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Microchemical Journal

devoted to the application of microtechniques in all branches of science

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Briefs

The Microdetermination of Certain Pyrimidine Derivatives. MONIER SHAKER AND G. W. YOUSSEF, *Biochemistry Department, Faculty of Veterinary Medicine, Cairo University, Giza, Cairo, Egypt.*

N-Bromosuccinimide is used for the determination of uracil, thymine, and sulfadiazine. The experimental error is ±2% within the limits of 10 mg and 100 μg.

Microchem. J. **19,** 339 (1974).

A Novel Combustion System for the Microdetermination of Nitrogen. H. TRUTNOVSKY, *Medizinisch-Chemisches Institut und Pregl-Laboratorium der Universitat Graz, A-8010 Graz, Austria.*

A method for the determination of nitrogen by combustion in a rapid stream of oxygen is described. The oxygen is produced electrolytically and the hydrogen produced simultaneously is used to reduce the oxides of nitrogen and to react with the excess oxygen. Any excess of hydrogen is oxidized by a small layer of copper oxide. Water vapor is utilized in the sweeping-out process, greatly reducing the amount of carbon dioxide necessary.

Microchem. J. **19,** 347 (1974).

Colorimetric Determination of Palladium(II) by Means of Promazine Hydrochloride. H. PUZANOWSKA-TARASIEWICZ, M. TARASIEWICZ, AND H. BASIŃSKA, *Technical University, Bialystok and Institute of Chemistry, Nicholas Copernicus University, Torun, Poland.*

In an acetate-buffered medium, palladium(II) forms two complexes with promazine hydrochloride, one orange, the other violet in color. The method described is based on the measurement of the absorbance at 540 nm.

Microchem. J. **19,** 353 (1974).

The Influence of Hydrogen-Bond Association on the Destruction of Hydroperoxides in the Autoxidation Process of Oleyl Alcohol, Oleic Acid, and Methyl Oleate. J. ŚLIWIOK, T. KOWALSKA, W. KOWALSKI, AND A. BIERNAT, *Institute of Chemistry, Silesian University, Katowice, Poland.*

The autoxidation process of the three compounds at various temperatures is considered with special stress laid upon the mechanism of cumulating and destruction of peroxidic products. Interpretation of the observed differences was based on the changed type of characters compared with the upper lines.

Microchem. J. **19,** 362 (1974).

Uramyldiacetic Acid (UDA) as Absorptiometric Agent. F. BERMEJO-MARTINEZ AND MERCEDES MOLINA-POCH, *Department of Analytical Chemistry and Analytical Chemisty Section of High Council of Scientific Research, Faculty of Sciences, Santiago de Compostela, Spain.*

The analytical behavior of uramyldiacetic acid as a potential chromogenic reagent for the spectrophotometric determination of trace metals is studied. Interesting reactions with a number of metals in neutral or acidic medium are reported.

Microchem. J. **19,** 373 (1974).

Tris(2,2′-bipyridyl)iron(II) Tetraphenylborate as a Solid Analytical Reagent in the Spectrophotometric Determination of Ag(I), Tl(I), and Hg(II). M. C. MEHRA AND P. O'BRIEN, *Chemistry Department, Universite de Moncton, Moncton, New Brunswick, Canada.*

Analytical applications of a solid reagent are described. It reacts selectively with Ag(I), Tl(I), and Hg(II) cations to give the colored 2,2′-bipyridyl iron(II) cation in solution, which is determined spectrophotometrically. The cations respond linearly in the 0–50-ppm range.

Microchem. J. **19,** 384 (1974).

Rapid Determination of Trace Amounts of Selenium(IV), Nitrite, and Nitrate by High-Pressure Liquid Chromatography Using 2,3-Diaminonaphthalene. GARRY L. WHEELER AND PETER F. LOTT, *Department of Chemistry, University of Missouri–Kansas City, Kansas City, Missouri 64110.*

Selenite, nitrite, and nitrate ions have been determined spectrophotometrically and fluorometrically using 2,3-diaminonaphthalene and high-pressure liquid chromatography. A fluorometric detector was constructed for the HPLC systems. The developed procedures were applied to the analysis of water and biological materials.

Microchem. J. **19,** 390 (1974).

Computers in Titrimetry. DANIEL JAGNER, *Department of Analytical Chemistry, University of Göteborg, Fack S-402 20 Goteborg 5, Sweden.*

The paper is chiefly a review of the "state of the art" and gives the major parts of an off-line titrator.

Microchem. J. **19,** 406 (1974).

Ultramicrodetermination of Nitrogen in Organic Compounds. I. Application of Interpolation in the Sealed Tube Method. KEIKICHI MIYAHARA, *Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan.*

A method is described for the decimilligram determination of nitrogen in organic compounds based on measuring by interpolation the volume of nitrogen collected in a capillary tube.

Microchem. J. **19,** 416 (1974).

Ultramicrodetermination of Nitrogen in Organic Compounds. II. A New Simple Nitrometer for the Sealed Tube Method. KEIKICHI MIYAHARA, *Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan.*

The nitrometer consists of a water-filled receiving chamber in which the capillary containing the collected nitrogen is placed, and a vertical graduated measuring tube. Nitrogen in the capillary is displaced into the measuring tube by water injected through a hypodermic syringe.

Microchem. J. **19,** 423 (1974).

Ultramicrodetermination of Nitrogen in Organic Compounds. III. Rise in Pressure of a Gas in a Nitrometer Due to the Capillary Phenomenon. KEIKICHI MIYAHARA, *Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan.*

The question of rise in pressure due to capillarity in a nitrometer with a tube of less than 1 mm bore is discussed and an equation given to correct such error.

Microchem. J. **19,** 429 (1974).

MICROCHEMICAL JOURNAL 19, 339–346 (1974)

The Microdetermination of Certain Pyrimidine Derivatives

MONIER SHAKER AND G. W. YOUSSEF

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Received April 18, 1973

INTRODUCTION

The pyrimidine or the 1,3-diazine ring occurs as such in the prosthetic group of some enzymes. Pyrimidine derivatives such as uracil and thymine occur in nucleic acids. Many sulfonamide drugs contain the pyrimidine ring in their structure, hence, arises the importance of determining these compounds.

Various colorimetric methods for determining thymine are described in literature (*6,11*), but there appears to be no report concerning the titrimetric determination of either uracil, or thymine. The present work involves the determination of uracil, thymine and sulfadiazine in their aqueous acidic solutions in quantities as low as 100 μg through titration against standard *N*-bromosuccinimide solution. Furthermore, the proposed method is used to determine sulfadiazine in tablets B.P.

MATERIALS AND METHODS

Equipment and Reagents

1. Microburet of 5-ml capacity graduated at 0.01 ml.
2. Graduated pipets of 1-, 2-, 5-, and 10-ml capacity.
3. Volumetric flasks of 100-ml capacity.
4. Standard *N*-bromosuccinimide solutions, e.g., 0.1% and 0.02% (w/v).
5. Methyl red alcoholic solution 0.04% (w/v).
6. Dilute sulfuric acid 10% (w/v).
7. Aqueous potassium bromide solution 2% (w/v).

Reaction Between N-Bromosuccinimide and Uracil in Acid Medium

Uracil (1.12 g, 0.01 mole) was dissolved in 25 ml of a cold solution of 10% HCl, then 1.78 g of *N*-bromosuccinimide (0.01 mole), suspended in 25 ml of distilled water, was added gradually at room temperature with shaking to the uracil solution whereby the reaction mixture

became clear. On cooling to 0°, a colorless precipitate was obtained. This was filtered and twice crystallized from hot water to give 5-bromouracil (yield, 1.3 g; 69%), melting at 295–298°C undepressed on admixture with an authentic specimen (*7*). The filtrate was distilled under reduced pressure and the solid residue, crystallized from benzene, proved to be succinimide, mp 125°C, undepressed on admixture with an authentic sample.

