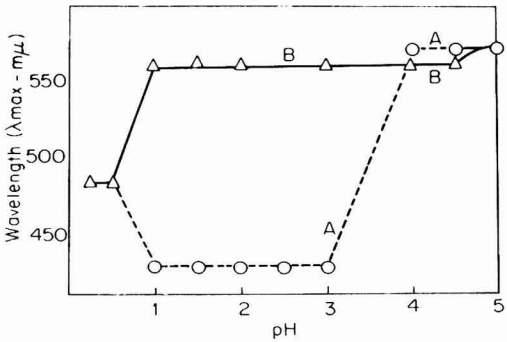
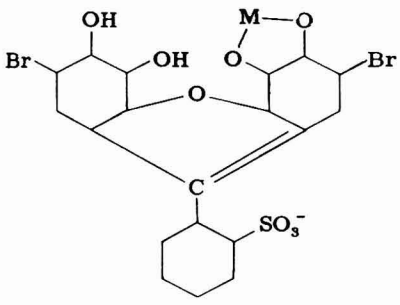
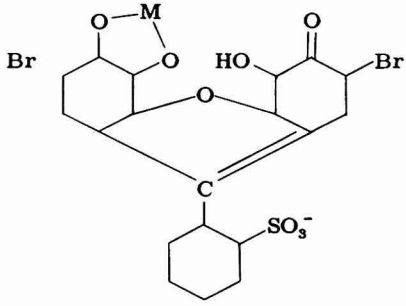


FIG. 2. Stability of the titanium(IV)–BPGR chelate with pH: titanium(IV), $C = 0.5 \times 10^{-4}$ *M* and BPGR, $C = 0.5 \times 10^{-4}$ *M*.

Structure of the chelate. There are two possible positions where the chelation can take place, i.e., between the quinoid oxygen and hydroxy group as shown in structure I and between the two hydroxy groups as shown in structure II. From the experimental facts it is observed that when the individual solutions of metal and ligand previously adjusted to the pH of study, mixed together, there is absolutely no fall in pH. Hence it is concluded that the chelation is taking place in the quinoid oxygen and the hydroxy group adjacent to it.



Structure I



Structure II

TABLE 2

WAVELENGTH OF MAXIMUM ABSORBANCE OF THE CHELATES

Metal chelates	pH of study	pH range of stability	λ_{max} of ligand (mμ)	λ_{max} of chelate (mμ)
Ti(IV)–BPGR	1.5	1.0–4.5	430	560
Zr(IV)–BPGR	0.6	0.3–1.5	430	540
Hf(IV)–BPGR	1.0	0.8–4.6	430	550

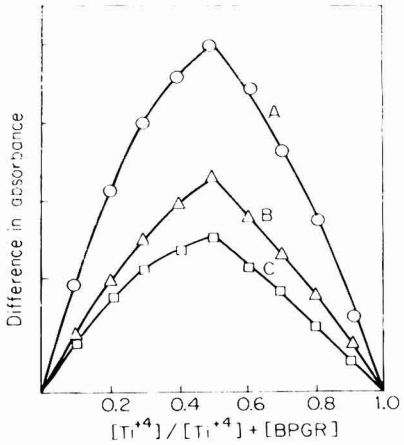


FIG. 3. Composition of the titanium–BPGR chelate by the method of continuous variation at pH 1.5 and 560 mμ (A) $C = 1.33 \times 10^{-4}$ *M*; (B) $C = 1.0 \times 10^{-4}$ *M*; (C) $C = 0.67 \times 10^{-4}$ *M*.

SUMMARY

The characteristics of thee colored chelates of titanium(IV), zirconium(IV), and hafnium(IV) with dibromopyrogallol red have been described. The studies include the determination of molar ratio by two different methods, the range of pH for the stability of the chelates and the evaluation of the conditional stability constants by

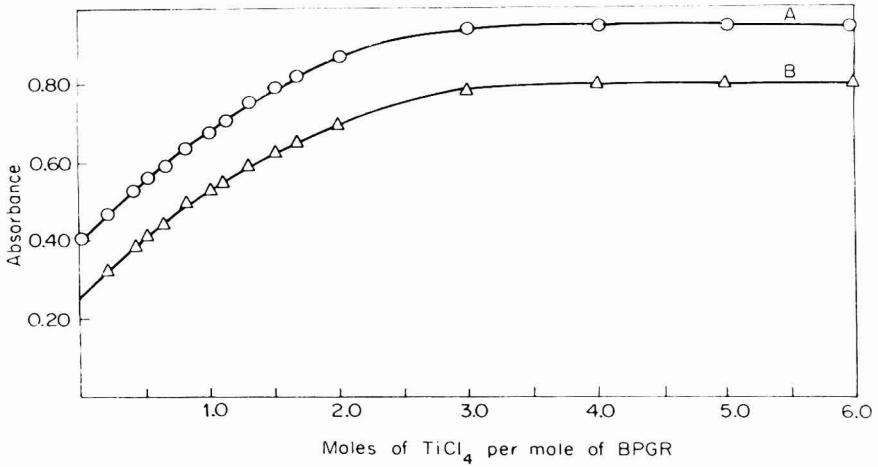


FIG. 4. Composition of the titanium–BPGR chelate by the mole ratio method at pH 1.5 and 560 mμ: (A) final concentration of BPGR $= 0.8 \times 10^{-4}$ *M*; (B) final concentration of BPGR $= 0.67 \times 10^{-4}$ *M*.

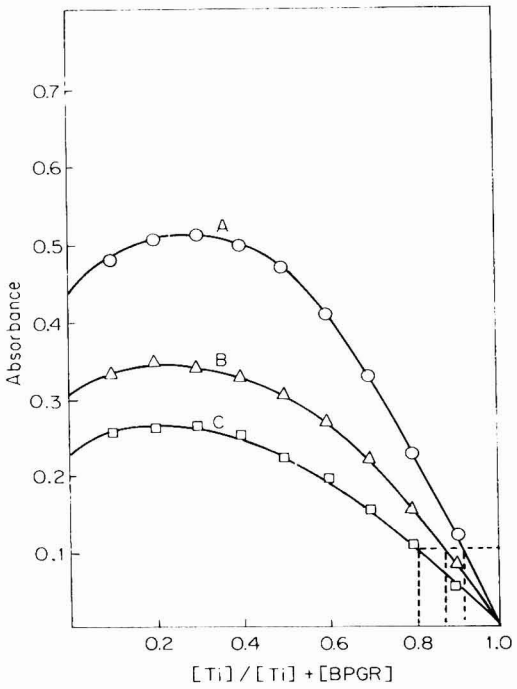


FIG. 5. Conditional stability constant of the titanium–BPGR chelate as evaluated by the method of Dey Mukherji and (5). Concentrations as in Fig. 3.

two methods, i.e., method of Dey and mole ratio method. The λ_{max} of the ligand was found to be at 430 mμ and that of the chelates of Ti(IV), Zn(IV), Hf(IV) were 560, 550, and 550 mμ, at the pH of study, i.e., 1.5, 0.6, and 1.0, respectively.

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TABLE 3

CONDITIONAL STABILITY CONSTANT OF THE CHELATES

Chelate	pH of study	Composition	log K at 25°C (1)	log K at 25°C (2)
Ti(IV)–BPGR	1.5	1:1	4.0073	5.0748
Zr(IV)–BPGR	0.6	1:1	3.5641	4.7086
Hf(IV)–BPGR	1.0	1:1	3.1948	4.3952

REFERENCES

1. Dagnall, R. M., Elghamry, M. T., and West, T. S. Analytical applications of ternary complexes. IV. Titration of macro and micro amounts of cyanide. *Talanta* **13**(12); 1967–1971 (1966).
2. Herrington, J., and Sneed, K. C., Spectrophotometric determination of the rare earths Y(III) and Ce(III) by bromopyrogallol red. *Anal. Chim. Acta* **22,** 180–184 (1960).
3. Jenickova, A., Malat, M., and Suk, V., Complexometric titrations (chelatomely) —bromopyrogallol red as a complexometric indicator. *Chem. Listy* **50,** 760–764 (1956).
4. Job, P., Spectrophotometric study of the formation of complexes in solutions and of their stability. *C. R. Acad. Sci.* **180,** 928–930 (1925); Formation and stability of inoranic complexes in solution. *Ann. Chim.* **9,** 113–203 (1928).
5. Mukherji, A. K., and Dey, A. K., Spectrophotometric and electrical conductance studies on chelate formation of uranl ion with ammonium aurin tricarboxylate. *Anal. Chim. Acta* **18,** 324 (1158); *J. Inorg. Nucl. Chem.* **6,** 314 (1958).
6. Pande, S. C., and Sangal, S. P., Pyrogallol red and dibromopyrogallol red as interesting chromogenic reagents. *J. Prakt. Chem.*, (in press).
7. Vosburg, W. C., and Cooper, G. R., The identification of complex ion in solutions by spectrophotometric measurements. *J. Amer. Chem. Soc.* **63,** 437–442 (1944).
8. Yoe, J. H., and Jones, A. L., Colorimetric determination of iron with disodium e, 2-dihydroxybenzene-3, 5-disulphonate. *Ind. Eng. Chem., Anal. Ed.* **16,** 111–115 (1944).

MICROCHEMICAL JOURNAL 17, 193–199 (1972)

Methods for the Isolation and Characterization of Constituents of Natural Products

XIV. Use of Iodine Monochloride for Detecting Unsaturation in Microgram Quantities of Colored Derivatives

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The detection of unsaturation in pure compounds isolated from natural products is an important prerequisite to their identification. At the microgram level, the noninstrumental methods which are currently favored in this regard include thin-layer adsorption chromatography on $AgNO_3$-impregnated plates (*6*) and bromination (*2–4*) also on thin-layer plates. For colored derivatives, principally 2,4-dinitrophenylhydrazones, only the $AgNO_3$ method has been extensively investigated (*1, 3, 5, 8*). Although this technique has been used with gratifying results in this laboratory, there have been instances when we were not always certain that a double bond existed based solely on the mobility of the unsaturated compound on a thin-layer plate impregnated with $AgNO_3$. An investigation of classical reagents for detecting double bonds has revealed that iodine monochloride is an efficient and relatively mild reagent for accomplishing this. A comprehensive study of the use of this reagent for the detection of unsaturation in colored derivatives of alcohols, carbonyls, amines, and acids is described below.

REAGENTS AND APPARATUS [2]

A 1% (w:v) solution of iodine monochloride (ICl) (Eastman Kodak Co., Rochester, N.Y. in CCl_4; melting point capillaries open at both ends (approx 1.5–2.0 mm o.d. and 100 mm long (Fisher Scientific Co., Silver Spring, MD, cat. no. 12-141). Thin-layer partition plates prepared according to Schwartz and Brewington (*7*) except that

[1] Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] Mention of specific commercial products does not imply endorsement.

1 ml of 85% H_3PO_4 was added to the slurry to stabilize the layer against flaking.

EXPERIMENTAL METHODS

Bend the capillary approximately at its center to an angle of 120–150°. Dip one end into the solution of ICl so that a column of liquid approximately 2–4 mm is retained in the capillary. Allow the liquid to fall to the bend and inject 1–3 μl of a CCl_4 solution of the derivative into the same end. Tilt the capillary so that the two solutions will mix. Immediately spot the contents directly from the capillary onto an area adjacent to a spot containing untreated derivative on a thin-layer partition plate and develop the plate immediately in an appropriate solvent. In most instances the effect of the addition of ICl to the double bond is apparent after the solvent has ascended a short distance and the plate can be removed. Exposure of the plate to diethylamine vapor or spraying with 1 *N* methanolic KOH can be used, if necessary, to intensify the spots.

RESULTS AND DISCUSSION

Table 1 lists the colored derivatives that were investigated. Included in the study were 2,4-dinitrophenylhydrazones, the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of alcohols, the α-methyl-2,4,6-trinitrophenylhydrazone derivatives of alkoxyacetaldehyde compounds, *N*-2,4-dinitrophenylamine derivatives and amides of pyruvic acid 2,6-dinitrophenylhydrazone. The percentage retardation was calculated from the respective R_f values of the treated and untreated derivatives. Approximately 1–5 μg of the derivative was used. However, 6×10^{-4} μmoles of an alcohol derivative and 7×10^{-4} μmoles of a 2,4-dinitrophenylhydrazone can still be halogenated and the spot detected on exposure of the plate to diethylamine vapor or by spraying with 1 *N* methanolic KOH.

It was first established that saturated compounds are not retarded when exposed to ICl. A large number of saturated derivatives (both carbonyls and alcohols) were subjected to the ICl procedure but not all of these are listed in Table 1. There is some destruction of both saturated and unsaturated 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds, but not of the alcohol derivatives. This destruction is manifested by a diminution in the intensity of the color of the spot of the treated compound. As a consequence, treatment of 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds with ICl should be carried out as rapidly as possible.

In all instances, where addition of ICl to the double bond took place,

TABLE 1

RETARDATION OF COLORED DERIVATIVES ON THIN-LAYER PARTITION PLATES AFTER REACTION WITH IODINE MONOCHLORIDE

	No. of double bonds	Retardation (%) in Hexane	Retardation (%) in Hexane:benzene (65:35)
Alcohols (as esters of pyruvic acid 2,6-dinitrophenylhydrazone)			
stearyl	0	0	
elaidyl	1	17	
vaccenyl	1	16	
oleyl	1	20	
linoleyl	2	51	
linolenyl	3	79	
11,14-eicosadien-1-ol	2	40	
cholestanol	0	0	
cholesterol	1	25	
lathosterol	1	0	
dihydrolanosterol	1	32	
lanosterol	2	67	
9,10-epoxystearyl	0	0	
10-undecen-1-ol	1		23
phytol	1		9
L-menthol	0		0
citronellol	1		13
L-isopulegol	1		42
linalool	2		54,74,83[a]
farnesol	3		52
β-phenylethanol	0		0
benzyl	0		0
3-phenyl-propan-1-ol	0		0
cinnamyl	1		20
furfuryl	2		92[b]
4-penten-1-ol	1		40
1-penten-3-ol	1		32
4-penten-2-ol	1		40
3-penten-2-ol	1		33
2-methyl-2-penten-1-ol	1		22
2,4-hexadien-1-ol	2		26
1,4-pentadien-3-ol	2		31,53[c]
1,6-heptadien-4-ol	2		44,93[c]
cis-3-hexen-1-ol	1		29
4-hexen-1-ol	1		29
4-hexen-3-ol	1		30
2-octen-1-ol	1		16
allyl	1		26
ricinoleyl	1		22[d]

TABLE 1 (continued)

	No. of double bonds	Retardation (%) in Hexane	Retardation (%) in Hexane-benzene (65:35)
Carbonyls (as 2,4-dinitrophenylhydrazones)			
stearyl	0	0	
oleylaldehyde	1	14	
2-hexadecenal	1	0	
tiglaldehyde	1		0
acrolein	1		0
2-ethyl-2-hexenal	1		0
trans-3-hexenal	1	0	0
cis-3-hexenal	1		60
cis-4-hexenal	1		50
cis-4-heptenal	1		47
cis-5-heptenal	1		38
7-octenal	1		33
nonanal	0		0
2-nonenal	1		0
trans-4-nonenal	1		36
trans-5-nonenal	1		36
cis-6-nonenal	1		28
trans-6-nonenal	1		23
trans-7-nonenal	1		33
8-nonenal	1		29
cis-7-decenal	1		31
2,4-octadienal	2		0
2,4-hexadecadienal	2		0
trans-2-*trans*-6-nonadienal	2		34
trans-2-*cis*-6-nonadienal	2		36
trans-2-*cis*-7-decadienal	2		34
cis-4-*cis*-7-decadienal	2		50
trans-2-*cis*-5-undecadienal	2		22
cis-5-*cis*-8-tetradecadienal	2	69	
2-ethyl-2-butyl-5-methyl-3,4-hexadienal	2		77
trans-2-*cis*-6-*cis*-9-pentadecatrienal	3	70	
trans-2-*cis*-5-*cis*-8-tetradecatrienal	3	67	
glycolaldehyde stearate	0	0	
glycolaldehyde oleate	1	35	
glycolaldehyde linoleate	2	81	
glycolaldehyde linolenate	3	86	
2-heptadecanone	0	0	
acetone	0		0
cholestan-3-one	0	0	
methyl-12-ketostearate	0	0	
ethyl pyruvate	0		0
mesityl oxide	1		0
1-hepten-4-one	1		0

TABLE 1 (continued)

	No. of double bonds	Retardation (%) in Hexane	Retardation (%) in Hexane:benzene (65:35)
2-nonen-4-one	1		0
testosterone	1		0
5-cholesten-3-one	1	0	
phorone	2		0
Alkoxyacetaldehyde compounds (as α-methyl-2,4,6-trinitrophenylhydrazones)			
octadecoxyacetaldehyde	0	0	
9-octadecenyloxyacetaldehyde	1	30	
9,12-octadecadienyloxyacetaldehyde	2	58	
Amines (as amides of pyruvic acid 2,6-dinitrophenylhydrazone)			
decyl	0		0
aniline	0		0
oleyl	1	20	
allyl	1		30
skatole	1		63
Acids (as *N*-2,4-dinitrophenyl-ethanolamine esters)			
lauric acid, ethanolamine ester	0	0	
palmitoleic acid, ethanolamine ester	1		20

[a] Three spots after treatment with ICl.

[b] Plate developed with hexane:benzene (2:1) saturated with stationary phase.

[c] Two spots after treatment with ICl.

[d] Plate developed with hexane:benzene (85:15). Ricinoleyl alcohol was investigated as the bis-derivative. This has zero R_f in hexane and the treated compound moves the same in hexane:benzene (65:35).

a sufficiently significant reduction occurred in the mobility of the treated derivative relative to the untreated derivative to warrant a conclusion regarding the unsaturated nature of the compound. Moreover, the complete absence of any unreacted derivative was always observed which helps to simplify the conclusion of whether a double bond is present. Preliminary experiments which involved spotting the derivative at the origin of a thin-layer plate and overspotting with the ICl solution always failed to completely halogenate the derivative. Despite the fact that the reaction had gone to a sufficient extent to detect a slower-moving component besides the original derivative, this approach was considered unsatisfactory because of the possibility of an unknown having zero mobility after halogenation. In these instances one might erroneously conclude that the compound was saturated.

A number of unsaturated derivatives failed to add ICl. All alk-2-enals, alk-2,4-dienals, and all α,β-unsaturated ketones were unchanged after treatment. The 2,4-dinitrophenylhydrazones of testosterone and Δ^5-cholesten-3-one also failed to react. *Trans*-3-hexenal (but not the *cis*-isomer) and 1-hepten-4-one likewise could not be halogenated. Thus, it appears that carbonyl compounds containing a single double bond or two double bonds conjugated with the hydrazone linkage and certain 2,4-dinitrophenylhydrazones containing a β, γ-bond will not react with ICl under the specified conditions. All unsaturated alcohol derivatives reacted satisfactorily except lathosterol (Δ^7-cholesten-3β-ol).

In some instances, in a given solvent system, derivatives containing more than one double bond are retarded to a significantly greater extent than are derivatives containing only one double bond. Thus, the derivatives of linoleyl and linolenyl alcohols and the glycolaldehyde esters of linoleic and linolenic acids are retarded to a greater extent than are oleyl and elaidyl alcohols and glycolaldehyde oleate, respectively. Similarly, lanosterol which differs from dihydrolanosterol by having a double bond in the side chain is retarded twice as much. Unfortunately, there were a number of exceptions. In the alcohols, 2,4-hexadien-1-ol is retarded as if it were a monoene. 1,6-heptadien-4-ol, and 1,4-pentadien-3-ol each gave two spots, one each of which was retarded like a monoene, the other as if it were a diene, suggesting that the addition of the halogen was incomplete for the second double bond.

The double bond in the pyrrolyl nucleus of skatole presumably reacted since the double bonds of the benzene ring are apparently inert to ICl under the prescribed conditions. At least one of the double bonds in furfuryl alcohol also reacted.

In several cases it was observed that derivatives which had added ICl to the double bond gave a different color than did the untreated derivative when the chromatogram was exposed to diethylamine vapor. This was especially true with the derivatives of alcohols which gave a reddish hue for the halogenated derivatives as opposed to a violet color for the untreated derivative. This color difference facilitated the analysis of ricinoleyl alcohol which was investigated as the *bis*-derivative. This compound, after treatment with ICl, moved identically to the untreated compound in hexane: benzene (65:35). Exposure of the plate to diethylamine vapor revealed the color difference. Separation was then effected in hexane: benzene (85:15).

A few trials were carried out to determine whether adsorption chromatography on silica gel G plates would effect significant separation of treated and untreated derivatives. However, the differences in

R_f were usually less than 5% and further study of this system was abandoned.

SUMMARY

A procedure is described for detecting unsaturation in the parent compound of colored derivatives at the microgram level using iodine monochloride. Included in the study were the 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds, the 2,6-dinitrophenylhyrazone derivatives of pyruvic acid esters of alcohols, the α-methyl-2,4,6-trinitrophenylhydrazone derivatives of alkoxyacetaldehyde compounds, the 2,6-dinitrophenylhydrazone derivatives of pyruvamides and the *N*-2,4-dinitrophenylethanolamine esters of fatty acids. Compounds which add iodine monochloride are sufficiently retarded on thin-layer partition plates to be classified as unsaturated. Carbonyl derivatives in which the double bond or bonds are conjugated with the hydrazone linkage and certain carbonyl compounds containing a double bond β,γ to the hydrazone linkage fail to be detected.

ACKNOWLEDGMENT

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REFERENCES

1. Badings, H. T., and Wassink, G., Separation and identification of aliphatic aldehydes and ketones by thin-layer chromatography of 2,4-dinitrophenylhydrazones. *Neth. Milk Dairy J.* **17**, 132–149 (1963).
2. Cargill, D. I., The separation of cholesterol from related stanols and stanones. *Analyst* **87**, 865–869 (1962).
3. Copious Peereboom, J. W., and Beekes, H. W., The analysis of mixtures of animal and vegetable fats. III. Separation of some sterols and sterol acetates by thin-layer chromatography. *J. Chromatogr.* **9**, 316–320 (1962).
4. Kaufmann, H. P., Makus, Z., and Khoe, T. H., Thin-layer chromatography of fats. VI. Hydrogenation and bromination on the plate. *Fette, Seifen, Anstrichm.* **64**, 1–5 (1962).
5. Meijboom, P. W., and Jurriens, G., Separation of positional and geometrical isomers of monoenoic aldehydes via the 2,4-dinitrophenylhydrazones. *J. Chromatogr.* **18**, 424–426 (1965).
6. Morris, L. J., Separation of lipids by silver ion chromatography. *J. Lipid Res.* **7**, 717–732 (1966).
7. Schwartz, D. P., and Brewington, C. R., Methods for the isolation and characterization of constituents of natural products. II. Separation of homologous series of esters of pyruvic acid 2,6-dinitrophenylhydrazone by thin-layer chromatography. *Microchem. J.* **12**, 1–6 (1967).
8. Urbach, G., Thin-layer chromatography of aliphatic 2,4-dinitrophenylhydrazones. *J. Chromatogr.* **12**, 196–208 (1963).

MICROCHEMICAL JOURNAL 17, 200–203 (1972)

Use of Ditelluratoargentate and Cerium(IV) in Microanalysis: Determination of Mixtures of Citric and Oxalic Acids and of Formic Acid and Methyl Alcohol

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INTRODUCTION

History regarding the discovery of Ag(III) has been described earlier (*1*).

In the present investigation, Ag(III) and Ce(IV) have been used as an oxidimetric reagent for estimation of mixtures of citric and oxalic acids, and formic acid and methyl alcohol.

Only citric acid is oxidized by Ag(III) to CO_2 and H_2O stage, whereas oxalic acid is unaffected. According to the following equation:

$$C_6H_8O_7 + 9\ O \rightarrow 6\ CO_2 + 4\ H_2O.$$

18 equivalents of the oxidant is consumed for complete oxidation of citric acid.

Both citric and oxalic acids are oxidized by Ce(IV) reagent in the presence of few drops of chromium sulfate as catalyst. Probably the following reaction takes place:

$$H_2C_2O_4 + O \rightarrow 2\ CO_2 + H_2O,$$
$$C_6H_8O_7 + 9\ O \rightarrow 6\ CO_2 + 4\ H_2O.$$

So 20 equivalents of oxidant are consumed for complete oxidation of oxalic and citric acids.

A combination of these two treatments provides a very simple method for determination of mixture of these two acids.

For the determination of mixture of formic acid and methyl alcohol; Ag(III) solution oxidized only formic acid to CO_2 and H_2O. Probably the following reaction takes place:

$$HCOOH + O \rightarrow CO_2 + H_2O.$$

So 2 equivalents of the oxidant are consumed for complete oxidation of formic acid.

TABLE 1

TITRIMETRIC DETERMINATION OF CITRIC ACID

Mixture solution	2.00×10^{-2} *M* Ag(III) used (ml)	Citric acid in mix. (mg)	
		Found	Present
1	1.12	0.239	0.240
2	2.25	0.480	0.480
3	4.52	0.963	0.960
4	5.63	1.200	1.200
5	6.78	1.451	1.445

While Ce(IV) oxidized both formic acid and methyl alcohol in presence of few drops of chromium sulfate as catalyst. Both formic acid and methyl alcohol are oxidized to CO_2 and H_2O as follows:

$$HCOOH + O \rightarrow CO_2 + H_2O,$$
$$CH_3OH + 3\ O \rightarrow CO_2 + 2H_2O.$$

So 8 equivalents of the oxidant are consumed for complete oxidation of formic acid and methyl alcohol.

From above two treatments, it is possible to determine the formic acid and methyl alcohol in their mixture.

EXPERIMENTAL METHODS

Reagents. All the chemicals used were of reagent grade.

Ag(III) solution was prepared and standardized as described (*1*).

Ceric sulfate solution (in 8 *N* H_2SO_4) was standardized against a standard solution of ferrous ammonium sulfate (in 1 *N* H_2SO_4) using *N*-phenylanthranilic acid as indicator (*2*).

Procedure. Five ml of the mixture solution was treated with an excess of Ag(III) at 80°C for 3 hours. The remaining Ag(III) was estimated as described (*1*). Same volume of the mixture solution was then

TABLE 2

TITRIMETRIC DETERMINATION OF OXALIC ACID

Mixture solution	1.00×10^{-2} *N* Ce(IV) used (ml)	Total citric and oxalic acid (mg)	Oxalic acid in mix. (mg)	
			Found	Present
1	6.75	3.070	2.830	2.830
2	8.50	3.000	2.520	2.520
3	12.02	2.860	1.900	1.890
4	13.76	2.780	1.580	1.570
5	15.51	2.720	1.270	1.260

TABLE 3

TITRIMETRIC DETERMINATION OF FORMIC ACID

Mixture solution	2.00×10^{-2} *M* Ag(III) used (ml)	Formic acid in mix. (mg)	
		Found	Present
1	0.50	0.234	0.235
2	0.75	0.345	0.345
3	1.00	0.460	0.460
4	1.49	0.687	0.690
5	2.01	0.926	0.920

refluxed for 60–90 minutes with an excess of ceric sulfate in the presence of concentrated sulfuric acid and a few drops of chromium sulfate. The unconsumed Ce(IV) was determined (*2*). The experiment was performed with different sets of mixture solution. Determination of citric and oxalic acids in mixture (Tables 1 and 2). Determination of formic acid and methyl alcohol in mixture (Tables 3 and 4).

RESULTS AND DISCUSSION

It has been observed that citric acid required 18 equivalents of oxidant for its complete oxidation to CO_2 and H_2O; whereas oxalic acid required 2 equivalents of oxidant for its oxidation to CO_2 and H_2O. Formic acid required two equivalents of oxidant; whereas methyl alcohol required 6 equivalents of oxidant for their complete oxidation. Tables 1 and 3 show that Ag(III) oxidized citric acid and oxalic acid, respectively; while Tables 2 and 4 show that Ce(IV) oxidized citric acid and oxalic acid; formic acid and methyl alcohol, respectively. Thus a method for determination of mixtures of citric acid and oxalic acid and formic acid and methyl alcohol has been described. The quantity of citric acid, oxalic acid, formic acid, and methyl alcohol have been calcu-

TABLE 4

TITRIMETRIC DETERMINATION OF METHYL ALCOHOL

Mixture solution	1.00×10^{-2} *N* Ce(IV) used (ml)	Total formic acid and methyl alcohol (mg)	Methyl alcohol in mix. (mg)	
			Found	Present
1	7.00	0.550	0.320	0.320
2	6.78	0.626	0.281	0.280
3	6.50	0.700	0.240	0.240
4	5.99	0.847	0.160	0.160
5	5.51	1.006	0.080	0.080

lated (mg) (as shown in Tables 1–4). The experiment was repeated several times and a deviation of $\pm$ 0.9% was obtained.

REFERENCES

1. Jaiswal, P. K., Oxidation of some carbohydrates with Ag(III). *Microchem. J.* **15**, 122–125 (1970).
2. Sharma, N. N., Oxidation of carbohydrates, *Fresenius' Z. Anal. Chem.* **154,** 340–344 (1957).

MICROCHEMICAL JOURNAL 17, 204–209 (1972)

Simultaneous Determination of Aluminum, Copper, Iron, and Manganese

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Among the various methods which are presently being used for simultaneous determination of metals, atomic emission and atomic absorption procedures find ready application because of their rapidity and accuracy. However, the usefulness of spectrophotometric methods are not to be ignored, for generally, they are less expensive and are often more sensitive. A review of the literature shows that numerous reagents have been reported for the spectrophotometric determination of aluminum, copper, iron and manganese individually, but few methods have appeared for the simultaneous determination of two or more of these elements in the presence of each other.

Middleton (*5*) reported on the simultaneous determination of iron and aluminum as their 8-hydroxyquinolinates, while Paul (*8*) described a procedure for the simultaneous determination of iron and aluminum by formation and extraction of tris(1,10-phenanthroline) iron(II) by chloroform, in the presence of perchlorate ions or trichloroacetic acid and subsequent determination of aluminum in the aqueous phase as its 8-hydroxyquinolinate complex. Nishimura and Imai (*7*) extracted iron(III) into diethyl ether from 6 *N* hydrochloric acid solutions, determined the separated iron after prior reduction with hydroxylamine, using the 1,10-phenanthroline procedure, and then proceeded to determine copper in the remaining aqueous phase with ammonium diethyldithiocarbamate. Iron and copper(I) were determined simultaneously by Schilt and Taylor (*9*) by complexing both elements with 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine and extracting them into isoamyl alcohol. The absorbance of the solvent phase was determined at 488 nm after which the extracted phase was treated with sodium cyanide to selectively convert the copper(I) complex into a cyanide complex, and the absorb-

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[2] Taken from a thesis submitted in partial fulfillment of the M.S. degree.

ance again measured at 488 and 555 nm. The loss of absorbance at 488 nm was directly proportional to the concentration of copper, and the final absorbance at 555 nm was directly proportional to the iron concentration. Dittel (*2*) determined manganese with diethylaniline in one aliquot of a sample containing manganese and copper, and then used a second aliquot and determined copper with hydroquinone in the presence of pyridine and hydrogen peroxide. A similar technique was employed by Arkangelskaya and Batalina (*1*) who used 2,2′bicinchoninic acid to determine copper and the permanganate method for the determination of manganese. Karkover and Barkovskii (*4*) have reported a procedure for the simultaneous determination of iron, copper and manganese, based on the complexation of copper with di-8-quinolyldisulfide at pH 5.0 and its extraction into chloroform while iron and manganese were determined as their 8-mercaptoquinolinates, the former at pH 4.0 and the latter at pH 9.0. Recently, Shah and Paul (*10*) described a procedure for the simultaneous determination of copper and manganese as their diethyldithiocarbamate complexes by selectively complexing copper at pH$<$1.0 in the presence of perchloric acid, then removing it by extraction into *n*-butyl acetate, and subsequently forming and extracting the managanese diethyldithiocarbamate from neutral solutions in the presence of perchlorate ions. Hitherto, no spectrophotometric procedure has been reported for the simultaneous spectrophotometric determination of aluminum, copper, iron and manganese in the presence of each other, but in view of the work of Paul (*8*) on the simultaneous determination of iron and aluminum, and of Shah and Paul (*10*) on the simultaneous determination of copper and manganese, the possibility existed that an appropriate combination of these two procedures might lead to a successful method for the simultaneous spectrophotometric determination of these elements. This paper reports such a method.

MATERIALS AND METHODS

Reagents. All reagents were of Analar Grade. Distilled water was used throughout. All reactions were carried out at room temperature except the preparation of the iron solution.

Iron Solution. Dissolve 0.0863 g of ferric ammonium sulfate(dodecahydrate) by heating in about 60 ml of water containing about 8 ml of concentrated hydrochloric acid, and dilute to 100 ml after cooling. Prepare further solutions from this by dilution.

Copper Solution. Dissolve 0.3929 g of copper(II) sulfate(pentahydrate) in water and dilute to 100 ml. Prepare further solutions from this by dilution.

Manganese Solution. Dissolve 0.3602 g of manganese(II) chloride-(tetrahydrate) in water and dilute to 100 ml. Prepare further solutions from this by dilution.

Aluminum Solution. Dissolve 3.3605 g of ammonium alum in water and dilute to 100 ml. Prepare further solutions from this by dilution.

Ammonium acetate buffer (pH 2.8), 0.1 *M* ammonia in 5 *M* acetic acid
1,10-phenanthroline solution (aqueous), 0.1% (w/v)
Sodium diethyldithiocarbamate solution (aqueous), 2% (w/v)
8-Hydroxyquinoline solution, 5% in 95% ethanol (w/v)
Hydroxylamine hydrochloride solution, 10% (w/v)
Ammonia solution (1:1)
Perchloric acid, 70%, sp gr 1.6
Chloroform
n-butyl acetate
isobutyl acetate
Carbon tetrachloride

RECOMMENDED SEQUENTIAL PROCEDURE FOR SIMULTANEOUS DETERMINATION

Iron Determination. Pipette 2.0 ml of test solution containing aluminum, copper, iron and manganese into a 50 ml separatory funnel and add 1.0 ml of hydroxylamine hydrochloride solution followed by 2.0 ml of acetate buffer. Mix the contents and leave to stand for 15 minutes to complete the reduction of the iron. Then, add 4.0 ml of 1,10-phenanthroline solution, mix by stirring and leave for 20 minutes for maximum color development. Add 1.0 ml perchloric acid and extract immediately with 10.0 ml of chloroform by shaking for 30 seconds. Transfer the aqueous phase into another 50 ml separatory funnel and read the absorbance of the organic phase at 490 nm against a similarly treated blank. Iron is thereby determined.