Reaction Between N-Bromosuccinimide and Thymine in Acid Medium

Finely powdered thymine (1.261 g, 0.01 mole) was suspended in 10 ml of 10% HCl solution and 1.78 g of powdered *N*-bromosuccinimide (0.01 mole) was added gradually and the mixture was shaken vigorously. The reaction mixture was then heated on a hot waterbath (60°C) till the solution became clear, filtered, and left to cool. Colorless prisms of 5-bromo-6-hydroxyhydrothymine, were obtained and showed no depression on melting admixed with an authentic sample (*3,9*), (≃216°C with decomposition). The filtrate was evaporated under vacuum and the residue, which was crystallized from benzene, proved to be succinimide.

Reaction Between N-bromosuccinimide and Sulfadiazine in Acid Medium

Sulfadiazine (1.25 g, 0.005 mole) was dissolved in 30 ml of 10% HCl and 100 ml glacial acetic acid was added. *N*-Bromosuccinimide (2.67 g; 0.015 mole) was dissolved in 100 ml hot distilled water. The cold *N*-bromosuccinimide solution was added gradually to the cold sulfadiazine solution with stirring. A colorless precipitate separated, filtered and crystallized from glacial acetic acid to give 2-(4-amino-3, 5-dibromophenylsulfonamido)-5-bromopyrimidine mp 269°C undepressed on admixture with an authentic sample (*10*) (Yield, 1.21 g; 50%). The filtrate was distilled under reduced pressure and the residue crystallized from benzene was identified as succinimide.

Validity of the Reaction for Quantitative Determination

The quantitative nature of the reaction between *N*-bromosuccinimide and either uracil, thymine, or sulfadiazine was checked.

A. In case of uracil. An accurately measured volume (e.g., 5 ml) of a solution containing 0.112 g (1 mmole) of uracil per 100 ml of 10% sulfuric acid was placed in a 75-ml conical flask, then 2 drops of methyl red indicator were added. The mixture was titrated with 0.178% (w/v) *N*-bromosuccinimide (1 mmole), which was added dropwise from a microburet, with continuous shaking until the red color of the indicator was just discharged and the titer used was

noted. A blank experiment was carried out and the reading was subtracted from the titer. It was found that the reaction was stoichiometric in acid medium at room temperature. The results of different runs were as follows:

Volume in ml of uracil solution (1 mmole/100 ml)				
5	4	3	2	1
Volume in ml of *N*-bromosuccinimide (1 mmole/100 ml):				
5.05	4.03	3.01	2	1.01

B. In case of thymine. An accurately measured volume (e.g., 10 ml) of a solution containing 0.126 g (1 mmole) of thymine per 100 ml of 10% sulfuric acid was placed in a 75-ml conical flask, then 1 ml of a 2% aqueous potassium bromide solution and two drops of methyl red indicator solution were added. The mixture was titrated as usual. A blank experiment was carried out and the reading was subtracted from the titer. The results of different runs were as follows:

Volume in ml of thymine solution(1 mmole/100 ml):					
10	8	6	4	2	1
Volume in ml of *N*-bromosuccinimide solution (1 mmole/100 ml):					
9.98	8.02	6.01	3.98	2	1.01

C. In case of sulfadiazine. An accurately measured volume, (e.g., 5 ml) of a solution containing 0.25 g (1 mmole) of pure sulfadiazine per 100 ml of 10% sulfuric acid was placed in a conical flask, then 20 ml of a mixture of equal volumes of glacial acetic acid and 10% sulfuric acid (to dissolve the tribromo derivative formed) was added followed by 1 ml of a 2% aqueous potassium bromide solution and 2 drops of methyl red indicator solution. The mixture was titrated as usual with a solution containing 0.178 g *N*-bromosuccinimide per 100 ml distilled water and the titer was noted. A blank experiment was simultaneously carried out and the reading was subtracted from the titer. A similar series of experiments were carried out with *N*-bromosuccinimide solution containing 0.534 g/100 ml (3 mmole). It was found that the reaction is stoichiometric in acid medium at room temperature. The results of different experiments were as follows:

Volume of sulfadiazine solution.	Titer of *N*-bromosuccinimide solution.	
(1 mmole/100 ml)	(1 mmole/100 ml)	(3 mmoles/100 ml)
5	14.95	5
4	11.95	4.01
3	9.05	3.01
2	6.02	2.0
1	3.02	1.01

PROCEDURE

Dissolve 100 mg of uracil, thymine, or sulfadiazine in 100 ml of 10% sulfuric acid. Transfer by means of a pipet a suitable volume (e.g., 5 ml) of this solution to a 75-ml conical flask. Add two drops of methyl red indicator solution. When determining thymine, 1 ml of a 2% aqueous potassium bromide must be added before titration and when determining sulfadiazine 1 ml of potassium bromide as well as a sufficient volume of a mixture of equal volumes of glacial acetic and 10% sulfuric acid must be added before titration. Shake the mixture continuously and run in the standard *N*-bromosuccinimide solution until the color of the indicator is just discharged. Carry out a blank experiment under parallel conditions without the pyrimidine derivative and subtract the blank value from the titer obtained before calculation. Calculate the pyrimidine derivative present from the expressions:

$$\text{Uracil present mg or } \mu g = V \times C \times 112.09/178.$$
$$\text{Thymine present mg or } \mu g = V \times C \times 126.12/178.$$
$$\text{Sulfadiazine present mg or } \mu g = V \times C \times 250/534,$$

where V is the titer of *N*-bromosuccinimide in ml and C is the concentration of the *N*-bromosuccinimide solution in mg or μg/ml.

RESULTS

Determination of Uracil

A 0.1 or 0.01% uracil solution in a 10% sulfuric acid was determined by the proposed method as if it was an unknown with 0.1 or 0.02% *N*-bromosuccinimide solution, respectively. The results are shown in Table 1.

Determination of Thymine

A 0.1% or 0.01% of thymine solution in 10% sulfuric acid was determined by the proposed method as if it was an unknown with 0.1% or 0.02% *N*-bromosuccinimide solution, respectively. The results are shown in Table 2.

Determination of sulfadiazine

A 0.1 or 0.01% sulfadiazine solution in 10% sulfuric acid was determined by the proposed method as if it was an unknown by the use of 0.2% or 0.02% *N*-bromosuccinimide solution, respectively. The results are shown in Table 3.

TABLE 1
RECOVERY OF URACIL BY THE PROPOSED METHOD

Volume of solution used (ml)		Uracil content (mg)	Titer of (0.1%) *N*-bromosuccinimide solution (ml)[a]	Uracil found (mg)	Error (%)
0.1%	10	10	16.10	10.13	1.30
	9	9	14.35	9.03	0.33
	8	8	12.85	8.09	1.12
	7	7	11.10	6.98	0.30
	6	6	9.55	6.01	0.17
	5	5	8.10	5.09	1.80
	4	4	6.45	4.06	1.50
	3	3	4.85	3.05	1.66
	2	2	3.15	1.98	1.00
	1	1	1.60	1.01	1.00
	(ml)	(μg)	(ml; 0.02%)[b]	(μg)	%
0.01%	10	1000	7.90	994	0.60
	9	900	7.25	912	1.33
	8	800	6.35	799	0.12
	7	700	5.60	705	0.71
	6	600	4.70	591	1.50
	5	500	3.95	497	0.60
	4	400	3.20	403	0.75
	3	300	2.35	296	1.33
	2	200	1.60	201	0.50
	1	100	0.80	101	1.00

[a] 1 ml of 0.1% *N*-bromosuccinimide ≡ 0.6292 mg uracil.
[b] 1 ml of 0.02% *N*-bromosuccinimide ≡ 125.84 μg uracil.