Copper Determination. To the separated aqueous phase, now iron free, but still containing an excess of perchloric acid, selectively form copper diethyldithiocarbamate by adding 2.0 ml of diethyldithiocarbamate solution. Extract immediately with 10.0 of *n*-butyl acetate by shaking for 30 seconds. Transfer the aqueous phase into another 50 ml separatory funnel and determine the absorbance of the *n*-butyl acetate layer after 5 minutes, at 440 nm against the similarly treated blank. Copper is thereby determined.

Manganese Determination. To the separated aqueous phase now iron and copper free, add ammonia solution until just slightly basic to a piece of litmus paper placed in the solution. Cool the contents

of the separatory funnel by standing at room temperature, then extract twice with 2 × 10 ml of chloroform. Discard the chloroform washings and add 2.0 ml of sodium diethyldithiocarbamate solution to selectively form the manganese diethyldithiocarbamate complex. Add 10.0 ml of isobutyl acetate and extract by shaking for 30 seconds. Remove the aqueous phase into another 50 ml separatory funnel and read the absorbance of the isobutyl acetate layer at 345 nm after 10 minutes, against the similarly treated blank. Manganese is thereby determined.

Aluminum Determination. To the remaining aqueous phase, iron, copper, and manganese free, add two drops of ammonia solution and 2.0 ml of 8-hydroxyquinoline solution. Mix and leave to stand for 30 minutes for maximum color development. Then, add 20.0 ml of carbon tetrachloride and extract by shaking for 30 seconds. Read the absorbance of the organic phase at 400 nm against the similarly treated blank. Aluminum is thereby determined.

RESULTS AND DISCUSSION

Applying the procedure as outlined, calibration graphs were obtained for all four elements in the following concentration ranges: iron, 0–20 μg/ml as Fe; copper, 0–30 μg/ml as Cu; manganese, 0–30 μg/ml as Mn; and aluminum, 0–80 μg/ml as Al. Except for the iron calibration graph, all the other calibration curves were constructed as if the preceding elements in the sequential procedure were present. Thus, in the construction of the copper calibration graph, copper was determined after the procedure for iron had been applied, for the manganese calibration graph, the procedures for both iron and copper were sequentially applied, and for aluminum, the procedures for iron, copper and manganese were all sequentially applied. The accuracy of the calibration graphs for each element was tested separately at various concentrations of the individual element in the absence of the other three. The results of these recovery experiments, recorded in Table 1, demonstrate that each calibration graph gave accurate and reproducible results.

To conclude these studies, the recoveries of iron, copper, manganese and aluminum from mixtures containing all four elements were determined by the procedure. The results of these experiments, recorded in Table 2, show that this method of simultaneous determination of aluminum, copper, iron and managanese is simple, rapid, accurate and reproducible.

1,10-Phenanthroline is known to complex with copper(I) in alkaline solutions of pH 8.3 (*3,6*). Since, in the present procedure, copper is determined in highly acidic aqueous solutions, the possibility of complexation of copper(I) with any excess of 1,10-phenanthroline which

TABLE 1

ACCURACY OF CALIBRATION GRAPHS FOR IRON, COPPER, MANGANESE AND ALUMINUM
(All concentrations listed below are in μg/2.0 ml.)

Iron present	Iron found	Copper present	Copper found	Manganese present	Manganese found	Aluminum present	Aluminum found
4	4	4	4	4	4	16	16
6	7	8	8	8	8	24	24
10	10	12	12	12	14	32	32
12	12	16	16	16	17	48	48
20	20	24	24	24	24	60	60
30	29	30	30	30	29	100	98

may be present after the removal of iron is obviated, and the results of Tables 1 and 2 support this conclusion.

For the successful determination of manganese by our sequential procedure, it is important that the neutralized aqueous phase remaining after removal of iron and copper be extracted twice with chloroform prior to the formation of the manganese diethyldithiocarbamate complex since, in the absence of such prior extraction, manganese diethyldithiocarbamate does not appear to be formed, as is evidenced by the fact that its color is not discharged. This effect is presently being further investigated.

SUMMARY

A simple, rapid, accurate and reproducible method for the simultaneous determination of aluminum, copper, iron and managanese is reported. The method is based on the sequential formation and extraction of tris(1,10-phenanthroline) iron(II), followed by the selective formation of copper diethyldithiocarbamate

TABLE 2

SIMULTANEOUS DETERMINATION OF IRON, COPPER, MANGANESE AND ALUMINUM IN MIXTURES OF EACH OTHER
(All concentrations listed below are in μg/2.0 ml.)

Mixtures	Iron present	Iron found	Copper present	Copper found	Manganese present	Manganese found	Aluminum present	Aluminum found
A	8	8	8	7	8	8	16	15
B	40	40	48	47	48	49	160	156
C	16	18	48	47	32	30	96	95
D	24	24	16	15	48	49	112	110
E	32	32	32	30	32	31	32	32
F	8	8	56	55	24	25	48	47

and its extraction by *n*-butyl acetate in the presence of perchloric acid. The remaining aqueous phase containing manganese and aluminum is neutralized with ammonia, extracted twice with chloroform, and manganese determined by formation and extraction of manganese diethyldithiocarbamate into isobutyl acetate, in the presence of perchlorate ions. Aluminum is then determined in the remaining aqueous phase as its 8-hydroxyquinolinate.

REFERENCES

1. Arkangelskaya, A. S., and Batalina, G. P. Difference photometric analysis of constructional and soldering alloys. *Zavod. Lab.* **34**(4), 408–409 (1968).
2. Dittel, F. Quantitative selective determination of very small amounts of manganese and copper in ash of biological substances by catalytic reactions. *Z. Anal. Chem.* **229**(3), 193–201 (1967).
3. Hoste, J. On a new copper specific group. *Anal. Chim. Acta*, **4**, 23–37 (1950).
4. Karkover, M. Z., and Barkovskii, V. F. A successive extraction–absorptiometric determination of iron, copper and manganese in aluminum. Cited from *Anal. Abst.* **16**, 1162 (1969).
5. Middleton, K. R. Spectrophotometric determination of iron and aluminum in leaves of the ribber tree. *Analyst* 89, 421–427 (1964).
6. Moss, M. L. and Mellon, M. G. Color reactions of 1,10 phenanthroline derivatives. *J. Ind. Eng. Chem. Anal. Ed.*, **14**, 931–933 (1942).
7. Nishimura, K., and Imai, T. Determination of impurities in high purity indium. III. Photometric determination of iron and copper. *Japan Analyst* **13**(8), 713–717 (1964).
8. Paul, J. Simultaneous determination of iron and aluminum in plant tissues and soil extracts. *Mikrochim. Acta*, **6**, 1075–1081 (1966).
9. Schilt, A. A., and Taylor, P. J. Simultaneous determination of iron and copper. *Anal. Chem.* **42**, 220–224 (1970).
10. Shah, S. M., and Paul, J. Simultaneous determination of copper and manganese by sodium diethyl dithiocarbamate. *Microchem. J.* **17**(1), 000 (1972).

MICROCHEMICAL JOURNAL 17, 210–213 (1972)

I. Direct Titrimetric Microdetermination of L-Histidine

II. Microdetermination of L-Histidine and L-Arginine Together in One Solution Without Separating

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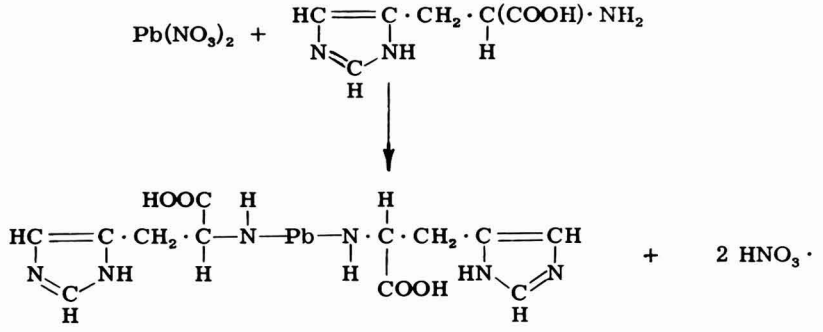
From current literature, it appears that there is neither direct titrimetric method for the determination of histidine separately nor in presence of other amino acids. However, histidine is determined by: high voltage electrophoresis (*1*), paper partition chromatography (*2*), a modified Knoop's reaction (*3, 6*), the influence of cations on the Ninhydrin reaction (*4*), photometrically (*5*), ion-exchange column (*7*), gas chromatography (*9*), and polarographically (*10*).

The aim of the present work was to find some simple, but accurate, method for the determination of histidine separately and in the presence of other amino acids. Histidine has been quantitatively determined in micro amounts by direct titration against lead nitrate solution separately and in the presence of L-arginine without separating. Potentiometric titrations show that lead forms a complex with L-histidine in the ratio of 1 : 2. Probably the following reaction takes place:



EXPERIMENTAL METHODS

Reagents used. L-Histidine, L-arginine, and chromazural red S (E. Merck, grade); lead nitrate, ferrous ammonium sulfate and sulfuric

acid (AnalaR B.D.H., grade); and eylenol orange and pyrogallo red. (B.D.H., grade).

Apparatus used. Micropipette and microburette were of least count = 0.01 ml.

L-Histidine, L-arginine, and lead nitrate standard solutions were prepared by dissolving the exactly weighed amount in distilled water. Standard ferrous ammonium sulfate solution was also prepared by dissolving the exactly weighed amount in 0.1 *N* H_2SO_4 solution. Xylenol orange, pyrogallol red, and chromazural red S solutions were prepared by dissolving 0.1 g in 100 ml of distilled water.

PROCEDURE

A. Direct Determination of L-*Histidine Separately*

A known volume of standard L-Histidine solution was placed in a beaker, diluted to 30 ml by adding distilled water and, then, few drops (2 to 3) of xylenol orange solution were added, and the whole solution became rose colored. The rose colored solution in the beaker was titrated against standard lead nitrate solution until the appearance of a pink color at the end point.

In the case where pyrogallol red solution was used as indicator, the change in color was from pink to purple at the end point on adding lead nitrate.

B. Simultaneous Microdetermination of L-*Histidine and* L-*Arginine*

Known volumes of standard L-histidine and L-arginine solutions were placed in a beaker, from a micropipette, and diluted to 30 ml by adding distilled water—which formed a solution mixture of the two amino acids. A few drops (2 to 3) of xylenol orange solution were added to the solution mixture, and it became light pink. Standard lead nitrate solution from a microburette was run in to titrate L-histidine first. At the end point, a deep pink purple color appeared.

In the same solution, at the second instant, few drops of chromazural red S were added and the whole solution became light yellow. Now, in order to titrate L-arginine (*8*), standard ferrous ammonium sulfate solution (in 0.1 *N* H_2SO_4) was added until the color changed to purple at the end point.

When pyrogallol red was used as indicator and was added in drops to the solution mixture, the whole solution became pink. L-Histidine, first was titrated against standard lead nitrate solution until the appearance of purple color at the end point. In the same solution, after titrating L-histidine, L-arginine was titrated against standard ferrous ammon-

ium sulfate solution, using chromazural red S as indicator until a violet color appeared at the end point.

RESULTS AND DISCUSSION

Results are given in Tables 1 and 2. L-Histidine and L-arginine were estimated in ranges of 0.3672×10^{-4} to 1.4688×10^{-4} mg/liters; and 3.4144×10^{-4} to 13.9987×10^{-4} mg/liter, respectively.

Table 1 shows that L-histidine has been separately estimated in micro amounts against lead nitrate solution using xylenol orange or pyrogallol red solution as indicator. Maximum error for L-histidine is 0.6%. Since the complex between lead nitrate and L-histidine is formed in the ratio of 1 : 2, in the calculations the observed values were multiplied by 2.

In the form of a mixture of L-histidine and L-arginine it is observed that L-histidine first was titrated, using either xylenol orange or pyrogallol as indicator. L-Arginine was always titrated in the same solution, after first titrating L-histidine, against ferrous ammonium sulfate using chromazural red S as indicator. Calculations for L-arginine were done in the same manner as that for L-histidine.

In these titrations, either separately or in mixtures, one of the most important precautions is that after every set of titrations the beakers must be washed well with sodium hydroxide solution and then, thoroughly, with distilled water (at least 10 times). By washing in this manner the colored complexes will not be able to stick to the sides of the titration beakers. In certain cases the eye needs training for a particular color by repeating the titration over and over again.

It is the only titrimetric technique, for the quantitative determination of amino acids either separately or in presence of others, available in the current literature—which is accurate, giving reproducible results, and less time consuming.

SUMMARY

L-Histidine has been quantitatively determined in micro amounts, separately and in a mixture with L-arginine, by titrating against lead nitrate solution, using

TABLE 1

MICRODETERMINATION OF L-HISTIDINE

L-Histidine 0.0008 *M* (ml)	$Pb(NO_3)_2$ 0.00103 *M* (ml)	Amount of L-histidine ($\times 10^{-4}$ mg/liter)		Error (%)
		Taken	Found	
0.2	0.08	0.3649	0.3672	
0.4	0.16	0.7298	0.7344	
0.6	0.24	1.0948	1.1016	0.6
0.8	0.32	1.4591	1.4688	

TABLE 2

MICRODETERMINATION OF L-HISTIDINE AND L-ARGININE IN ONE SOLUTION

L-Histidine 0.0008 *M* (ml)	$Pb(NO_3)_2$ 0.00103 *M* (ml)	Amount of histidine ($\times 10^4$ mg/liter) Taken	Found	L-Arginine 0.02 *M* (ml)	$FeSO_4(NH_4)_2SO_4$ 0.0098 *N* (ml)	Amount of Arginine ($\times 10^4$ mg/liter) Taken	Found
0.8	0.32	1.4597	1.4688	0.1	0.1	3.484	3.4144
0.6	0.24	1.0948	1.1016	0.2	0.2	6.968	6.8288
0.4	0.16	0.7298	0.7344	0.3	0.3	10.452	10.5844
0.2	0.08	0.3649	0.3672	0.4	0.4	13.936	13.9987

xylenol orange or pyrogallol red as indicator. The complex between lead and L-histidine is formed in the ratio of 1:2. Maximum error for L-histidine is 0.6%. L-Histidine with L-arginine was first titrated either with xylenol orange or pyrogallol red as indicator and then, in the second phase of titration, L-arginine was titrated using chromazural red S as indicator.

REFERENCES

1. Atfield, G. N., and Morris, C. J. O. R., The determination of amino acids by high-voltage electrophoresis. *Protides Biol. Fluids, Proc. Colloq.* **8,** 78 (1960).
2. Auclair, J. L., and Dubreuil, R., Simple ultramicromethod for the quantitative method for the quantitative estimation of amino acids by paper partition chromatography. *Can. J. Zool.* **30,** 109 (1952).
3. Hunter, A., A method of histidine determination based upon a modified Knoop reaction. II. *J. Biol. Chem.* **216,** 391 (1955).
4. Meyer, H., and Riklis, E., Influence of cations in the ninhydrin reaction for the determination of amino acids. *Nature (London)* **172,** 543 (1953).
5. Michel, M. C., Procedure for the microdetermination of *o*-amino nitrogen. *Amino Acides Peptides Proteines Cahier* **4,** 63 (1960).
6. Reifer, I., Microdetermination of histidine based on Knoop method. *Rocz. Chem.* **27,** 134 (1953).
7. Sandegren, E., Ljungdahl, L., and Wittasek, N., Determination of amino acids with ion-exchange columns. *Mitt. Versuchssta. Gaerungsgewerbe Wien.* **6,** 72 (1952).
8. Saxena, O. C., Direct titrimetric microdetermination of L-arginine. I. Direct estimations of L-arginine and DL-valine; L-arginine and DL-alanine; and L-arginine, DL-valine, and DL-alanine together in one solution without separating. *Microchem. J.* **15,** 000 (1970).
9. Wanger, J., and Rausch, G., Gas chromatographic separation and determination of amino acids. II. Gas chromatographic separation and determination of amino acids in the form of methyl esters of hydroxyacids. I. *Fresenius' Z. Anal. Chem.* **194,** 350 (1963).
10. Wenger, P. E., Monnier, D., and Faraggi, S., The determination of histidine. I. A polarographic study and determination dinitrifluorobenzene. *Anal. Chim. Acta* **13,** 89 (1955).