Assay of Sulfadiazine Tablets BP

One tablet BP was finely pulverized and dissolved in a 10% sulfuric acid solution and was assayed by the proposed method. The results are given in Table 4.

DISCUSSION

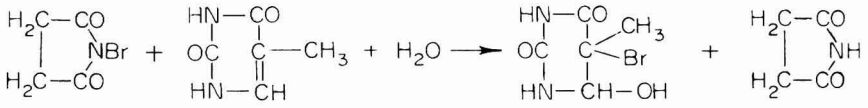
The proposed method is based on the fact that *N*-bromosuccinimide readily and quantitatively brominates an aqueous acidic solution of uracil, thymine, or sulfadiazine and is itself irreversibly reduced to succinimide. In case of uracil, the reaction is quantitative in aqueous acidic medium at room temperature (1 mole uracil to 1 mole *N*-bromosuccinimide); 5-Bromouracil was isolated from the reaction mixture and identified.

TABLE 2
RECOVERY OF THYMINE BY THE PROPOSED METHOD

Volume of solution taken (ml)		Thymine content (mg)	Titer of (0.1%) *N*-bromosuccinimide solution (ml)[a]	Thymine found (mg)	Error (%)
0.1%	10	10	14.00	9.92	0.80
	9	9	12.60	8.93	0.77
	8	8	11.20	7.93	0.87
	7	7	9.80	6.94	0.85
	6	6	8.50	6.02	0.33
	5	5	7.05	4.99	0.20
	4	4	5.60	3.97	0.75
	3	3	4.20	2.98	0.67
	2	2	2.78	1.97	1.50
	1	1	1.40	0.99	1.00
	(ml)	(μg)	(ml; 0.02%)[b]	(μg)	%
0.01%	10	1000	7.10	1006	0.60
	9	900	6.30	893	0.78
	8	800	5.60	793	0.87
	7	700	4.90	694	0.85
	6	600	4.20	595	0.83
	5	500	3.50	496	0.80
	4	400	2.80	397	0.75
	3	300	2.10	298	0.67
	2	200	1.40	198	1.00
	1	100	0.70	99.2	0.80

[a] 1 ml of 0.1% *N*-bromosuccinimide ≡ 0.70842 mg thymine.
[b] 1 ml of 0.02% *N*-bromosuccinimide ≡ 141.68 μg thymine.

In case of thymine, the reaction is quantitative for the molecular ratio of 1 mole thymine to 1 mole *N*-bromosuccinimide; 5-bromo-6-hydroxyhydrothymine was isolated from the reaction mixture and identified. The mechanism of the reaction could be formulated as follows:



The reaction with sulfadiazine proceeds quantitatively at room temperature in aqueous acidic medium for the molecular ratio of three molecules of *N*-bromosuccinimide to one molecule of sulfadiazine. 2-(4-amino-3,5-dibromophenyl sulfonamido)-5-bromopyrimidine was isolated from the reaction mixture and identified. The reaction could be formulated as follows:

TABLE 3
RECOVERY OF SULFADIAZINE BY THE PROPOSED METHOD

Volume of solution used (ml)	Sulfadiazine content (mg)	Titer of (0.2%) *N*-bromosuccinimide solution (ml)[a]	Sulfadiazine found (mg)	Error (%)
10	10	10.60	9.92	0.80
9	9	9.50	8.89	1.22
8	8	8.50	7.96	0.50
7	7	7.35	6.88	1.71
6	6	6.40	5.99	0.16
0.1% 5	5	5.30	4.96	0.80
4	4	4.20	3.93	1.75
3	3	3.15	2.95	1.66
2	2	2.10	1.97	1.50
1	1	1.05	0.98	2.00
(ml)	(μg)	(ml; 0.02%)[b]	(μg)	%
10	1000	10.65	997	0.30
9	900	9.55	894	0.66
8	800	8.55	801	0.13
7	700	7.40	693	1.00
0.01% 6	600	6.50	609	1.50
5	500	5.35	501	0.20
4	400	4.20	393	1.75
3	300	3.20	300	—
2	200	2.15	201	0.50
1	100	1.05	98	2.00

[a] 1 ml of 0.2% *N*-bromosuccinimide ≡ 0.9363 mg sulfadiazine.
[b] 1 ml of 0.02% *N*-bromosuccinimide ≡ 93.63 μg sulfadiazine.

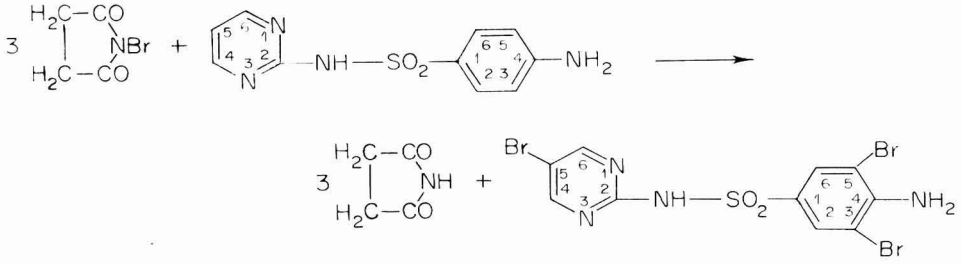


TABLE 4
ASSAY OF SULFADIAZINE TABLETS

	Content (mg)	Found (mg)	Error (%)
Sulfadiazine tablets (BP)	500	508	1.6

N-Bromosuccinimide is an effective brominating agent (*1*), capable of brominating sulfonamides (*2*) as well as pyrimidine bases (*8*) and can decolorize methyl red in aqueous acidic medium (*5*). However, bromination of uracil, thymine, or sulfadiazine is effected quantitatively in acid medium prior to the decolorization of methyl red indicator. Any slight excess of *N*-bromosuccinimide causes the methyl red to be decolorized and consequently the end-point can be easily detected. Also, in case of thymine and sulfadiazine, addition of potassium bromide solution renders the end point more sharp. Methyl red has been used previously as an indicator in titrations involving the use of *N*-bromosuccinimide (4).

SUMMARY

N-Bromosuccinimide has been used in a new titrimetric method for the microdetermination of uracil, thymine, and sulfadiazine. The reaction between these compounds and *N*-bromosuccinimide in dilute aqueous solutions is discussed. The determination is carried out within the limits of 10 mg to 100 μg where the experimental error did not exceed $\pm 2\%$.

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A Novel Combustion System for the Microdetermination of Nitrogen

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INTRODUCTION

The combustion of the sample for the gasometric determination of nitrogen in organic compounds has undergone a significant change since the pioneer work of Dumas. Combustion in oxygen has been made the mode in recent years. One drawback to the use of oxygen is the requirement for large quantities of reduced copper to remove excess oxygen from the system and the necessity of using greater and greater quantities of potassium hydroxide. It was expedient, therefore, to find a convenient substitute for the copper layer.

An unsuccessful attempt toward this was made by Kirsten (*1*) who tried to replace the copper layer with boiling sulfur. He was unable to obtain acceptable results and later it could be shown (*3*) that, under the conditions of an oxygen-in-sulfur flame, carbon dioxide was reduced to carbon monoxide and thus the readings of the nitrometer gave no sense. Attempts to replace the copper layer by various preparations of nickel (*2*) were unsuccessful.