MICROCHEMICAL JOURNAL **17**, 214 (1972)

A Glass Sprayer for Thin-Layer or Paper Chromatography [1]

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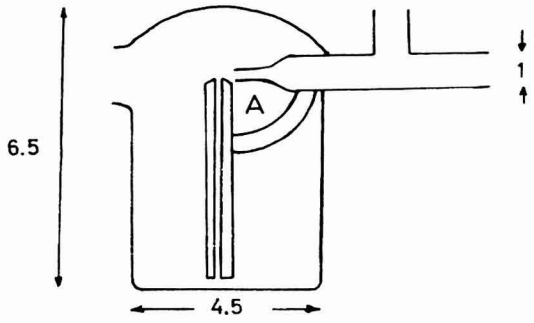
A model of a glass sprayer built in these laboratories that has been in use several years with excellent results is shown in the figure, dimensions given in cm.

The apparatus is built of Pyrex glass and one of the things the glass blower has to take into account before putting the spray system *A* inside the glass envelope, is to try blowing it by mouth. If the angle of attack of the air jet is correct, on the 0.8–1.0 mm thick walled capillary, a very fine spray should be obtained when the bottom of the capillary tube is put into contact with water.

The upper opening in the T tube is to control the amount of air by means of the thumb.



[1] Contribution No. 346.

MICROCHEMICAL JOURNAL 17, 215–219 (1972)

New Method for Isolation of Carrier Free Sulfur-35 and Phosphorous-32 from Neutron Activated Potassium Chloride

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Carrier free sulfur-35 and phosphorous-32 are induced in neturon irradiated potassium chloride target through the nuclear reactions $^{35}Cl(n,p)\ ^{35}S$ and $^{35}Cl(n,\alpha)\ ^{32}P$. This has been considered as one of the basic routes for production of both carrier free ^{35}S and ^{32}P radioisotopes (*1* and *5*). The main radiochemical procedures so far reported for their separation from an irradiated KCl target are based on recrystallization of the target with hydrogen chloride gas, simultaneous separation of the different radioactive species using activated alumina column, and anion exchange elution chromatography as well (*1* and *5*). In the present work, a new method for isolation of both carrier free sulfur-35 and phosphorus-32 from neutron irradiated potassium chloride target is developed. This method is based on the extraction chromatographic technique using the organic solvent, *N*-lauryl-trialkylmethyl amine, loaded on hydrophobic Celite as stationary phase and potassium chloride solution as mobile phase.

EXPERIMENTAL METHODS

Apparatus. For the radiochemical investigations, a glass column of 1 cm in diameter and 30 cm in length have been developed. The column was washed with 2% silane solution in alcohol and dried before use to prevent any adsorption of radioactivity on the wall of the column.

Reagents and solutions. The solvent (*N*-lauryl-trialkylmethyl amine) used is of A.R. grade and obtained from B.D.H. and used without any purification. Celite No. 365 is supplied from Manville Co., U.S.A. All other reagents are of A.R. grade chemicals.

Preparation of the stationary phase. The celite was made hydrophobic by mixing with 5% solution of dimethyldichlorosilane (silane) in alcohol, followed by drying at 150°C for 2 hours. To achieve homogeneous mixing and distribution of the organic solvent on the Celite, a mixture of 5% (V/W) of used solvent to celite was mixed together with

excess alcohol. The mixture was then allowed to dry by standing overnight at 80°C with interrupted stirring.

Distribution coefficient determination. The distribution data were determined using the column technique. The used column was packed with 10 g of the dried stationary phase (5%; v/w), equilibrated with used eluant and milligram quantities (10 mg) of PO_4^{3-} and SO_4^{2-} anions were loaded on the stationary phase from minimal volume of water. The column was eluted with chosen eluant using a flow rate of 0.82 ml/minute and 1-ml fractions of the effluents solution were collected. These fractions were analyzed gravimetrically for sulfate and/or phosphate anions by precipitation as barium sulfate and magnesium ammonium phosphate, respectively. From the maximum elution position of each anion (v), the volume of the stationary phase (i), and the free column volume (f); the distribution coefficient was determined using the expression (*3*):

$$D = \frac{V}{i} - f$$

Working procedure. 150 mg of A.R. grade potassium chloride was irradiated in the 2 MW research reactor-UARRI-, for 48 hours with an activation neutron flux of 1.3×10^{13} n/cm^2. second. After irradiation, the induced 12.5 h potassium-42 radioactivity was allowed to cool for 3 days before treating. The target was then dissolved in minimum water; and 1 ml of H_2O_2 (30%) was added to oxidize any phosphorous and sulfur radioactivities to the phosphate and sulfate forms, respectively. Excess of peroxide was removed by boiling and the target solution was then adjusted to 40-ml volume with water (ca. 0.05 *M* KCl solution). The solution was allowed to pass through a column of 1-cm diameter and 30-cm length of 10 g 50% (v/w) of the used extractant loaded on the hydrophobic Celite (as previously mentioned), using a flow rate of 0.25 ml/minute. After elution of the 40 ml of neutron irradiated target, the column was then washed with 10 ml of 0.05 *M* inactive KCl followed by 20 ml of double distilled water to elute the residual ^{35}P activity. Sulfate activity was then eluted by 30 ml of 1 *M* HCl solution. A typical elution profile for the eluted phosphorus-35 and sulfur-32 radioactivities is given in Fig 1.

RESULTS AND DISCUSSION

The distribution of sulfate and phosphate anions on used column in the concentration range of 10^{-2} to 0.05 *M* KCl solution is presented in Fig. 2. It is clear that a linear double logarithmic relation is obtained

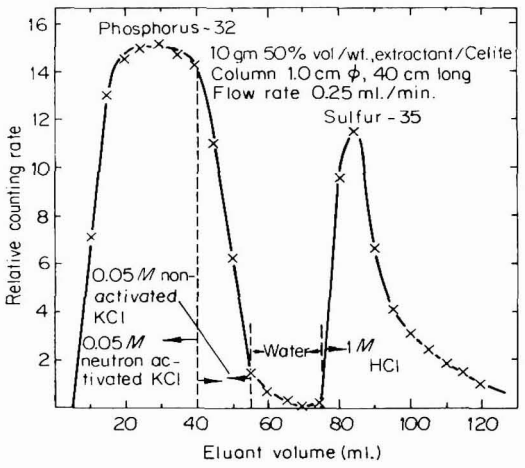

FIG. 1. Typical separation profile of phosphorus-32 and sulfur-35 radioactivities.

between the distribution coefficient and the eluant concentration with higher uptake for SO_4^{2-}. The difference in D values between different anions seems to be great enough to affect their possible separation. This difference is manifested with dilute eluant solution rather than high concentration of KCl. To increase the capacity of the stationary phase, 50% of the extractant to Celite ratio was used in the working procedure instead of the experimented ratio. In this case, the adsorption of sulfate is not affected with the eluting action of 0.05 M KCl up to 150 mg of potassium chloride target. The phosphorous activity produced by

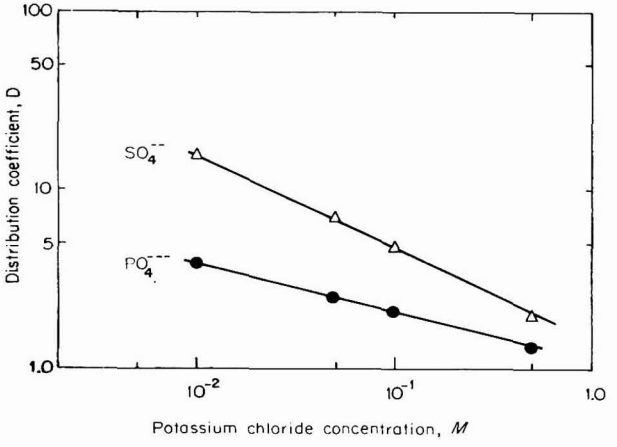

FIG. 2. Distribution of sulfate and phosphate anions.

the (n,p) reaction of chlorine was therefore continuously washed down the column leaving carrier free sulfur-35 firmly adsorbed on the stationary phase. After washing with inactive 0.05 *M* KCl and water to remove residual ^{32}P activities, 1 *M* HCl was found sutiable eluant to generate ^{35}S radioisotope. The purity of the phosphorus and sulfur fractions were checked by aluminum absorption curves which gave more than 98% radiochemical pure ^{32}P and ^{35}S. The purity was further checked by the addition of sulfate and phosphate carriers to a representative sample of ^{32}P and ^{35}S fractions, respectively. Any sulfur activity in the ^{32}P fraction was then separated with the added carrier as barium sulfate and compared with the original radioactivity in solution. The same procedure was carried out on the ^{35}S fraction with the separation of phosphorous as magnesium ammonium phosphate. This procedure gave more than 98% purity for both fractions. It is worth mentioning that the stability of the developed column was proved by the constancy of the elution profile up to 5 times.

The higher uptake of SO_4^{2-} than PO_4^{3-}, could be best explained on the similarity between the extraction mechanism of amine extractants and its analog the anion exchange resin. In the system studied, the bivalent and/or the trivalent anions are exchanged with the monovalent chloride ions. In this case, the main interactions affecting preferential uptake of anions are ion–water, water–water, ion–ion, and ion–extractant matrix interactions (*2* and *4*). Since the potassium chloride concentration used is relatively small in the concentration range investigated, the main interactions of significant importance are those of ion–water and water–water interactions. Related to ion–water interaction, the highly charged anion will tend to form primary hydration shell and its solvation is expected to be great. On the other hand, for the less charged anion, SO_4^{2-}, solvation will be relatively limited. This will lead to preferential solvation of PO_4^{3-} in the aqueous phase leaving the less charged anion, SO_4^{2-}, in the less solvating stationary phase. In parallel, the water–water interaction will favor the highly charged anion to be less generated on the stationary phase. This is mainly dependent on the hydrogen bonded structure of pure water which is disturbed with soluble ions (*4*). In this case, the pure water structure is expected to oppose inclusion of molecular ions. Nevertheless, highly charged ions will cause considerable polarization to achieve inclusion in such structure, while less charged ions will have not enough charge density to cause this effect and the water structure will push the less charged anion in the organic phase. In the present case, the anion volume is of second importance compared to the difference in the charge of the anions investigated.

SUMMARY

The distribution of phosphate and sulfate anions on a developed extraction chromatographic column was investigated. A working procedure for separation of carrier free ^{32}P and ^{35}S from neutron activated potassium chloride target was adapted. The procedure proved to be adequate and efficient for production of high chemical and radiochemical pure sulfur-35 and phosphorus-32 radioactivities. The distribution behavior of the studied species was discussed in the light of the different interactions affecting the preferential generation of the anionic species.

REFERENCES

1. Abdel-Rassoul, A. A., and Abdel-Aziz, A., Further studies on the production of carrier free sulfur-35 from pile irradiated potassium chloride targets. Z. *Anorg. Allg. Chem.* **330,** 91 (1964).
2. Abdel-Rassoul, A. A., Abdel-Aziz, A., and Aly, H. F., Sequential separation of sulfur-35 species from neutron activated potassium chloride. *J. Inorg. Nucl. Chem.* **31**, 3043 (1969).
3. Aly, H. F., and Latimer, R. M., Variables affecting ion exchange for inter-actinide separations, *J. Inorg. Nucl. Chem.* **29,** 2041 (1967).
4. Diamond, R. M., and Whitney, D. C., *In* "Ion Exchange" (J. A. Marinsky, ed.), Chap. 8. Dekker, New York (1966).
5. "Manual of radioisotope production." *Tech. Rep. Ser., Int. At. Energy Ag.* **63,** (1966).

MICROCHEMICAL JOURNAL 17, 220–230 (1972)

The Study of the Electrochemical Oxidation Mechanism of Diphenylamine Derivatives in Dipolar Aprotic Solvents

II. The Electrochemical Oxidation of 4-Aminodiphenylamine in Acetonitrile

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INTRODUCTION

The application of electrochemical techniques to study the oxidation mechanism of aromatic amines is well established (*1*). In this respect, many problems present themselves, which result in ambiguities in defining a reaction mechanism precisely. The most frequent problems—besides those arising from changes in the electrode surface conditions—are the follow-up chemical reactions after the primary electron transfer (*2–5*), the reactions that may occur through more than one pathway (*6, 7*), and further oxidation, which may lead to products that are difficult to identify (*8*).

4-Aminodiphenylamine is expected to exhibit many of the above-mentioned difficulties when electrooxidized. Although not thoroughly investigated, this compound has been repeatedly mentioned as an intermediate in the electrooxidation of aniline both in aqueous media (*3, 4, 8*), as well as in acetonitrile (*5, 9*). In both media emeraldine-type final products were suggested. In acetonitrile unidentified final oxidation products are also reported (*10*). Dvorák *et al.* studied the behavior of the depolarizer in acetonitrile at the rotating platinum electrode (*11*). Its oxidation occurred in two successive waves of unequal height. No mechanism was given; instead, probable electrode surface effects, polymerization, and follow-up chemical reactions were suggested.

Accordingly it was of interest to throw some more light on the electrooxidation mechanism of this compound in acetonitrile. The diagnostic criteria given by Nicholson and Shain (*12, 13*) together with the effect

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of addition of acids and bases on the polarograms are expected to be indicative. Techniques followed in this work are: cyclic voltammetry, macroscale electrolysis at controlled potential, and voltammetry at a rotating platinum electrode.

EXPERIMENTAL

Rotating Pt electrode experiments were performed using a conventional Radiometer PO4 Polarograph in conjunction with a Metrohm IR-compensator, type E446. Rotation speed was kept at 680 rpm. Cyclic voltammetric curves at a sweep rate of 0.013 V/sec were also recorded by this instrument.

Macroscale electrolysis was performed using a Tacussel Potentiostat Type ASA4-SHT2. The number of electrons transferred in the electrochemical reaction was estimated by measuring the amount of gas liberated in a hydrazine sulfate coulometer (*14*).

Cyclic voltammetry at scan rates between 0.1 V/sec and 10 V/sec were performed using a Chemtrix SSP-2 Polarographic Analyzer System. All experiments were carried out in practically water-free acetonitrile solutions containing 0.25 *M* $LiClO_4$ as base electrolyte. All other experimental details, preparation of solutions and materials have been described elsewhere (*15*).

RESULTS AND DISCUSSION

Voltammetry at rotating Pt electrode. The oxidation of 4-aminodiphenylamine in neutral solutions exhibits two well-defined anodic waves which show the linear relation between the limiting current and $w^{1/2}$ ($w = 2\pi n$, n = rotation speed). The first shows a slightly larger height and slope than the second. The $E_{1/2}$ of both waves are $E_{1/2}^1 = +0.065$ V and $E_{1/2}^2 = +0.565$ V vs. Ag/0.01 *M* Ag^+ electrode.

Addition of perchloric acid to the solution has no effect on the oxidation potential of either wave. Instead the height of the first wave decreases and that of the second wave increases, but not to the extent to compensate for a constant total height (Curves 1–3, Fig. 1A). In the presence of 10^{-2} *M* acid (one equivalent/mole depolarizer), while the first wave almost disappeared, the second exhibited slight splitting. The $E_{1/2}^3$ of the new wave (third) is $\sim +0.7$ V, Curve 4, Fig. 1A. Finally, the last two waves merged and oxidation only occurred at the potential of the third wave when the acid content of the solution was 3×10^{-2} *M* (3 eq/mole depolarizer). In the meantime the total height of the waves decreases by increasing the acid content of the solution. This behavior suggests that more than one form of the depolarizer is undergoing oxidation, and the height of the different waves correspond to the concen-

tration of the forms present. This case recalls the behavior of nitrosophenols polarographically reduced in methanol (*16*). The decrease in total height of the polarogram as a result of an increase of acid content of the solution is due, as will be confirmed later, to an increase in concentration of the protonated form of the depolarizer.