It was decided to use gaseous hydrogen as reductant for the oxides of nitrogen and the excess oxygen. Hydrogen and oxygen can be produced electrolytically in equivalent volumes and high purity. The water vapor produced will then act as a sweeping gas for the small layer of copper oxide which serves as a buffer in the case of flow delays of one gas and as oxidant for the free hydrogen which corresponds to the oxygen used to combust the sample. Carbon dioxide is necessary in only minute amounts as the nitrogen has only to be swept from the point where the water vapor condenses to the nitrometer and not out of a large and porous copper layer.

Even the first experimental set-up gave excellent results on all kinds of organic compounds. The present state of the development of a compact unit is described below.

EXPERIMENTAL

The general layout is shown in Fig. 1. The furnaces are a standard Heraeus U unit with a split-type second-long burner. The combustion tube consists of quartz except the part before the movable burner which is made of Pyrex and connected with a heat-shrinkable tubing after insertion into the furnaces. This connecting technique has proved to produce seals of high quality permitting complicated tubes to be mounted without difficulty (*4*). The cobaltocobaltic oxide and the copper oxide are quite coarse, grain size approximately 1 mm to keep the flow resistance of the tube sufficiently small. Ignited BTS catalyst is used as copper oxide on carrier as it does neither sinter nor shrink even on repeated oxidation-reduction cycles. The T-shaped part of the combustion tube where the minute hydrogen–oxygen flame burns is a capillary with 2 mm i.d. and 8 mm o. d. With this small volume where a mixture of oxygen and hydrogen may be present, no danger of an explosion exists. The outer surface reaches a temperature of about 500°C. Flashback-stops are inserted in the lines from the electrolytic cell. The valves are Swiss-made Lucifer valves, all connections are made with copper tubing except a short length of thick-walled polyethylene tubing connected between the cap of the combustion tubing and the solenoid valve EX. A small water trap (2 ml) with a stopcock is inserted into the line to the nitrometer which can be any model. The oxygen flow is 50 ml/min, the hydrogen flow 100 ml/min. The carbon dioxide, which is used for the final sweep out and during the standby to prevent the invasion of air, is set to 40 ml/min with needle valve and flowmeter.

The electrolytic cell is shown in Fig. 2. It consists of a glass vessel with a concentric glass-spacer to separate the anodic from the cathodic compartment. The electrodes are made of stainless-steel gauze which proved to be resistant in an alkaline electrolyte. For the elec-

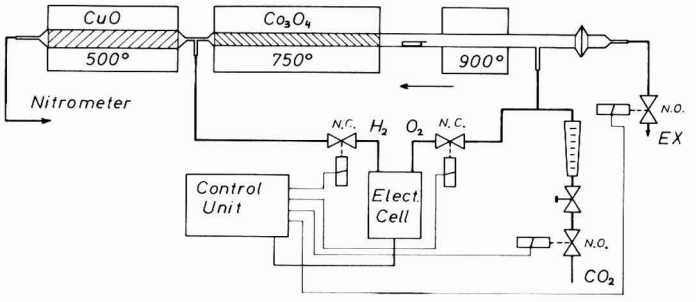

FIG. 1. Diagram of set-up.

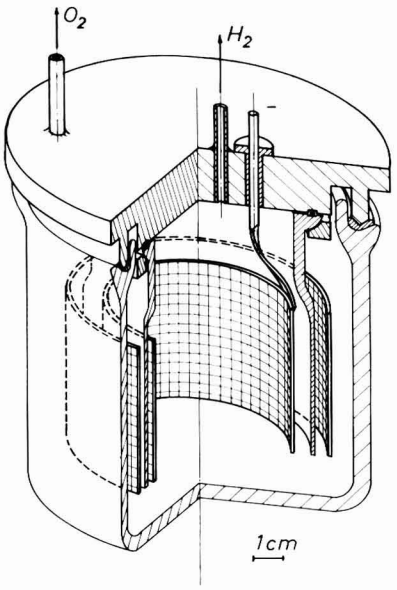

FIG. 2. Electrolytic cell.

trolyte, the same 50% potassium hydroxide as used for the nitrometer was employed with good results. The cover of the vessel is made of steel and all connections are made to it. The current of 12.5 A causes a voltage drop of about 6 V, the temperature of the cell increases due to the dissipated power to approximately 60°C but the surface is sufficiently large to prevent higher temperatures. An earlier construction with small-volume, fritted glass diaphragm, and gold electrodes needed special electrolyte coolers to prevent boiling and gave rise to serious trouble.

The electronic control circuit (Fig. 3) opens and closes the valves and switches on the current for the electrolysis. This current can be adjusted and is kept constant independent from the cell resistance. The circuit is started by switching on the transport of the movable burner, EX and CO_2 valve are closed, O_2 and H_2 valves are opened and the electrolysis current switched on. This continues until a settable time (approx 30 sec) has elapsed since the movable burner has stopped. Then the electrolysis current is switched off, the CO_2 valve is opened and the O_2 and H_2 valves are closed to prevent absorption of CO_2 by the caustic electrolyte. The carbon dioxide flows for a further time, for example, 45 sec, through the combustion tube. This is sufficient to bring all nitrogen quantitatively to the nitrometer.

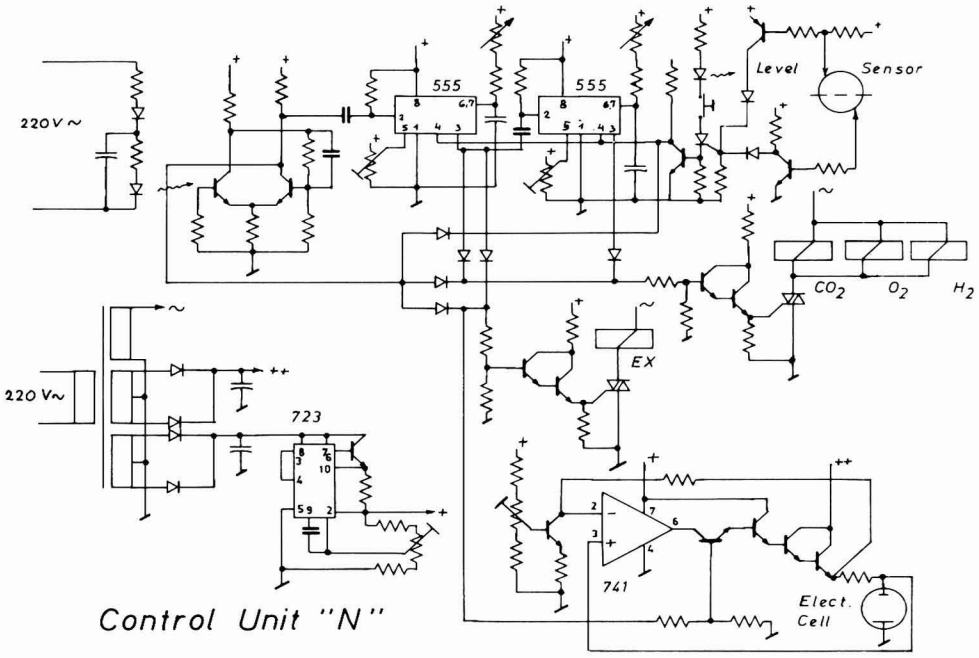

FIG. 3. Electronic control circuit.

Thereafter the valve EX is opened and the carbon dioxide is bypassed to the atmosphere. In this standby position the next sample can be loaded. The control circuit simultaneously monitors the electrolyte level within the electrolytic cell. Should the level in the cathodic compartment fall below a definite level due to consumption of water or rise above another preset level due to a high flow resistance of the oxidation catalyst layer, a red signal light is turned on and the circuit immediately goes to standby. By a switch the valve EX can be closed during the standby if for any reason a prolonged carbon dioxide flow is desirable, for example, after regeneration of the copper oxide layer.