On the other hand, the presence of increasing amounts of diphenylguanidine in solution causes the development of a prewave at *ca* 0.16 V less positive than the oxidation potential of the original first wave. The increase in the prewave height by increasing base content is accompanied—although not stoichiometrically—by a decrease in the height of the original second wave, Curves 1–5 Fig. 1B. Addition of more base causes also the decrease of the original first wave height, Curve 6 Fig. 1B. Finally, when the solution contained 10^{-2} *M* base, oxidation was almost complete at the potential of the prewave. More base addition did not affect the oxidation pattern most probably due to equilibrium effects. This is represented by the irregularities exhibited at the

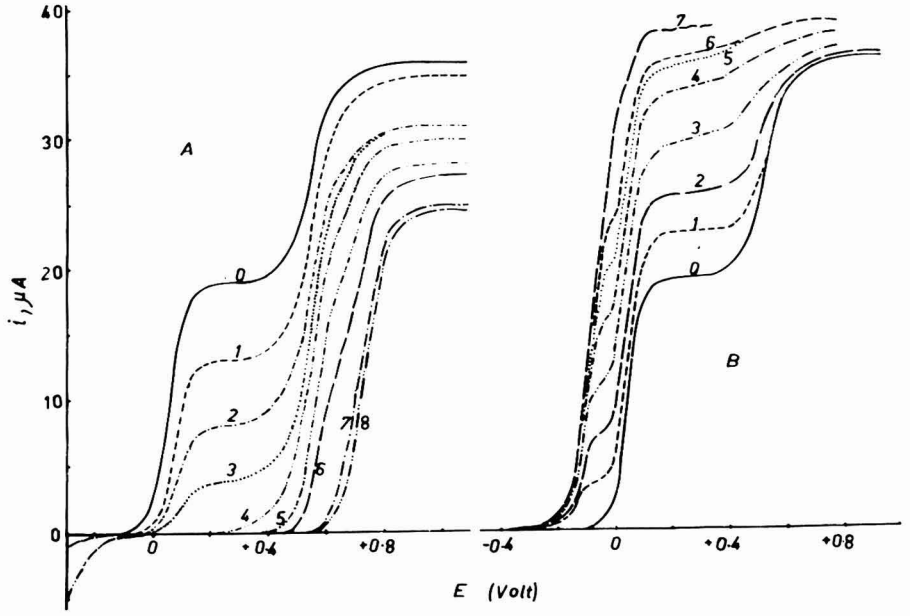


FIG. 1. Effect of addition of (A) $HClO_4$ and (B) diphenylguinidine on the current–potential curves of 10^{-2} *M* 4-aminodiphenylamine in AcN at the rotating Pt electrode. (A) 0: 0.0; 1: 2.5; 2: 5.0; 3: 7.5; 4: 10.0; 5: 15.0; 6: 20.0; 7: 30.0; 8: 40 × 10^{-3} *M* $HClO_4$. (B) 0: 0.0; 1: 2.5; 2: 5.0; 3: 7.5; 4: 10.0; 5: 12.5; 6: 15.0; 7: 20.0 × 10^{-3} *M* diphenylguanidine.

top of the polarogram, Curve 7 Fig. 1B. Here, however, the addition of base just confirms the inference that more than one form of the depolarizer is subject to oxidation, and the behavior in the presence of the base is the counterpart of that in the presence of acid. In this case not only the depolarizer cannot be protonated, but also the removal of a proton from the oxidizing species becomes easy and a cation radical is not formed. Hence, the diffusion of uncharged particles takes place with larger D values, and the total height of the polarogram is higher than in absence of the base.

Macroscale Electrolysis

Controlled potential oxidation of 4-aminodiphenylamine in acetonitrile at +0.2 V resulted in a deep bluish-violet solution and the transfer of 1.02 ± 0.02 electrons. Completing the electrolysis in the same solution at +0.8 V indicated a further transfer of 1.43 ± 0.03 electrons per molecule. A total of 2.4 electrons is transferred when electrolysis is done in one step at +0.8 V. It is clear that the number of electrons involved in oxidation in the macroscale electrolysis does not correspond to the ratio of the wave heights recorded at the rotating Pt electrode. However, in separate experiments macroscale elctrolysis was completed at the potential of the first step (i.e., +0.2 V), then increasing amounts of either acid or base were added to the solution and their polarograms were recorded. The following macroscale electrolysis was completed at +0.8 V in the acidified solutions and at +0.1 V in the basic solutions. In the first case 1.82 electrons were further transferred, while only 1.48 electrons were measured in the second. The polarograms of all these solutions are shown in Fig. 2. The curves clearly show that the proton availability in solution determines not only the oxidation potential of the depolarizer but also the number of electrons associated with the oxidation process. This last effect probably indicates the extent to which the oxidized material undergoes polymerization and further oxidation (*8, 9, 17*). Regarding the dependence of the oxidation potential on proton availability—polarograms recorded after complete electrolysis at the potential of the first step—the presence of acid caused oxidation at the second wave potential to be more difficult and reduction of the first-wave oxidation products more easy (Curves 3, 4, Fig. 2). The countereffect occurred when increased amounts of the base were added (Curves 5–9, Fig. 2). Here, since the protons are not available, no reduction wave is observed. Moreover, when excess base is present, two effects can be observed on the curves of Fig. 2. The first is that oxidation, instead of occurring at +0.565 V, occurred at

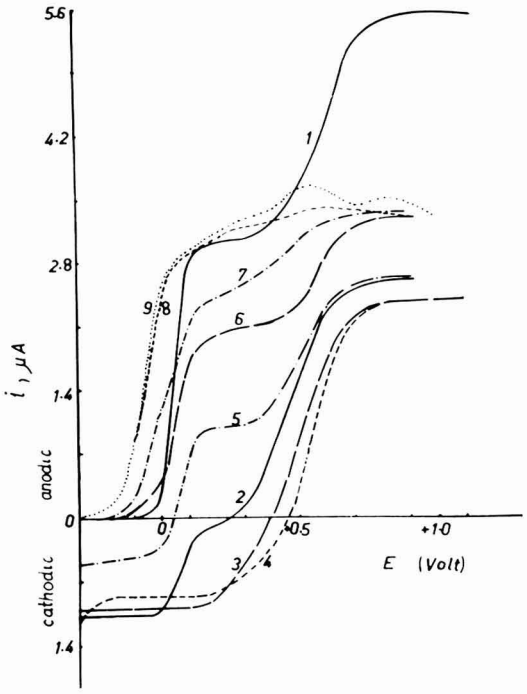
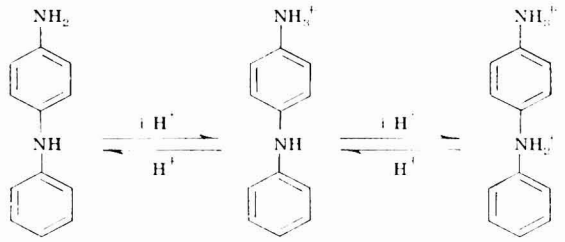


FIG. 2. Effect of addition of $HClO_4$ and diphenylguanidine on the current–potential curves of 10^{-3} *M* depolarizer at rotating Pt electrode at different stages of macroelectrolysis (1) Before electrolysis, (2) after complete electrolysis at +0.2 V, (3) and (4) same as (2), where 5 and 10×10^{-3} $HClO_4$ is added, respectively, (5)–(9) same as (2), but containing 0.5, 1.0, 1.5, 2.5, and 4.0×10^{-3} *M* diphenylguanidine, respectively.

~ -0.07 V. Secondly, the height of the wave (original second wave) has increased to an extent such as to reach that of the original first oxidation wave.

From these results, it is concluded that the oxidation of 4-aminodiphenylamine in acetonitrile can be such that two molecules participate in the process. The first is oxidized with the transfer of two electrons at the potential of the first wave, the second is just functioning as proton acceptor. This latter protonated molecule undergoes oxidation at the potential of the second wave. This supposition can be further accepted if one realizes that acetonitrile cannot act as a base to accept protons because the depolarizer is known to be a stronger base (*18*). This mode of oxidation explains the presence of two unequal oxidation waves and, unless for protonation effects, the two waves would be equal in height. Moreover, it explains the development of a third wave according to the

amount of acid or base added to the solution. The addition of acid is expected to cause the following equilibrium to exist:



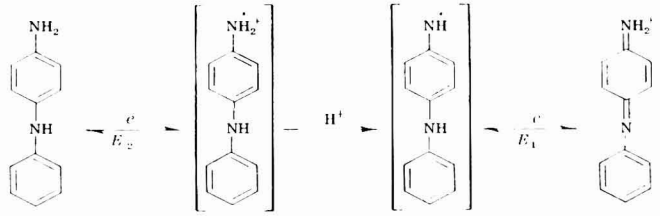
(I) (II) (III)

In neutral solution the two waves correspond to the oxidation of species I and II. In moderate acidity the three waves correspond to the above three forms. Species III predominates in the presence of excess acid and oxidation occurs at the potential of the third step.

On the other hand, the prewave that occurs in the presence of base can reasonably be anticipated to an electron transfer and proton removal (by the base) occurring simultaneously. This state of affairs recalls the reduction of organic compounds that consume protons when proton donors are added (*19*), i.e., ease of electrochemical process. This behavior is confirmed, after macroscale electrolysis of the first step, where further oxidation at rotating Pt electrode could be already achieved at + 0.1 V in the presence of excess base.

Cyclic Voltammetry

The transfer of two electrons in one act in either of the oxidation steps in aprotic solvents is kinetically not probable. It is cyclic voltammetry which proved its potentiality in such problems. Thus, cyclic voltammograms recorded at a stationary Pt electrode over the potential range −0.3–+0.7 V at sweep rates from 0.1 to 10 V/sec, exhibited two reversible redox couples (Fig. 3) corresponding to the two anodic waves obtained at the rotating Pt electrode. When the anodic current function ($ip_a/v^{1/2}$) of the first peak is plotted vs. v (scan rate), it is found that



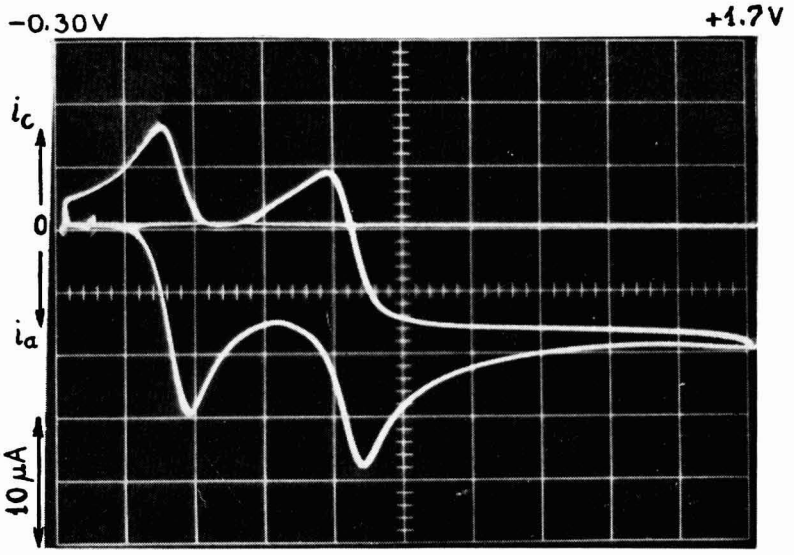


FIG. 3. Cyclic voltammetric curve of 10^{-2} *M* depolarizer in neutral solution at scan rate 0.2 V/sec and in the range −0.3 to +1.7 V.

this function decreases as the polarization rate increases in a manner similar to that described by Nicholson and Shain (*12, 13*) and also presented by Adams (*20*). This behavior is an indication of a follow-up chemical reaction after the primary electron transfer and suggests an ECE mechanism, where $E_2 > E_1$. This mechanism further supports the conclusion that each of the polarographic oxidation waves obtained at the rotating Pt electrode corresponds to the transfer of two electrons.

Chemical Reversibility

This is best demonstrated by cyclic voltammetric curves obtained either at a slow scan rate in the presence of acid or base, or in neutral solution at increasing scan rates. In the first case a slow potential scan rate of 0.013 V/sec was applied, which is just enough for the protons resulting from the oxidation process to migrate away from the electrode surface. In such case the calculated current ratio (*21*) (ip_c/ip_a) of the first peak is found to be 0.511 and 0.648 in the absence and presence of one equivalent of acid, respectively. This increased ratio is an indication that the added acid suppresses both deprotonation of the oxidizing species and also supplies protons for the reduction half cycle, i.e., chemical reversibility is approached. In the presence of the base complete chemical irreversibility is observed, i.e., no cathodic half-cycle is obtained. (Fig. 4).

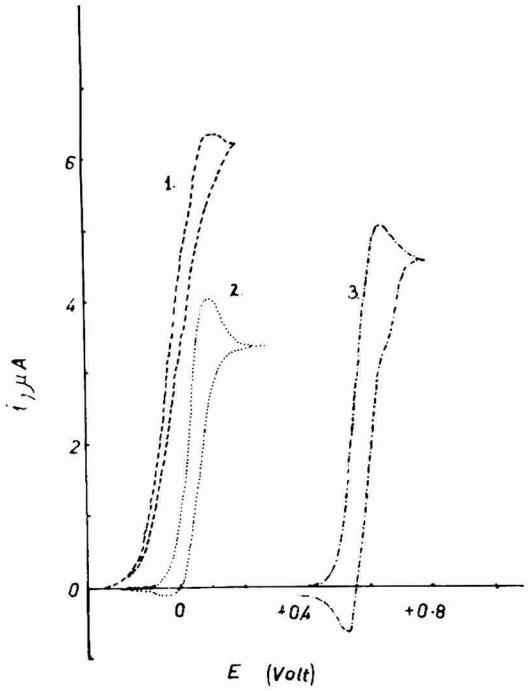


FIG. 4. Cyclic voltammetric curves of 10^{-2} *M* depolarizer at 0.013 V/sec in presence of (1) 10^{-2} *M* base, (2) neutral, (3) 10^{-2} *M* $HClO_4$.

In the second case, the application of increased scan rates is expected to show increased chemical reversibility. From experiments the ratios of (ip_c/ip_a) are calculated and tabulated as function of scan rate in Table 1.

In conclusion, the oxidation mechanism of 4-aminodiphenylamine in acetonitrile can be as follows: The first wave (or peak) accounts for a primary one-electron transfer to give rise to a cation radical. This latter undergoes irreversible follow-up deprotonation to give a radical, then another electron transfer accounting for an ECE mechanism. The eliminated proton from the follow-up reaction is neutralized by an unoxidized

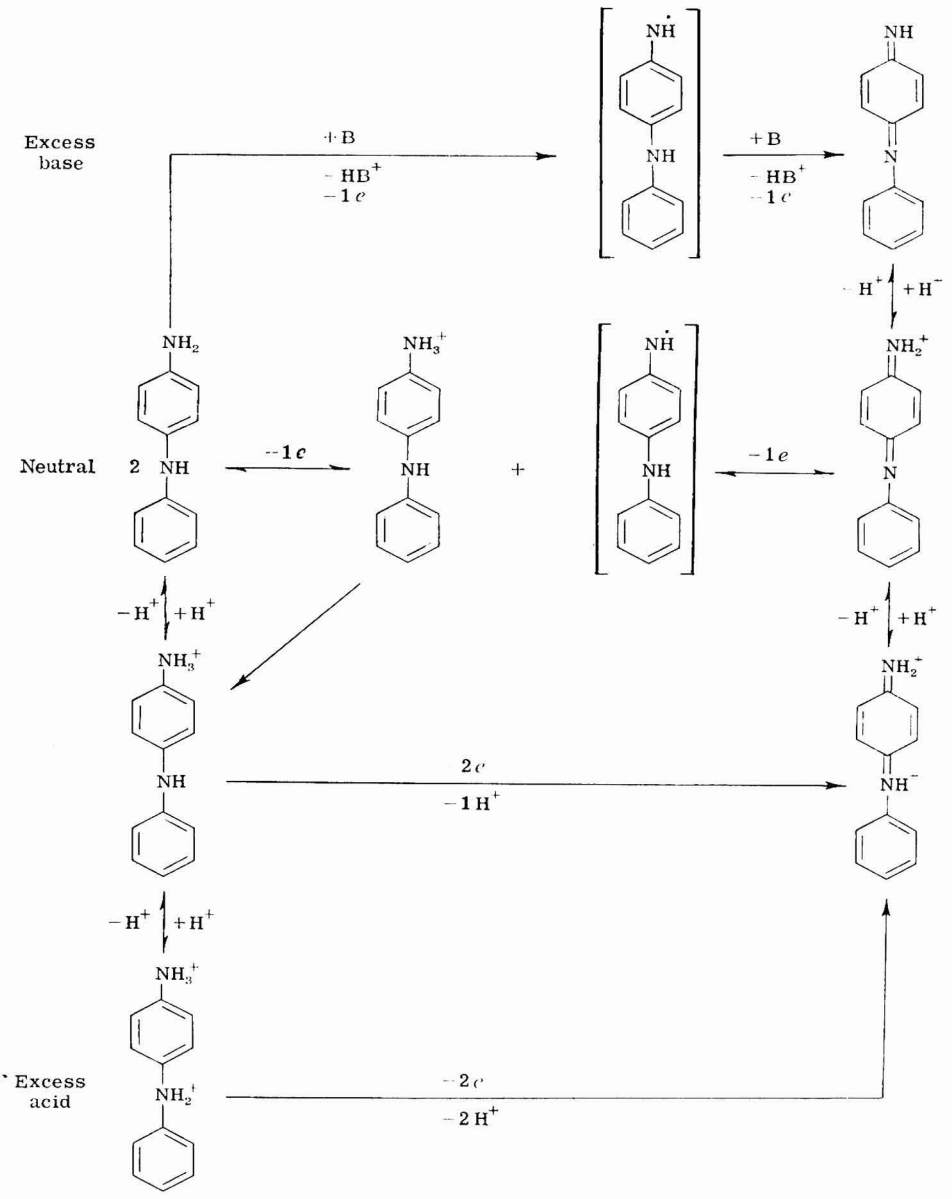
TABLE 1

ip_c/ip_a	Scan rate (V/sec)
0.577	0.05
0.796	0.25
0.817	0.50
0.825	1.00
0.844	2.50

depolarizer molecule which undergoes complete oxidation at the potential of the second wave (peak).

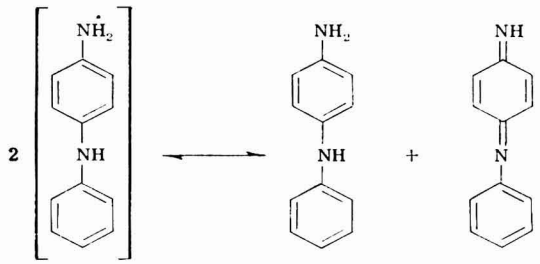
In scheme I a general representation of the chemical and electrochemical reactions that are expected to take place during the oxidation of 4-aminodiphenylamine in neutral, acidic, and basic solutions is shown:

Scheme I



It may be inferred that the two oxidation waves or peaks of 4-aminodiphenyl amine correspond to two one-electron processes, where a cation radical and a dication radical are the respective products. This mechanism cannot be correct as it does not explain, by any means, the presence of three interdependable waves when the compound is oxidized in the presence of increasing amounts of either acid or base. Moreover, a decrease in the current of the first peak as the rate of polarization was increased would have not occurred. Accordingly, the oxidation potentials of the various waves are determined, not by the oxidation state as required by the above supposition, but rather by the charge status of the different species undergoing oxidation. These, when arranged in the order of their ease of oxidation are: easily deprotonated depolarizer molecule (in presence of base) > (neutral molecule) > single charged > double charged (in the presence of acid).

It may also be claimed that the uncharged radical resulting after the follow-up reaction may undergo disproportionation regenerating the original material as:



This reaction is expected to be significant in the presence of base. However, in this case, the development of a prewave overshadows any effect of disproportionation on the first wave and the treatment of Saveant (*24*) could not be applied. In this case, although disproportionation could not be completely excluded, yet it is believed that if it exists it will not affect the picture to any considerable extent.

SUMMARY

The electrochemical oxidation of 4-aminodiphenylamine was investigated in acetonitrile. Voltammetry at a rotating Pt electrode, controlled potential electrolysis and cyclic voltammetry were the techniques followed. The oxidation occurred in two steps, the first involves the complete oxidation of one molecule through an ECE mechanism. The ejected proton—in the follow-up reaction—is received by the other molecule which undergoes oxidation at the potential of the second step. This result was confirmed from the effect of acid and base addition on the oxidation patterns.

Depending on the acidity of the medium, the number of electrons transferred during macroscale electrolysis ranged between 2.4 and 2.8. The excess fraction

above two electrons indicates the extent to which the oxidized product undergoes polymerization and further oxidation.

REFERENCES

1. Adams, R. N., "Electrochemistry at Solid Electrodes." Dekker, New York, 1969.
2. Smith, J. R. L., Norman, R. O. C., and Walker, W. M., *J. Chem. Soc. B*, 269, 1968.
3. Bacon, J., and Adams, R. N., *J. Amer. Chem. Soc.* **90**, 6596 (1968).
4. Daniels, D. G. H., and Saunders, B. C., *J. Chem. Soc.* 2112 (1951).
5. Wawzonek, S., and McIntyre, T. W., *J. Electrochem. Soc.* **114**, 1025 (1967).
6. Papouchado, L., Bacon, J., and Adams, R. N., *J. Electrochem. Soc.* **24**, 000 (1970).
7. Leedy, D. W., Ph.D. thesis, University of Kansas, 1968.
8. Mohilner, D. M., Adams, R. N., and Argersinger, W. J., Jr., *J. Amer. Chem. Soc.* **84**, 3618 (1962).
9. Breitenbach, M., and Heckner, K. H., *J. Electroanal. Chem.* **29**, 309 (1971).
10. Nelson, R. F., Ph.D. thesis, University of Kansas, 1966 (cited in Ref. *1*).
11. Dvořák, V., Němec, I., and Zýka, J., *Microchem. J.*, **12**, 324, (1967).
12. Nicholson, R. S., and Shain, I., Anal. Chem. 36, 706 (1964).
13. Nicholson, R. S., and Shain, I., *Anal. Chem.* **37**, 178 (1965).
14. Page, J. A., and Lingane, J. J., *Anal. Chim. Acta* **16**, 175 (1957).
15. Farsang, G., Kovács, M., Saber, T. M. H., Ladányi, L. (submitted to the J. Electroanal. Chem.).
16. Mustafa, H. S., Ph. D. thesis, University of Beograd (1970).
17. Millar, Ian T., and Springall, H. D., "A Shorter Sidgwick's Organic Chemistry of Nitrogen," Chap. 4. Clarendon, London, 1969.
18. Charlot, G., and Trémillon, B., "Chemical Reactions in Nonaqueous Solvents and Melts," 1st Ed., Chaps. I and II. Pergamon Press, Oxford, 1969, translated to English.
19. Shams El-Din, A. M., Saber, T. M. H., and Abed, N. M., *J. Electroanal. Chem.* **21**, 377 (1969).
    Saber, T. M. H., Ali, M. I., and Abed, N. M., *J. Electroanal. Chem.* **28**, 177 (1970).
20. Seo, E. T., Nelson, R. F., Fritsch, J. M., Marcous, L. S., Leedy, D. W., and Adams, R. N., *J. Amer. Chem. Soc.* **88**, 3498 (1966).
21. Nicholson, R. S., *Anal. Chem.* **38**, 1406 (1966).
22. Mastragostino, M., Nadjo, L., and Saveant, J. M., *Electrochim. Acta* **13**, 721 (1968).

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Application of Acid–Base Indicator Character in the Detection of Isomers and Homologous Compounds

Detection of 6-Chloro-o-cresol in the Presence of o-Cresol and 4-Chloro-o-cresol

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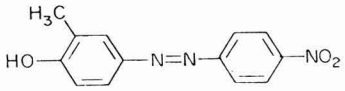
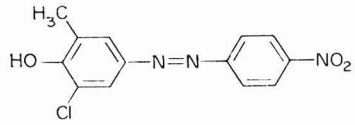
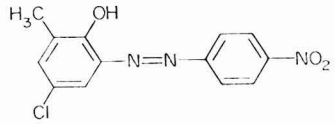
Chlorination of *o*-cresol yields 4-chloro-, 6-chloro- and 2,4-dichloro-*o*-cresol. The detection of the compound described here is based upon the differences in acid–base character of their azo derivatives. From the above-mentioned compounds 6-chloro-*o*-cresol and *o*-cresol couple rapidly with diazonium salt, 4-chloro-*o*-cresol to a slight extent, but 2,4-dichloro-*o*-cresol does not couple. On this basis it should be possible to detect, for example, 6-chloro- in the presence of 4-chloro-*o*-cresol, but not in that of *o*-cresol.

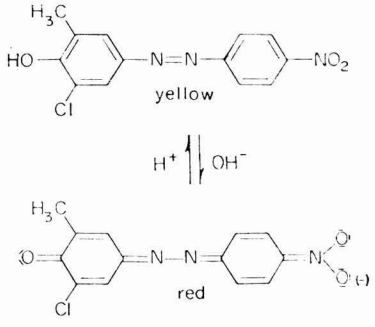
In alkaline medium *o*-cresol and 6-chloro-*o*-cresol yield with diazotized *p*-nitroaniline the same intense color, but in neutral medium 6-chloro-*o*-cresol couples faster. In this case *o*-cresol gives an orange color, 6-chloro-*o*-cresol an orange-red, and 4-chloro-*o*-cresol a yellow one. This difference in coupling velocity is not enough to detect 6-chloro-*o*-cresol in the presence of *o*-cresol. According to our experiments this color difference will be greater after dilution of the solutions with ethanol, viz., 6-chloro-*o*-cresol yields a red color, *o*-cresol and 4-chloro-*o*-cresol a yellow one. To find out its cause, azo compounds have been prepared from *o*-cresol, 6-chloro- and 4-chloro-*o*-cresol, with diazotized *p*-nitroaniline. All three azo compounds are indicator acids, the pK variations of which in water–ethanol mixture are shown in Table 1. The pK of the indicator prepared from *o*-cresol is 0.4 greater than that of the 6-chloro-*o*-cresol derivative. This difference is small and cannot be caused by the various effects in the coupling reaction. On the other hand it is essential with respect to detection, that the pK value of the indicator prepared from *o*-cresol increases to a slight extent in ethanolic medium, whereas that of the 6-chloro-*o*-cresol derivative decreases. In this manner the difference in the pK values of both compounds increases in aqueous ethanol to 2.4.

TABLE 1

VARIATION OF pK OF AZO COMPOUNDS IN AQUEOUS ETHANOL

Azo compound	mp (°C)	Solvent	Apparent pK
	200	water 75% ethanol	8.40 9.20
	167	water 10% ethanol 50% ethanol	8.00 7.00 6.80
	195	water 25% ethanol 50–75% ethanol	11.10 10.00 9.40

When a weak acid carries no charge or is negatively charged, its pK value increases in ethanolic medium (*1*). Phthalein indicators and, to a slight extent, the azo compound prepared from *o*-cresol show similar behavior. On the other hand the behavior of the nitronic acid originating from 6-chloro-*o*-cresol is irregular; this is due to the electronegative chlorine atom of the molecule. The pK value of this indicator decreases in aqueous ethanol to such an extent that this indicator shows in ethanolic medium its alkaline color well below the neutral point, too; this is the basis of the detection. Consequently when *o*-cresol and its chloro derivatives are coupled in neutral medium with diazotized *p*-nitroaniline and the solution is diluted with ethanol, both *o*-cresol and 6-chloro-*o*-cresol couple, the latter a little faster, but in neutral ethanolic medium, the indicator based upon *o*-cresol shows its acidic yellow color, while that of 6-chloro-*o*-cresol its alkaline red color:



Under the circumstances 4-chloro-*o*-cresol couples with diazotized *p*-nitroaniline only to a slight extent, and therefore does not disturb the detection of 6-chloro-*o*-cresol. In addition, the indicator based upon 4-chloro-*o*-cresol has a large p*K* also in ethanolic medium, so it shows its acidic color in neutral medium.

EXPERIMENTAL METHODS

Preparation of Azo Compound from o-Cresol and Diazotized p-Nitroaniline

Dissolve 0.54 g of *o*-cresol in 5 ml of *N* sodium hydroxide.

Dissolve 0.69 g of *p*-nitroaniline in 6.5 ml of 2 *N* sulfuric acid under heating, then add 20 ml of water. After cooling the solution, a small amount of crystalline product separates. Add 0.345 g sodium nitrite in 3 ml of water all at once, mix the solution at 30° with glass rod, while the largest part of the precipitate is dissolved. Filter the diazonium salt into the *o*-cresol solution prepared previously. Filter off the separated orange product, wash with water to neutrality and dry it at 100°, mp 198°; crystallized from ethanol, mp 200°.

In the same way prepare the appropriate azo compound from 6-chloro-*o*-cresol and diazotized *p*-nitroaniline, which orange red substance has a mp of 167°. The azo derivative yielded from 4-chloro-*o*-cresol and diazotized *p*-nitroaniline has a brown color, mp 195°.

Detection of 6-Chloro-o-cresol in the Presence of o-Cresol and 4-Chloro-o-cresol

Dissolve 0.54 g of *p*-nitroaniline in 5 ml of 65% sulfuric acid and make up to 100 ml with water. To 5 ml of this solution add 1 ml of 17% sulfuric acid and 5 ml of sodium nitrite (0.34 g/100 ml). Dilute this diazonium salt to tenfold. Add 1 ml of this diazotized *p*-nitroaniline to 1 ml (max. 10 mg) of ethanolic 4-chloro-*o*-cresol, then add 0.3 ml of 2 *M* sodium acetate and 5 ml of 96% ethanol. An orange color appears, which changes to red or orange-red in the presence of 6-chloro-*o*-cresol. A blank test is advisable. With this method 0.2% of 6-chloro-*o*-cresol is detectable in the presence of 4-chloro-*o*-cresol.

Similarly the reaction yields with 1 ml (10 mg) *o*-cresol a yellow color, which turns red or orange in the presence of 6-chloro-*o*-cresol. Limit of identification: 0.2% 6-chloro-*o*-cresol in *o*-cresol.

REFERENCES

1. Bertin, C. Acid–base reactions in mixtures of water and other solvents. *Anal. Chim. Acta* **7**, 105–126 (1952).

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Methods for the Isolation and Characterization of Constituents of Natural Products

XV. Application of a Periodic Acid Column for Locating Double Bond Position

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Common methods for locating double bond position are essentially variations of four reactions—ozonolysis (*2, 4, 6*), oxidation with osmium tetroxide (*9, 10*), oxidation with peroxy acids (*8, 17*), and oxidation with potassium permanganate in the presence of periodic acid or its salts (*1, 5, 7, 11*). Apparently little attention has been paid to the report that periodic acid itself can be used to locate double bonds (*3*). The lack of interest in this reaction may stem from the authors' statement that although the cleavage of the double bond with periodic acid proceeds smoothly with water-soluble substances, some difficulty might be encountered with water-insoluble substrates. We noted, however, while studying the regeneration of some 2,4-dinitrophenylhydrazone derivatives of unsaturated carbonyl compounds with periodic acid impregnated on magnesium sulfate, that some oxidation of double bonds occurred (*13*). The aldehydes resulting from the oxidation could be readily identified by converting them to 2,4-dinitrophenylhydrazones and subjecting these to thin-layer chromatography. Subsequently it was learned that ethylenic unsaturation in a number of classes of water-insoluble compounds could be satisfactorily located by this technique.

This report describes the method as applied to microgram quantities of fatty acids, their methyl esters, alcohols, and carbonyl compounds. In addition, the location of the double bond in the parent compound of the 2,4-dinitrophenylhydrazone derivatives of unsaturated aldehydes and in the 2,6-dinitrophenylhydrazone derivatives of the pyruvic acid esters of unsaturated alcohols has been accomplished and is also described.

APPARATUS AND MATERIALS [1]

Dowex 50W × 8, anhydrous magnesium sulfate and CCl_4 were supplied by J. T. Baker Chemical Co., Phillipsburg, NJ. The CCl_4 gave a negligible blank and was used as received. Paraperiodic acid was obtained from the G. Frederick Smith Co., Columbus, Oh; disposable Pasteur pipets (145 × 7 mm o.d.) served as inexpensive columns. They were cut just below the crimp to facilitate insertion of column materials and were plugged with a small wad of glass wool.

EXPERIMENTAL

The method is comprised of four steps: (1) oxidation of the ethylenic bond on passage of a CCl_4 solution of the compound through a column of $MgSO_4$ impregnated with periodic acid; (2) formation of 2,4-dinitrophenylhydrazone derivatives of the carbonyl fragments as they emerge from the periodic acid column, (3) removal of excess 2,4-dinitrophenylhydrazine on an ion-exchange resin; and (4) thin-layer chromatography of the derivatives. The entire procedure takes approximately 2 hr.

Preparation of Periodic Acid Column

A volume of 1 ml of saturated aqueous periodic acid is ground with 5 g of $MgSO_4$ in a mortar until the mixture appears homogeneous. This is achieved by scraping the mortar several times with a spatula during the grinding process. The powder is sieved (80-mesh screen) and the material passing is stored at −18°C. If the powder is returned to the freezer immediately after each use, it is stable for up to one year.

A column is prepared by transferring 0.5 g of powder to a Pasteur pipet. It is packed by tapping on a bench top and then completely wetted with CCl_4. Air bubbles are removed by stirring with a wire. When the powder has resettled to a uniform packing, the flow rate should be slower than 20 min/ml of CCl_4. Approximately 30 min/ml is desired and this can be achieved by slight tamping.

Preparation of 2,4-Dinitrophenylhydrazine Column

Celite impregnated with a phosphoric acid solution of 2,4-dinitrophenylhydrazine is prepared (*14*) and stored at −18°C. About 0.4 g of the mixture is packed under moderate pressure in a Pasteur pipet. Prior to use, 2-column volumes of benzene followed by 2-column volumes of CCl_4 are added to remove impurities.

[1] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Preparation of Dowex-50 Column

A Pasteur pipet is packed by pushing the end into the resin (used as received) so that a column approximately 30 mm in length will result when the resin is pushed lightly onto the glass wool plug. The resin is washed with 2-column volumes of CCl_4 prior to use.

Column Setup for Reaction

The columns are stacked from top to bottom as follows: periodic acid, 2,4-dinitrophenylhydrazine, and Dowex-50 with a 4-ml vial acting as receiver. The tips of the two top columns are shortened, if necessary, so that the tip just touches the top of the bed of the subsequent column.

Procedure for Locating Double Bond

The compound to be investigated (minimum 5 μg) is applied to the top column in 1 ml of CCl_4. When this has entered completely, the sides of the tube are washed with about 0.25 ml of CCl_4. This is followed by a column volume of CCl_4. The periodic acid column is removed and a column volume of CCl_4 is added to the next column and it is removed. The last column (Dowex-50) is then washed with a column volume of CCl_4.

Identification of Oxidation Products

The effluent from the column system (3–3.5 ml) is evaporated to dryness under a stream of N_2, the residue taken up in about 50 μl of benzene and an appropriate volume spotted on an alkaline thin-layer partition plate (*15*) along with authentic derivatives. The plate is developed with hexane. If, however, insufficient movement of the spots is observed, the plate may be developed with a more polar system such as hexane: benzene 63:35 (*15*).

Alkaline plates were used in this procedure because very small amounts (< 0.5 μg) of a hydrazone are readily visible. Neutral plates (*15*) may also be used and should be used if more polar hydrazones are expected. On neutral plates the spots may be intensified by spraying with methanolic KOH.