PROCEDURE

A sample is weighed in a platinum boat and inserted into the combustion tube. The tube is closed by the cap and the nitrometer adjusted to zero. The transport of the movable burner is switched on and the sample is burned in a flow of pure oxygen. The time for this depends on the speed and the starting position of the movable burner. All steps are carried fully automatic until the nitrometer is to be read.

The blank is determined exactly in the same manner but without sample, it is in the range of 3–5 μl for 4 min electrolysis time. This is sufficiently small to permit trace determinations. If the copper oxide layer is reduced after several analyses, blanks consuming no copper oxide, it is easily reoxidized by opening the stopcock at the water trap, closing the carbon dioxide needle valve, and introducing a flow of air or technical oxygen into the combustion tube. As those gases do not attack the oxidation catalyst, no precautions are necessary. The reoxidation of a 15-cm layer of copper oxide (BTS) can be done in about 3 min. Thereafter the tube is swept out for about 10 min with carbon dioxide and is again ready for use. Copper oxide does not absorb oxygen as metallic copper does with hydrogen; therefore, no increased blank occurs after the regeneration. As the volume of the hydrogen line is much smaller than of the combustion tube, hydrogen arrives always prior to oxygen at the copper oxide layer. Thus, even after reoxidation of the copper oxide, metallic copper is present before the gases of combustion reach it and no oxides of nitrogen can pass unreduced.

The results obtained by this method are excellent, especially compounds with low nitrogen content and C-methyl groups can be analyzed without difficulties.

DISCUSSION

In the future some technical detail shall be studied, especially the possibility to avoid the use of carbon dioxide completely, sweeping with water vapor quite into the nitrometer. Also an automatic reoxidation of an even smaller copper oxide layer after each analysis and the application of this method for the analysis of large samples of technical products are intended. In preliminary experiments samples up to 100 mg benzoic acid could be combusted without difficulties or increased blank by merely allowing sufficient time for the carbon dioxide to sweep out the air as the platinum boat was filled completely and by reducing the speed of the travel of the movable burner.

SUMMARY

A method for the determination of nitrogen by combustion in a fast flow of pure oxygen is described. The oxygen is produced electrolytically and the hydrogen produced simultaneously is used to reduce the excess oxygen and oxides of nitrogen. A short layer of copper oxide oxidizes the hydrogen which remains uncombined due to the oxygen consumption of the sample. This layer is swept out by the water vapor; thus, only minute amounts of carbon dioxide are used, reducing the consumption of potassium hydroxide. The small layer of copper oxide can easily be reoxidized with air or technical oxygen. A virtually unlimited excess of oxygen during the combustion makes this method especially useful for samples difficult to combust and for trace determinations.

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Colorimetric Determination of Palladium (II) by Means of Promazine Hydrochloride

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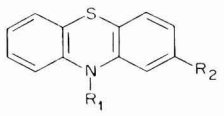
INTRODUCTION

As has been pointed out in our previous works (*3, 4, 14*) phenothiazine derivatives exhibit a number of interesting analytical properties due to their characteristic structure (the presence of chemically active sulfur and nitrogen atoms and of substituents in positions 2 and 10).



Of these properties, the most important are the liability to oxidation by means of many oxidizing agents (for example $Ce(SO_4)_2$, NH_4VO_3, $K_2Cr_2O_7$, $KBrO_3$, KJO_3, KJO_4, $NaNO_2$, H_2O_2, etc), with the formation of colored compounds and the ability of the formation of compounds with a number of acidocomplexes of metals, sparingly soluble in water but easily soluble in some organic solvents.

The above-mentioned properties are the basis of application of phenothiazine derivatives as reagents for spectrophotometric and gravimetric determinations of some metals (*2–4, 13*) and as indicators in oxidation-reduction methods (*1, 12*).

In this work, which is a continuation of previous studies on the application of phenothiazine derivatives in analytical chemistry, the formation of colored complexes of palladium(II) with promazine hydrochloride (PM), and the use of these complexes for the spectrophotometric determination of palladium(II) are discussed.

Palladium(II) ions show high ability to formation of complexes and react practically with reagents of all types. Owing to this fact, the number of methods proposed for the spectrophotometric determination of palladium(II) is very large. The more important methods have been discussed in monographs (*9, 11*). The majority of the methods

of palladium(II) determination take advantage of organic reagents. Noteworthy are organic reagents containing nitrogen and sulfur, with which palladium(II) forms the most stable complexes. Another group comprises heterocyclic compounds containing sulfur, nitrogen, or oxygen atoms in the ring. Characteristic of reagents of this group is the ability to react with palladium ions in acidic, alkaline, and neutral media. The complexes of palladium formed with the above-mentioned reagents are stable exclusively in aqueous solutions containing water miscible organic reagents (*16*). The absorption spectra of these complexes do not exhibit absorption bands in the visible region hence the analytical measurements are generally carried out at $\lambda = 380$ nm (*5,10*). The selectivity of the elaborated methods of palladium(II) determination with the aid of these reagents is not high. Platinum metals, gold, iron, copper and other elements (*11*) interfere with the determination. In practice the selectivity and sensitivity of the methods can be enhanced by carrying out extraction of the complexes formed, or by using masking agents.

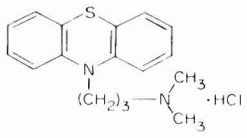
We considered it purposeful to examine the possibility of application of phenothiazine derivatives to the analysis of palladium(II). Phenothiazine derivatives belong to the above-discussed group of reagents due to the presence of sulfur and nitrogen atoms in the ring. Preliminary experiments showed that, in acetate buffer medium, palladium(II) ions react with phenothiazine derivatives with the formation of stable colored complexes. The absorption maxima of the complexes appear at $\lambda = 460$ and 540 nm. The reaction of palladium(II) with phenothiazine derivatives is relatively sensitive and selective.

In these studies promazine hydrochloride was used as a representative of phenothiazine derivatives.

EXPERIMENTAL

Reagents and Apparatus

Palladium chloride, 0.01 *M* solution in 1 *N* HCl. The concentration of the solution was determined gravimetrically by means of dimethylgloyoxime (*15*).



Promazine hydrochloride, i.e. [10-(3-dimethylamine-propyl)-phenothiazine]. HCl manufactured by EGYT-Budapest, 0.01 *M* solution.

The concentration of the solution was determined gravimetrically by means of silicotungstic acid (*6*).

Acetate buffer of pH $\approx$ 2.0. Dissolve 25 g of sodium acetate in water, add 75 ml 1 *N* HCl and fill up with water to 500 ml (*8*).

A Spectrophotometer Spekol (Carl Zeiss, Jena) 1-cm cells.

Spectrophotometric Examination of the Formation of Palladium(II) *Complexes with Promazine Hydrochloride*

It has been found that palladium(II) ions form with promazine hydrochloride two complexes of orange and violet colors. The conditions of the formation of the complexes have been established and the effects of the medium, time, concentration, and reagent ratio have been examined.

The Effect of the Reaction Medium

The effect of the concentration of acids (HCl, H_2SO_4, HNO_3), bases (NaOH, NH_4OH), and buffers (acetate, phosphate, and ammonium buffers) on the formation of complexes of palladium(II) ions with promazine hydrochloride has been examined.

It has been found that the complexes are formed in acidic and neutral media and not in basic media. In solutions acidified with hydrochloric acid, independently on the acid concentration, a mixture of two complexes—the orange one and the violet one—was obtained. In solutions acidified with sulfuric acid the mixture of complexes is formed at acid concentrations up to 2 *N*. In the concentration range 2 *N*–3 *N* H_2SO_4 only the violet complex is formed. At higher H_2SO_4 concentrations the violet complex precipitates. Nitric acid cannot be used for acidification because above a concentration of 0.02 *N* it oxidizes promazine hydrochloride forming an orange product. Of the buffers examined, the acetate buffer turned out to be the most suitable. In this buffer in the pH range 2.0–6.0 both complexes, the orange and the violet ones, can be obtained.