RESULTS AND DISCUSSION

The compounds subjected to the oxidation, the expected fragments, and the fragments identified are listed in Tables 1 and 2. Reproductions of thin-layer chromatograms are shown in Figs. 1 and 2. In this study, 25 μg of the compound were passed over the columns, and one-fifth of the residue was analyzed by thin-layer chromatography. However, the double bond in several of the compounds was satisfactorily located

using the 5 μg for the oxidation and either all or part of the residue for thin-layer chromatography. On samples of 5 μg or less, a solvent blank should be run.

The colored substrates (Table 2, Fig. 2) as a rule showed more background than did colorless substrates (Table 1, Fig. 1), but a reliable determination of double bond position could still be made on 5 μg. The colorless substrates generally gave only the expected product, although some minor spots (about 2%) always were detected. In general, cleaner chromatograms were obtained from substrates containing the double bond located near the center of the chain.

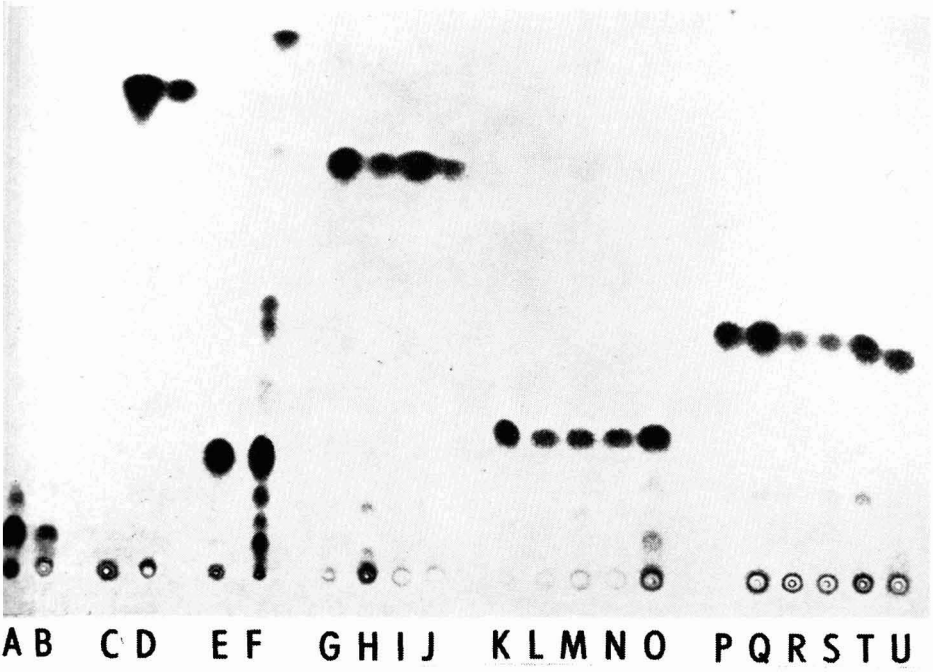

FIG. 1. Thin-layer partition chromatogram of unsaturated compounds oxidized on a periodic acid column. Spots are control 2,4-dinitrophenylhydrazones and 2,4-dinitrophenylhydrazone derivatives of carbonyl fragments produced by the oxidation. (A) propanal, (B) *cis*-3-hexen-1-ol products, (C) pentadecanal control, (D) methyl-*cis*-5-eicosenoate products, (E) hexanal control, (F) methyl-12-octadecenoate products, (G) dodecanal control, (H) methyl-*cis*-6-octadecenoate products, (I) petroselinic acid products, (J) methyl petroselinate products, (K) heptanal control, (L) vaccenyl alcohol products, (M) methyl-*trans*-vaccenate products, (N) methyl-*cis*-vaccenate products, (O) methyl palmitoleate products, (P) nonanal control, (Q) selachyl alcohol products, (R) methyl nervonate products, (S) elaidyl alcohol products, (T) oleyl alcohol products, (U) methyl oleate products.

Oxidation of compounds with terminal unsaturation either did not take place or else the formaldehyde escaped detection. Several of these were tried including methyl-10-undecenoate and 10-undecenoic acid. Citronellol and linalool, both of which would produce acetone, did not oxidize. No other compounds that would yield a ketone fragment were investigated.

A number of unsaturated 2,4-dinitrophenylhydrazones also failed to yield the expected fragment. Tiglaldehyde (2-methyl-2-butenal)-2,4-dinitrophenylhydrazone did not react to yield acetaldehyde. Since tiglaldehyde, citronellol and linalool all have a methyl group attached to

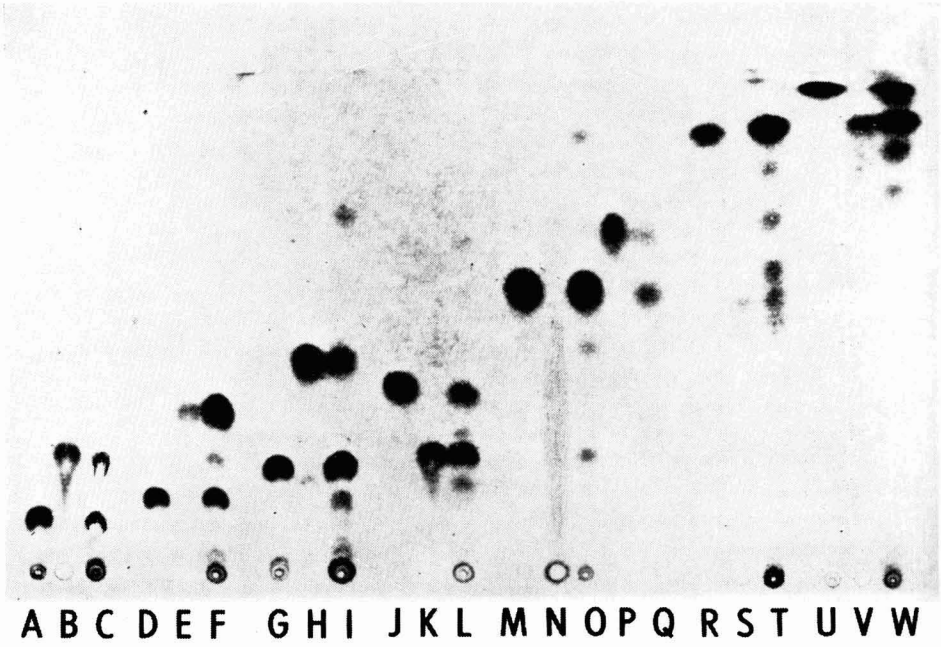

FIG. 2. Thin-layer chromatogram of unsaturated colored derivatives oxidized on a periodic acid column. Spots are control 2,4-dinitrophenylhydrazones and the 2,4-dinitrophenylhydrazone derivatives of carbonyl fragments produced in the oxidation. (A) propanal control, (B) 4-*cis*-heptenal-2,4-dinitrophenylhydrazone, (C) oxidized B, (D) butanal control, (E) 2-ethyl-hex-2-enal-2,4-dinitrophenylhydrazone, (F) oxidized E, (G) pentanal control, (H) 4-*trans*-nonenal-2,4-dinitrophenylhydrazone, (I) oxidized H, (J) heptanal control, (K) 2-nonenal-2,4-dinitrophenylhydrazone, (L) oxidized K, (M) nonanal control, (N) 9-octadecenyloxyacetaldehyde-α-methyl-2,4,6-trinitrophenylhydrazone, (O) oxidized N, (P) oleyl alcohol ester of pyruvic acid 2,6-dinitrophenylhydrazone, (Q) oxidized P, (R) dodecanal control, (S) 2,4-hexadecadienal-2,4-dinitrophenylhydrazone, (T) oxidized S, (U) tetradecanal control, (V) hexadec-2-enal-2,4-dinitrophenylhydrazone, (W) oxidized V.

TABLE I

COLORED DERIVATIVES SUBJECTED TO OXIDATION ON A COLUMN OF $MgSO_4$ IMPREGNATED WITH PERIODIC ACID

Compounds	Source	Fragments expected	Identified
Esters			
Methyl nervonate	NCP[a]	Nonanal, methyl 14-formyl tetradecanoate	Nonanal
Methyl-*cis*-vaccenate		Heptanal, methyl 10-formyl decanoate	Heptanal
Methyl-*trans*-vaccenate		Heptanal, methyl 10-formyl decanoate	Heptanal
Methyl palmitoleate		Heptanal, methyl 8-formyl octanoate	Heptanal
Methyl petroselinate		Dodecanal, methyl 5-formyl pentanoate	Dodecanal
Methyl oleate	H[b]	Nonanal, methyl 8-formyl octanoate	Nonanal
Methyl-*cis*-5-eicosenoate	NMN[c]	Pentadecanal, methyl 4-formyl butyrate	Pentadecanal
Methyl-*cis*-6-octadecenoate		Dodecanal, methyl 5-formyl pentanoate	Dodecanal
Methyl-12-octadecenoate		Hexanal, methyl 11-formyl undecanoate	Hexanal
Methyl-10-undecenoate	A[d]	Formaldehyde, methyl 9-formyl decanoate	None
Acids			
Petroselenic	NCP	Dodecanal, 5-formyl pentanoic acid	Dodecanal
Oleic	H	Nonanal, 8-formyl octanoic acid	Nonanal
10-Undecenoic	A	Formaldehyde, 9-formyl nonanoic acid	None
Alcohols			
Elaidyl	NCP	Nonanal, 9-hydroxy nonanal	Nonanal
Oleyl	H	Nonanal, 9-hydroxy nonanal	Nonanal
Vaccenyl	NCP	Heptanal, 11-hydroxy undecanal	Heptanal
Selachyl	F[e]	Nonanal, 9-octadecenyloxy acetaldehyde	Nonanal
Cinnamyl	MCB[f]	Benzaldehyde, glycolaldehyde	Benzaldehyde
Linalool	A	Acetone, formaldehyde, 4-ketopentanal	None
Citronellol	A	Acetone, 4-methyl-6-hydroxy hexanal	None
Aldehydes			
Crotonaldehyde	A	Acetaldehyde, glyoxal	Acetaldehyde, crotonaldehyde
Cinnamaldehyde	EK[g]	Benzaldehyde, glyoxal	Benzaldehyde, cinnamaldehyde

[a] Nu Chek Prep, Elysian, MN.
[b] Hormel Institute, Austin, MN.
[c] Northern Marketing and Nutrition Laboratories, USDA, Peoria, IL.
[d] Aldrich Chem. Co., Milwaukee, WI.
[e] Fluka, Buchs, Switzerland.
[f] Matheson, Coleman & Bell, East Rutherford, NJ.
[g] Eastman Kodak, Rochester, NY.

TABLE 2

COLORED DERIVATIVES SUBJECTED TO OXIDATION ON A COLUMN OF $MgSO_4$ IMPREGNATED WITH PERIODIC ACID

Compound	Fragments expected	Identified [b]
Aldehydes (as 2,4-dinitrophenylhydrazones)		
Oleic	Nonanal, nonanedial	Nonanal [a]
2-Ethyl-hex-2-enal	Butanal, α-keto butanal	Butanal
Hexadec-2-enal	Tetradecanal, glyoxal	Tetradecanal
Hept-2-enal	Pentanal, glyoxal	Pentanal
cis-4-Heptenal	Propanal, butanedial	Propanal
cis-5-Heptenal	Acetaldehyde, pentanedial	Acetaldehyde [c]
Non-2-enal	Heptanal, glyoxal	Heptanal
trans-4-Nonenal	Pentanal, butanedial	Pentanal
trans-5-Nonenal	Butanal, pentanedial	Butanal
trans-6-Nonenal	Propanal, hexanedial	Propanal
trans-7-Nonenal	Acetaldehyde, heptanedial	Acetaldehyde [c]
cis-3-Hexenal	Propanal, propanedial	Not oxidized
Tiglaldehyde	Acetaldehyde, α-keto propanal	Not oxidized
Hexadec-2,4-dienal	Dodecanal, but-2-en-dial, glyoxal	Dodecanal
trans-2-*trans*-6-nonadienal	Propanal, butanedial, glyoxal	Propanal
Alcohols (as esters of pyruvic acid 2,6-dinitrophenylhydrazone)		
Oleyl	Nonanal, mixed derivative of 9-hydroxy nonanal	Nonanal
Cinnamyl	Benzaldehyde, mixed derivative of glycolaldehyde	Benzaldehyde
The α-methyl-2,4,6-trinitrophenylhydrazone of octadec-9-enyloxyacetaldehyde	Nonanal, mixed derivative of 8-formyloctyloxyacetaldehyde	Nonanal

[a] Some decanal also detected.

[b] Unoxidized colored substrates by virtue of their color are also seen on the plate.

[c] Several other unidentified spots on chromatogram.

one of the carbons of the double bond, it may indicate that this type of structure in general will not be oxidized on the periodic acid column.

The oxidation of the 2,4-dinitrophenylhydrazone of *cis*-3-hexenal proceeded quite well but the *trans* isomer did not react at all.

Yields of the carbonyl resulting from the oxidation were usually about 30–40% of theory. The yield was determined spectrophotometrically on the 2,4-dinitrophenylhydrazone of the aldehyde identified in the products of the oxidation from 25 μg of substrate. The yield, within limits, was dependent on the flow rate of the periodic acid column. A relatively rapid flow rate (20 min/ml) gave lower yields, and flow rates from 30–60 min/ml gave maximal yields. Flow rates slower than 60 min/ml did not increase the yield, but rather a small decrease was noted.

Besides CCl_4, other solvents including $CHCl_3$, CH_2Cl_2, benzene, cyclohexane and *n*-hexane were tried. All compounds underwent oxidation most readily in the nonpolar solvents. For example, selachyl alcohol (3-(9-octadecenyloxy)-1,2-propanediol) gave about 94% 9-octadecenyloxyacetaldehyde and 6% nonanal when CH_2Cl_2 was used as solvent. In CCl_4 no 9-octadecenyloxyacetaldehyde was observed, and the yield of nonanal increased to about 35% of theory. CCl_4 was the preferred solvent over the slightly less polar hexane because it gave a negligible blank without purification. Both hexane and cyclohexane were suitable solvents for carrying out the oxidation but gave high blanks despite being rendered carbonyl and alcohol free (*12, 14*).

Supports other than $MgSO_4$ were also tested. No oxidation took place on Celite 545, Analytical Grade Celite, Microcel T-38 or on glass micro beads. Celite and glass beads as a support for periodic acid oxidation of *vic*-glycols have also failed (16). Although $CaSO_4$ was suitable as a support to a certain extent, it proved to be inferior to $MgSO_4$.

Observations made during the course of this work indicated that under a given set of conditions, the ethylenic linkage in free fatty acids was more readily oxidized than in the other classes studied. The methyl esters appeared to be next, followd by the alcohols and carbonyls. This order of reactivity was determined by running the column at suboptimal speeds, that is, at a faster flow rate than 30 min/ml, and determining the yield of aldehyde produced from the oxidation of the double bond of the various classes studied.

It should be mentioned that a number of olefinic hydrocarbons including all possible unbranched monounsaturated decenes, and 9-octadecene were checked in both CCl_4 and hexane and found not to oxidize.

On the basis of the failure of Celite and glass as supports in the oxidation, the order of reactivity of the various classes studied, the observa-

tion that oxidation proceeds better in nonpolar solvents, and the inability of olefinic hydrocarbons to oxidize, the data suggests that adsorption may be a prerequisite for oxidation.

The procedure afforded the detection only of the carbonyl fragment produced on the hydrocarbon side of the double bond. The carbonyl fragment on the more polar side went undetected or unrecognized. The ester bond in the methyl esters of aldehydic acids produced from the oxidation of the fatty acid methyl esters are thought to be hydrolyzed by the periodic acid column (13). The 2,4-dinitrophenylhydrazones of other polar fragments such as aldehydic acids, hydroxyaldehydes and dialdehydes are either too insoluble in CCl_4 to be removed from the column or do not move in the thin-layer chromatography system. The method as described, therefore, is limited to the identification of only the carbonyl fragment from the hydrocarbon side of the double bond in monoethylenic compounds, and the hydrocarbon side of the ultimate double bond in polyunsaturated compounds.

Isomerism was observed in the 2,4-dinitrophenylhydrazones of most of the aldehydes produced from the oxidation when the hydrazones were spotted soon after they were formed. This was not a serious limitation in this study with pure or relatively pure starting compounds since the main isomer on the chromatogram always moved identically to the authentic expected fragment. It was noted, however, that if the hydrazones from the oxidation were stored dry for 48 hr or longer prior to chromatography, the faster-moving (weaker) isomer reverted quantitatively to the other form. This phenomenon should simplify interpretation of chromatograms that may be less readily interpretable, such as those obtained from impure starting material.

Although there are several limitations to the method, a number of advantages can also be claimed: (1) the procedure is relatively mild, (2) an ozonizer or gas chromatograph is not required; (3) short chain aldehydes, particularly acetaldehyde, if produced can be detected readily whereas this might be located under the solvent peak in gas chromatography, and (4) double bonds in colored derivatives can be located. To our knowledge this is the only method which can directly do this.