The Effect of Time

The effect of time on the absorbance of the colored complexes of palladium(II) with promazine hydrochloride in solutions acidified with H_2SO_4 and in acetate buffer has been examined.

It has been found that in sulfuric acid solutions the complexes are unstable and changes in the absorbance of solutions of the violet complex and of the mixture of complexes take place after 5–10 min and 30–60 min, respectively. In acetate buffer the stability of the complexes is relatively high and no changes in the absorbance of the violet and the orange complexes were observed during 3 days. In ace-

tate buffer medium, independently on the initial concentrations of the reagents and their molar ratio the violet complex does not precipitate. In further studies acetate buffer medium was used.

The Effect of Reagent Ratio

The results of the examination of the effect of the reagent ratio are shown in Fig. 1. The data indicate that in the system Pd(II)—PM(where PM stands for promazine hydrochloride) the absorbance increases with increasing excess of promazine hydrochloride with respect to palladium(II). This increase in absorbance is accompanied by a shift of the absorption maximum toward longer wavelengths.

The results obtained indicate that in the system Pd(II)—PM two complexes are formed—an orange one for which $\lambda_{max} = 460$ nm and the violet one for which $\lambda_{max} = 540$ nm.

The type of the complex formed depends on the ratio of Pd(II):PM. For example, in acetate buffer medium (pH = 2) at a constant palladium(II) concentration equal to 2×10^{-4} *M* and a Pd(II):PM ratio of 1:1 the orange complex is formed (Fig. 1, curve 1); at a 2- to 50-fold excess of PM with respect to Pd(II) a mixture of

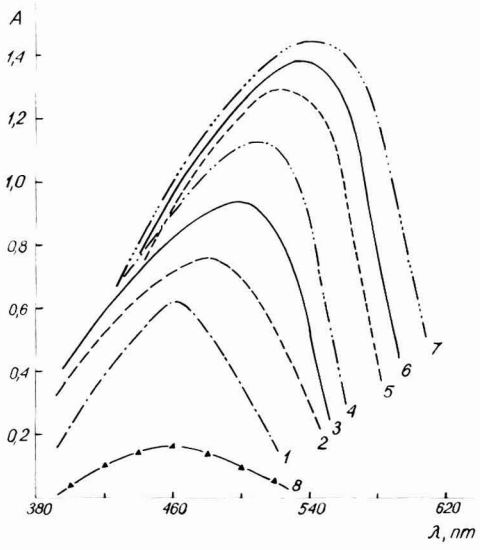


FIG. 1. Absorption curves performed in the system Pd(II)-PM at different molar ratio of Pd(II):PM, (where PM stands for promazine hydrochloride). $C_{Pd} = 2.0 \times 10^{-4}$ *M*. (1) Pd:PM = 1:1; (2) Pd:PM = 1:4; (3) Pd:PM = 1:10; (4) Pd:PM = 1:20; (5) Pd:PM = 1:40; (6) Pd:PM = 1:55; (7) Pd:PM = 1:60. Acetate buffer of pH ≈ 2.0, (8) $PdCl_2$, $C = 1 \times 10^{-3}$ *M*.

complexes is formed (Fig. 1, curves 2–5) while at higher excess of PM the violet complex is formed (Fig. 1, curves 6 and 7).

The Composition of the Complex

The composition of the complexes in the system Pd(II)—PM was established using Job's method of isomolar series as well as the method of equilibrium shift.

Examination of the composition of the orange complex using Job's method was carried out in the medium of acetate buffer, by mixing isomolar solutions of $PdCl_2$ and PM and measuring the absorbance at $\lambda = 460$ nm (Fig. 2, curves 1, 2). Examination of the composition of the violet complex by means of Job's method was carried out in sulfuric acid medium of an acid concentration of 2.5 *N* (Fig. 2, curves 3 and 4) by measuring the absorbance of isomolar solutions of $PdCl_2$ and PM at $\lambda = 540$ nm. In this case the acetate buffer could not be used, because in this buffer the violet complex is formed first at a 50-fold (or higher) excess of promazine with respect to Pd(II). On the other hand, in the medium of 2.5 *N* sulfuric acid, independently on the reagent ratio, only the violet complex is formed.

As follows from Fig. 2, the isomolar curves of the orange complex show absorption maxima at a molar ratio of Pd:PM = 1:1 while those of the violet complex at a ratio of 1:2.

The composition of the complexes was confirmed by the method of equilibrium shift. Solutions of $PdCl_2$ of a concentration of $1.5 \times$

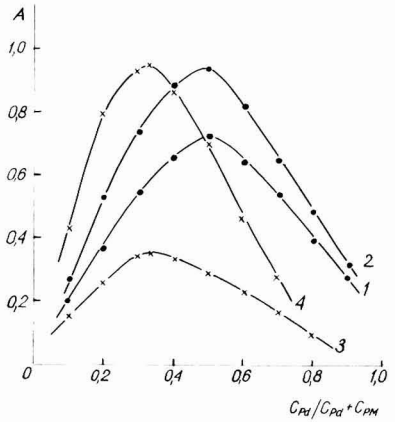


FIG. 2. Job's curves for isomolar solutions of $PdCl_2$ and PM. (1) $\Sigma C = 1 \times 10^{-3}$ *M*, (2) $\Sigma C = 1.2 \times 10^{-3}$ *M*. Acetate buffer of pH ≈ 2.0, $\lambda = 460$ nm. (3) $\Sigma C = 5 \times 10^{-4}$ *M*, (4) $\Sigma C = 7 \times 10^{-4}$ *M*, $C_{H_2SO_4} = 2.5$ *N*, $\lambda = 540$ nm. Absorbance was measured at once after added solution of promazine.

10^{-4} *M* were titrated in acetate buffer medium (of pH $\approx$ 2) with a solution of promazine chloride. The results are presented in Fig. 3.

The tangents of the straight lines in the coordinate system

$$\log \frac{A_x}{A_0 - A_x} - \log [\mathrm{PM}]$$

are approximately equal to 1 and 2. Such course of the curves confirms the formation of the orange complex of a molar ratio of Pd:PM = 1:1 as well as of the violet complex of a ratio of 1:2.

On the basis of the spectral studies the formation of the complexes can be presented as follows

$$\mathrm{Pd^{2+}} + \mathrm{C_{17}H_{20}N_2S} \longrightarrow \underset{\text{orange complex}}{\mathrm{Pd(C_{17}H_{20}N_2S)^{2+}}} \qquad (1)$$

$$\mathrm{Pd^{2+}} + 2(\mathrm{C_{17}H_{20}N_2S}) \longrightarrow \underset{\text{violet complex}}{\mathrm{Pd(C_{17}H_{20}N_2S)_2^{2+}}} \qquad (2)$$

Further studies on the reaction mechanism and the structure of the complexes are in progress.

The Instability Constants of the Complexes

The instability constants of the palladium(II) complexes with promazine hydrochloride were determined with the aid of Bjerrum's

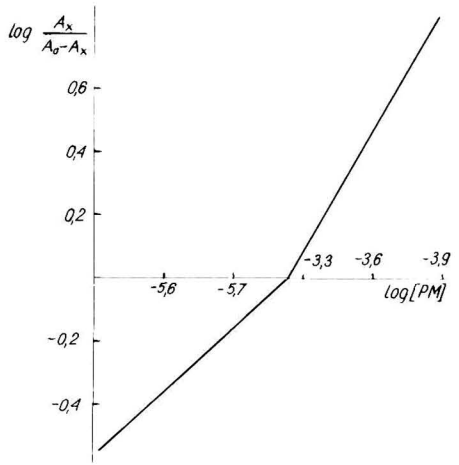


FIG. 3. The dependence's curve log $[A_x/(A_0 - A_x)]$ on promazine hydrochloride concentration, where A_0-absorbance of the solution after complexation all palladium(II), A_x increasing absorbance before complete complexation palladium(II) ions. $C_{Pd} = 1.5 \times 10^{-4}$ *M*, acetate buffer of pH $\approx$ 2.0.

method of corresponding solutions" (*7*). The concentration of the unbound ligand $[L]$ (in this case promazine) and the Bjerrum's function n were calculated from the following equations:

$$\bar{n} = \frac{C_L - [L]}{C_M} \tag{3}$$

$$\bar{n} = \frac{C'_L - C''_L}{C'_M - C''_M}, \tag{4}$$

where: C_M — total concentration of the metal and
C_L — total concentration of the ligand.

The gradual instability constants of the complexes examined in acetate buffer medium of pH $\simeq$ 2 amount to

$K_1 = (1.12 \pm 0.02) \times 10^{-4}$; $pK_1 = 3.95$
$K_2 = (8.51 \pm 0.02) \times 10^{-4}$; $pK_2 = 3.07$, respectively.

The overall instability constants take values $p\beta_1 = 3.95$, $p\beta_2 = 7.02$.

Determination of Palladium(II)

The formation in acetic buffer medium of the intensively colored, stable, violet complex of palladium(II) with promazine hydrochloride of a molar ratio of Pd(II):PM of 1:2 has been advantageously used for the spectrophotometric determination of palladium(II).

Procedure: To 25-ml flasks the following reagents were added successively: 0.5–4.0 ml of 1×10^{-3} *M* $PdCl_2$ solution, 8 ml of acetate buffer of pH $\simeq$ 2, 6 ml of 5×10^{-2} *M* solution of promazine

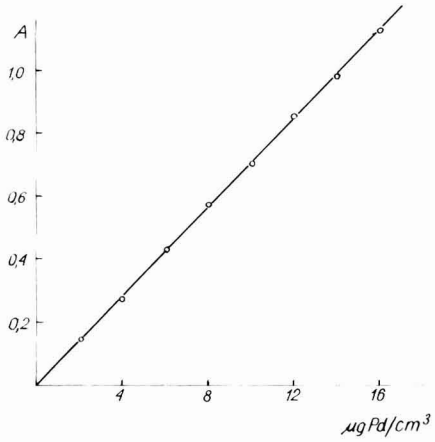


FIG. 4. The dependence of absorbance on palladium(II) concentration, $\lambda = 540$ nm, $l = 1$ cm.

hydrochloride. The flasks were filled with water up to the mark. Then the absorbance at $\lambda = 540$ nm was measured by means of a Spekol spectrophotometer, using palladium(II) solution in acetate buffer as reference solution. It has been found that in the concentration range 2–17 μg/1 ml the dependence of absorbance on Pd(II) concentration is linear and conforms to the Beer's law (Fig. 4). The error of the determination with respect to the gravimetric method amounts to $\pm 2\%$. The time of the determination is 20 min. Iron(III), Ce(IV), Pt(IV), V(V), Cr(VI), and HNO_3 interfere with the determination.

SUMMARY

In acetate buffer medium palladium(II) ions form with promazine hydrochloride (PM) two complexes: an orange one of a formula $[Pd(C_{17}H_{20}N_2S)]^{2+}$ ($\lambda_{max} = 460$ nm, $\epsilon = 4.5 \times 10^3$, at 20°C and pH = 2) and a violet one of a formula $[Pd(C_{17}H_{20}N_2S)_2]^{2+}$ ($\lambda_{max} = 540$ nm, $\epsilon = 8.8 \times 10^3$ at 20°C and pH $\simeq$ 2).

The values for instability constants determined by Bjerrum's method amount to $pK_1 = 3.95$; $pK_2 = 3.07$; $p\beta_1 = 3.95$; $p\beta_2 = 7.02$, respectively.

A colorimetric method of the determination of palladium(II) has been elaborated. The method consists in a measurement of the absorbance of the violet complex of palladium(II) with promazine hydrochloride at $\lambda = 540$ nm. The method permits the determination of 2–17 μg Pd/ml with an error of $\pm 2\%$. The time of the determination is 20 min. Iron(III), Ce(IV), Pt(IV), V(V), Cr(VI), and HNO_3 interfere with the determination.

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The Influence of Hydrogen-Bond Association on the Destruction of Hydroperoxides in the Autoxidation Process of Oleyl Alcohol, Oleic Acid, and Methyl Oleate

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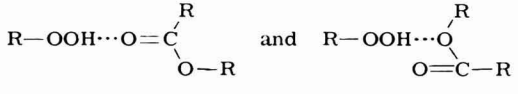
INTRODUCTION

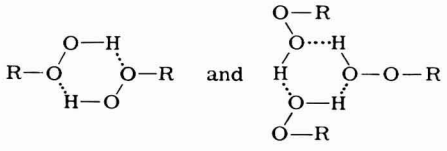
The oxidation of higher aliphatic compounds having a double bond with the molecular oxygen (i. e., autoxidation) plays a significant role in numerous technological processes. According to the present investigations (*1, 3, 4, 7, 9*), dealing with autoxidation of monounsaturated aliphatic and alicyclic compounds, the primary products of this reaction are mainly hydroperoxides.

Lately some attention was paid to the influence of hydrogen bonds on the course of autoxidation. The molecules of organic compounds, having sufficiently "acidic" hydrogen atoms, like alcohols, acids, and hydroperoxides, can associate with one another or with the molecules of the reaction products (*5*). These interactions seem to be seriously responsible for the further development of the discussed process. Some specific features of autoxidation, considered in detail for the selected compounds, were explained with the help of association patterns:



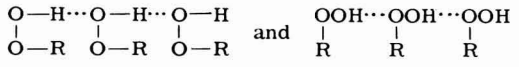
(5)

associates of hydroperoxides with esters



(5)

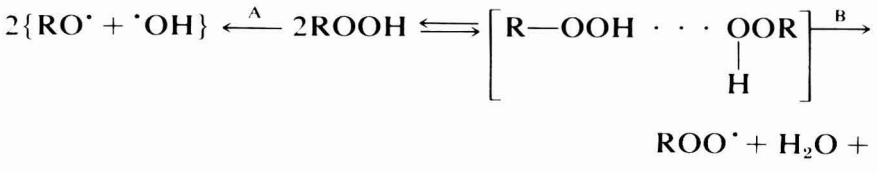
dimeric and trimeric associates of hydroperoxides



(5)

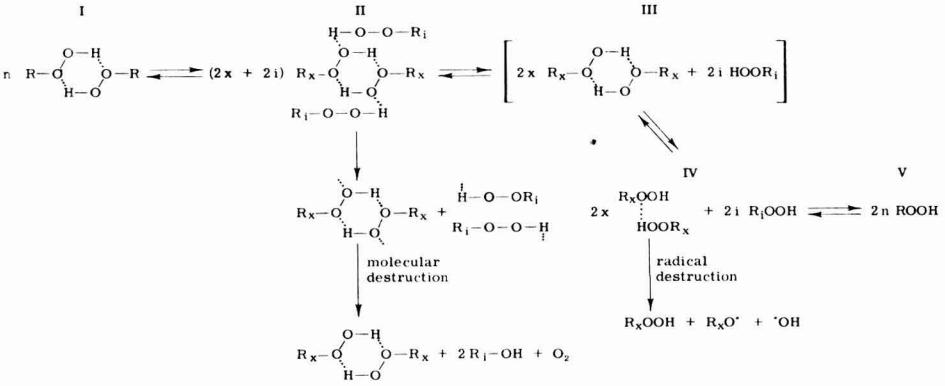
chain associates of hydroperoxides

The investigations of kinetics of the liquid phase autoxidation (9) showed that the destruction of hydroperoxides depends on the reaction conditions and is subject either to the monomolecular mechanism A, or to the bimolecular mechanism B:



$$ROO^{\cdot} + H_2O + {}^{\cdot}OR.$$

This destruction may be interpreted taking into assumption the presence of linear and cyclic associates in the discussed systems (5):



Nevertheless, one found no comparison of the autoxidation process for a number of substances, which differ with the functional groups only, where the observed divergences in the course of the process might be explained with the hydrogen-bond association. Therefore, the purpose of this work was to determine the influence of association on the autoxidation of oleyl alcohol, oleic acid, and methyl oleate (mainly on the yields of peroxidic and hydroperoxidic products of the process). The a/m substances differ with the aptitude of their functional groups to associate and therefore the influence of hydrogen bonds ought to be particularly evident from the very beginning of autoxidation.