SUMMARY

The ability of a microcolumn of periodic acid impregnated on magnesium sulfate to effect oxidation of ethylenic unsaturation was studied. The aldehyde, produced on the hydrocarbon side of the double bond in 30–40% yield, was converted to a 2,4-dinitrophenylhydrazone on a microderivatizing column and identified by thin-layer chromatography. The method was applied to unsaturation in fatty acids, their methyl esters, alcohols, aldehydes, and colored derivatives of the latter two classes. Double bonds in a variety of positions in the chain were

successfully located, but terminal unsaturation or unsaturation with a methyl group on either carbon of the double bond could not be characterized. The procedure was routinely run on 25 μg but can still be successfully executed on 5 μg of a pure compound.

ACKNOWLEDGMENTS

F. R. Earle, Northern Marketing and Nutrition Research Division, U. S. Department of Agriculture, Peoria, IL. kindly donated several of the unsaturated methyl esters. Dr. P. J. Meijboom, Unilever, Vlaardingen, The Netherlands, supplied the 2,4-dinitrophenylhydrazones of the unsaturated aldehydes of known configuration.

REFERENCES

1. Bergelson, L. D., Dyatlovitskaya, E. V., and Voronkova, V. V., Complete structural analysis of fatty acid mixtures by thin-layer chromatography. *J. Chromatogr.* **15**, 191–199 (1964).
2. Beroza, M. and Bierl, Barbara, Rapid determination of olefin position in organic compounds in microgram range by ozonolysis and gas chromatography. *Anal. Chem.* **39**, 1131–1135 (1967).
3. Chatterjee, A. and Majumdar, S. G., Use of periodic acid for detecting and locating ethylenic unsaturation. *Anal. Chem.* **28**, 878–879 (1956).
4. Davison, V. L. and Dutton, H. J., Microreactor chromatography. Quantitative determination of double bond position by ozonization–pyrolysis. *Anal. Chem.* **38**, 1302–1305 (1966).
5. Downing, D. T. and Greene, R. S., Rapid determination of double bond positions in monoenoic fatty acids by periodate–permanganate oxidation. *Lipids* **3**, 95–100 (1968).
6. Edwards, H. M., Localization of unsaturation in fatty acids by identification of 2,4-dinitrophenylhydrazones derived from ozonides by thin-layer chromatography. *Lipids* **1**, 1–5 (1966).
7. Grimmer, G. and Jacob, J., Optimierung der Oxydativen Spaltung mit MnO_4^-/IO_4^-. I. Mitteilung: Monofettsäuren. *Z. Naturforsch.* **24b**, 565–568 (1969).
8. Kenner, G. W. and Stenhagen, E., Location of double bonds by mass spectrometry. *Acta Chem. Scand.* **18**, 1551–1552 (1964).
9. McCloskey, J. A. and McClelland, Martha J., Mass spectra of isopropylidine derivatives of unsaturated fatty esters. *J. Amer. Chem. Soc.* **87**, 5090–5093 (1965).
10. Perkins, E. G. and Argoudelis, C. J., Determination of double bond position in polyunsaturated fatty acids using combination gas chromatography–mass spectrometry. *Lipids* **4**, 619–621 (1969).
11. Rudloff, E. von, Periodate–permanganate oxidations. IV. Determination of the position of double bonds in unsaturated fatty acids and esters. *J. Amer. Oil Chem. Soc.* **33**, 126–218 (1956).
12. Schwartz, D. P., Quantitative microdetermination of alcohols as esters of pyruvic acid 2,6-dinitrophenylhydrazone. *Anal. Biochem.* **38**, 148–157 (1970).

13. Schwartz, D. P. and Brewington, D. R., Methods for the isolation and characterization of constituents of natural products. XIII. Regeneration of 2,4-dinitrophenylhydrazones on a periodic acid column. *Microchem. J.* **17**, (1), 63–67 (1972).

14. Schwartz, D. P. and Parks, O. W., Preparation of carbonyl-free solvents. *Anal. Chem.* **33**, 1396–1398 (1961).

15. Schwartz, D. P., Shamey, J., Brewington, C. R. and Parks, O. W., Methods for the isolation and characterization of constituents of natural products. X. New and improved methods for the analysis of carbonyl 2,4-dinitrophenylhydrazones and 2,4-dinitrophenylosazones. *Microchem. J.* **13**, 407–417 (1968).

16. Schwartz, D. P., Weihrauch, J. L. and Burgwald, L., Methods for the isolation and characterization of constituents of natural products. A periodic acid column procedure for the oxidation of *vic*-glycols, epoxides and α-hydroxy acids at the micromole level. *Anal. Chem.* **41**, 984–986 (1969).

17. Vacherow, M. J., Michel, G. and Guilluy, R., Location of double bonds in unsaturated acids by mass spectrometry. *Bull. Soc. Chim. Biol.* **51**, 177–185 (1969).

MICROCHEMICAL JOURNAL 17, 245–249 (1972)

Book Reviews

Laboratory Manual for College Chemistry, Semimicro Qualitative Analysis Edition. 4th Ed. By WILLIAM T. SMITH AND JESSE H. WOOD. Harper and Row, New York, 1971. iv + 247 pages. $5.95.

The book contains the usual format of a short introduction to each experiment followed by the description of how to perform the experiment and then sheets for answering the questions and working problems. The selection of experiments is well taken, covers a wide range and allows the book to be used in courses differing within a wide range.

Points of possible physical danger and those important for getting good results are always well stressed, and the student should have no trouble to sail successfully through the work. In some cases one feels even a little bit too smoothly. Case in point, for example the experiment on determining the density of an unknown. The arrangement of the required data, like "volume of water," "vol of water and sample," etc. is in the exact order as being required for the calculations and leaves no doubt as to what to do. Thus the evaluation of the experiment boils down to a mere placing of numbers in the right place and does not force the student to think about what to do with what number and when and how.

The subtitle "Semimicro Qualitative Analysis Edition" is quite misleading. One would expect a laboratory manual for performing what the subtitle indicates and on a thorough and broad basis. Instead the book contains 207 pages of the common general chemistry type experiments including some quantitative analysis like a titration and only about 40 pages devoted to actual qualitative analysis. Of those 40 pages, roughly half are working sheets. While there is no doubt about the benefit the student can gain from working unknowns in qualitative analysis, there is considerable doubt in the reviewer's mind that the average student can handle all the 5 classical analytical groups plus the anions in such a condensed form. Especially, when thinking of the often very deplorable state of pretraining from high schools. However, like so much in present day education, and for all analytical education, also, this is a question of personal preference and belief. The thoughts offered should not detract from the fact that the book is a useful one; the necessity of a fourth edition after a relatively short period speaks for itself.

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Microtechniques-6. International Union of Pure and Applied Chemistry. Plenary Lectures, VIth International Symposium on Microtechniques. Symposium editor, G. KAINZ. Butterworths, London, 1971. 74 pp. $6.00.

The five papers in this volume, containing the proceedings of the International Union of Pure and Applied Chemistry on microtechniques held in conjunction

with the Austrian Society for Microchemistry and Analytical Chemistry at Graz, Austria, on Sept. 7–11, 1970, cover a broad range of topics. Three of the papers, namely Inorganic Microanalysis, Organic Microanalysis, and Utilization of Modern Methodology in Structure Elucidation of Organic Compounds are in German, while the last 2 on the Determination of Very Small Amounts of Materials by the Techniques of Atomic Absorption and Atomic Fluorescence Spectroscopy, and Modern Trends in Radiochemical Methods are in English.

Of all the papers in this little volume, the most interesting, in this reviewer's opinion, is the one dealing with the determination of very small amounts of elements by atomic absorption and atomic fluorescence spectroscopy. This contribution describes a technique of ultramicrotrace analysis in which both principles are combined. Trace constituents are analyzed in samples of ultramicro proportions *viz* 1 μl. A flameless technique of atomic absorption and atomic fluorescence is evolved which allows amounts of some elements to be detected down to 10^{-15} g and to be determined with a precision of 2–3% at 10^{-14} g levels. The samples are evaporated on a resistively heated graphite rod and the temperature is then swept up to about 2600°C by increasing the voltage to about 12 V. This produces a transient cloud of atoms which are studied by a fast response detection system for atomic absorption or fluorescence signals. The technique is absolutely specific for each element and allows most elements to be determined with a sensitivity far surpassing that of X-ray fluorescence and challenging and sometimes even surpassing neutron activation analysis, mass spectrometry, and electron probe microanalysis using apparatus at a fraction of the cost and generally available, with only slight modification, in almost every inorganic trace analysis laboratory.

To sum up, it is always difficult to review the proceedings of a symposium. Each paper must be considered as an entity and the overall coverage and quality of the meeting must be considered. The chief shortcomings of the book are the tantalizing snippets of knowledge which whet the appetite for more. The organizers were well aware of this. They were faced with the inevitable problem of rapid publication or loss of timeliness, and made the only decision. A greedy reader, however, may have wanted more when the list of titles and authors is read. The papers presented are state of the art reviews and the rapid presentation of these achieves the avowed interest of the Symposium. I recommend this comprehensible and useful book to anyone involved in the analysis of trace elements.

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Electron Microscopy and Plant Ultrastructure. By A. W. ROBARDS. McGraw-Hill, Maidenhead, England, 1970. x + 298 pp. $9.75.

During the past decade there has been an ever increasing number of books either dealing with the technical aspects of equipment and techniques associated with the preparation and observation of biological specimens for the electron microscope or atlases primarily concerned with the ultrastructure of selected biological preparations. Various recent books dealing with cytology and cell biology integrate structure and function at the subcellular level, drawing on information obtained from still rapidly expanding areas of research. However, except for sporadic, highly technical review papers, this book appears to be among the first published works which provide an overall view of electron microscopy as it applies to plants in general (*1, 2, 3*).

As presented in the preface, the aim of this book is to provide for the general understanding of the techniques and results achieved by electron microscopy without having to be concerned with the specific and detailed information necessary to the specialist and user. As the title of the book implies, it is divided into two parts: electron microscopy, three chapters; and plant ultrastructure consisting of nine chapters dealing with cellular organelles and the cell wall, one chapter on the variation in higher plant cells, and four chapters dealing with the algae, fungi, bacteria and blue-green algae, and viruses, respectively.

The book is well-written and contains many excellent line drawings and diagrams. There are numerous electron micrographs, some of which could have been better produced. The author provides a good, up-to-date bibliography at the end of each chapter and a practical glossary. The author does a good job of condensing and simplifying the concepts and techniques related to electron microscopy. The chapters dealing with cellular organelles, the cell wall, and the variation in higher plant cells are each relatively short but do present recent information clearly and understandably. The last four chapters are each written by a specialist in that particular area and, like the other chapters, give a general view but with pertinent detail.

The book is recommended for use at either the undergraduate or graduate level and should be a useful resource book for biologists not directly involved in electron microscopy.

REFERENCES

1. Buvat, R., "Plant Cells," 256 pp. McGraw-Hill, New York, 1969.
2. Juniper, B. E., Cox, G. C., Gilchrist, A. J., and Williams, P. R., "Techniques for Plant Electron Microscopy," 108 pp. Blackwell, Oxford, 1970.
3. Ledbetter, M. C., and Porter, K. R., "Introduction to the Fine Structure of Plant Cells," 188 pp. Springer-Verlag, Berlin, 1970.

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Treatise on Analytical Chemistry, Part I, Vol. 9. Edited by I. M. KOLTHOFF AND P. J. ELVING, Wiley-Interscience, New York, 1971. xv + 552 pp. $24.95.

This book represents a continuation of Part I, Theory and Practice, of this well known Treatise. The general topics for consideration, (1) Radioactive Methods and (2) Application of Measurement are treated by five and three chapters, respectively.

The first chapter, "Radioactive and Isotopic Methods of Analysis: Nature, Scope, Limitations, and Interrelations," (40 pages) by H. L. Winston acts as an introduction to the very basic principles of radioactivity and nuclear considerations. This is followed by a rather brief description of how radionuclides may be produced. Winston has also written the second chapter, "Nuclear Radiations: Characteristics and Detection (38 pages). After discussing the interaction of nuclear radiation with matter, radiation detectors are covered, particularly the more elegant solid state detectors. A short section then outlines readout instrumentation. The two chapters act as reasonable survey introduction to radioactive methods, but I would have been pleased to see the author flesh out his material somewhat more.

Analytical chemists have been making great progress in developing techniques and hardware which are sufficiently selective as to eliminate many previously necessary separations. However, many cases in radiochemical methods still require separations, making the next chapter, "Radiochemical Separations" (44 pages), by C. E. Crouthamel and R. R. Heinrich very useful. Peculiar problems are associated with such techniques; a variety of separation approaches are described to handle these, such as solvent extraction, ion exchange, volatilization, and electrochemical methods. The Radioactive Methods section is completed by two interesting and quite informative chapters, "Tracer Techniques" (68 pages) by William Seaman and "Activation Analysis" (58 pages) by Vince Guinn. Seaman explains the selection and preparation of tracers and their subsequent application in isotopic dilution, indicator analysis, and radiometric analysis. Guinn treats the important topic of activation analysis with justice. A reader unfamiliar with the technique could obtain a good introduction to both the capabilities and, to a lesser degree, the limitations of activation analysis.

The second section of this volume is introduced with a detailed chapter by R. W. King, "Combination of Physical and Chemical Properties for Characterization and Analysis" (90 pages). An extensive discussion centering about hydrocarbons is followed by a description of pertinent physical methods and applications. In the next chapter, R. K. Skogerboe and G. H. Morrison have attempted to cover "Trace Analysis: Essential Aspects" in 26 pages. In the words of the authors, "only the highlights of the subject" are treated. The volume closes with a chapter by F. H. Stross and J. H. Bradley, "Determination of Purity" (69 pages), an apt topic to follow trace analysis. As a general survey and outline of the many physical and chemical methods which may be brought to play on this problem, the chapter is adequate.

Each new volume in this series is always a welcome addition. There seem to be many areas which could be justifiably enlarged upon, but the comprehensive scope of the Treatise may not allow this. Complaints about individual chapters or topics are insignificant compared to the overall value of this monumental work.

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Manometric & Biochemical Techniques. 5th ed. Edited by W. W. UMBREIT, R. H. BURRIS, AND J. F. STAUFFER. Burgess, Minneapolis, 1972. v + 387 pp. $12.95.

This book consists of 16 chapters, written by a total of 11 contributors, including the 3 editors. The titles of the various chapters are as follows: Constant Volume Manometry—the "Warburg"; Carbon Dioxide and Bicarbonate; Direct and Indirect Methods for Carbon Dioxide; Calibration of Respirometers; Useful Techniques in Manometry; Constant Pressure Manometry; Differential Manometry—the "Barcroft"; Continuous Measurement of Dissolved Oxygen; Methods for Preparation and Study of Tissues and Enzymes; The Homogenate Technique; Methods for the Isolation of Particulate Components of the Cell; Zonal Centrifugation; Manometric & Chemical Estimation of Metabolites and Enzyme Systems; Spectrophotometry; Design of Chromatographic Procedures; Polyacrylamide Gel Electrophoresis. The chapters are followed by a section consisting of 17 pages of references, arranged alphabetically.

The chapters have been written in discussion fashion and give few experimental details, even where methods are being described. The book is rich in derivation of formulas in regard to the theoretical discussions. In connection with this n, the number of moles, has been omitted from the equation for the Ideal Gas Law, on page 1. The equation should read:

$$PV = nRT.$$

Generally speaking, this book is a valuable reference one for those already working in the field. It is not a text on "how-to-do-it."

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Synthetic Peptides, Vol. 2. By GEORGE R. PETTIT. Van Nostrand Reinhold, New York, 1971. vii + 190 pp. $15.50.

The last few years have seen a marked increase in the number of papers published on peptide synthesis. The annual rate of about 200 was reached in 1970. Dr. Pettit did workers in this important area a great service when he published Vol. 1 of "Synthetic Peptides" in 1970 [see *Microchemical Journal*, **16**, 329, (1971)].

Now he has extended the survey to October, 1971. Volume 2 is set up in the same way as Volume 1 and 219 publications have been consulted. As with Volume 1, the references, which deal primarily with small-scale manipulations, are of great value to microchemists.

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Erratum

Vol. **16**, No. 3 (1971), in the article, "Simultaneous Microchemical Analysis of Glycerol, Fatty Acid, and Phosphorus in Complex Lipids," by DeWayne Townsend, Brian Livermore, and Howard Jenkin, pp. 456–466:

Page 458, line 6 from the bottom, ". . . 25 ml of 2.2 *N* HCl. . . ." should read: ". . . 25 μl of 2.2 *N* HCl. . . ."

Announcements

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Further details will appear in the June issue of
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Reverse Osmosis

By S. SOURIRAJAN
Division of Chemistry
National Research Council of Canada
Ottawa, Canada

This monograph presents a review of the science and engineering of reverse osmosis — a new and versatile field of separation technology in chemical process engineering. This general separation technique is applicable in the separation, concentration, and fractionation of inorganic and organic substances, both in aqueous and nonaqueous solutions in either the liquid or the gaseous phase. This book examines the reverse osmosis process as applied to saline water conversion, and to water treatment in general. It discusses the preparation and performance of reverse osmosis membranes, parameters of process design, separation of mixed inorganic solutes and organic liquid mixtures, and it provides suggestions for future research and development.

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