EXPERIMENTAL

The substrates to our experiment were: oleyl alcohol ("Fluka," Switzerland), oleic acid (B.D.H., England) and methyl oleate (synthesized by esterification of oleic acid).

The autoxidation of oleyl alcohol, oleic acid, and methyl oleate was conducted at three temperatures: 40, 50, and 60°C for 14 days. The 30-g samples of each compound were placed in Petri scales (diameter of a scale 8.5 cm).

The measurements of peroxide number were performed daily, according to Polish standards (*8*).

In purpose to determine the amounts of unoxidized substances in the investigated samples, the adsorption TL chromatography was applied. These analyses were led in the intervals of a few days, and the procedure was as follows: The chromatographic glassplates (10 × 20 cm) were covered with Kieselgel G (E. Merck, Darmstadt), the thickness of a layer being 0.5 mm; then they were dried at room temperature for 24 hr and at last activated at 110°C for 45 min. The oleyl alcohol, oleic acid, and methyl oleate samples were prepared as 1% benzene solutions and developed in the amounts of 50 μl (0.445 mg). The mobile phase was composed of benzene and methanol in a volume ratio of 9:1 (*10*). The chromatograms were evoked with a 5% solution of ammonium molybdate in concentrated sulfuric acid.

The NMR measurements were performed for the unoxidized samples and those after 14 days of oxidation at 50 and 60°C. The NMR spectra were registered for the 28% (% by wt) carbon tetrachloride solutions of the a/m samples. The carbon tetrachloride used was previously distilled over P_2O_5 and stored over the molecular sieves. The Varian type XL-100 apparatus (Varian, Analytical Instruments Division, USA) was used and the working conditions were as follows: frequency, 100.1 MHz; temp, 20°C; sweep width, 1300 Hz; time, 500 sec; tube o.d., 5 mm; spin rate, 28 rps; filter, 2; spectrum amplification, 16, 20; number of scans, 4.

The results of peroxide number measurements are shown in Fig. 1.

In the case of oleyl alcohol, the higher the temperature of oxidation, the higher the level of the peroxide number obtained. At 60°C the yield of cumulated peroxides was the greatest, at 50°C, smaller, and at 40°C, the smallest. Oleic acid showed the contrary behavior: at the lowest temperature it cumulated the highest amounts of peroxides and vice versa. The oxidation of methyl oleate ran similarly to that of alcohol, but the peroxide number values were, respectively, higher.

Table 1 illustrates the decrease of chromatographic spots' surfaces for the samples of alcohol, acid, and ester after 12 days of oxidation.

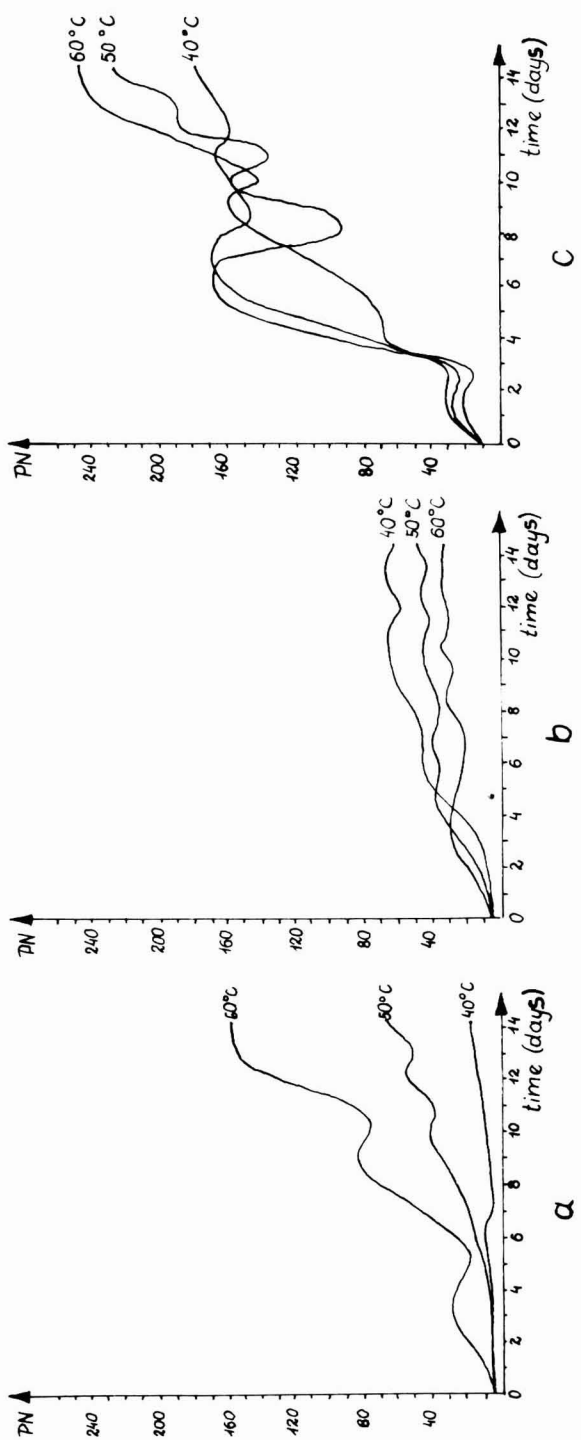


FIG. 1. The peroxide number (PN) values vs time of oxidation at 40, 50, 60°C for: (a) oleyl alcohol: (b) oleic acid: (c) methyl oleate.

TABLE 1
THE DECREASE OF CHROMATOGRAPHIC SPOTS' SURFACES FOR THE SAMPLES OF OLEYL ALCOHOL, OLEIC ACID, AND METHYL OLEATE AFTER 12 DAYS OF OXIDATION AT 40, 50, AND 60°C

Substance	% Decrease at		
	40°C	50°C	60°C
Oleyl alcohol	18	26	40
Oleic acid	22	38	50
Methyl oleate	21	25	29

One stated that autoxidation induced the decrease of chromatographic spots' surfaces. In addition, the higher temperature influenced the quicker decrease of these surfaces than did the lower one. The greatest transformation of the original material was noticed in the case of oleic acid. The decrease of oleyl alcohol was less ef-

TABLE 2
EVALUATION OF THE NMR SPECTRA OF PURE OLEYL ALCOHOL, OLEIC ACID, AND METHYL OLEATE

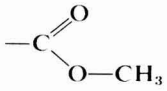
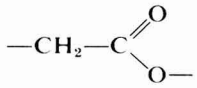
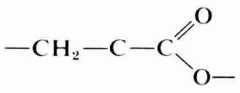
Chemical shift (δ ppm)	Multiplicity	No. of protons	Chemical representation
11.78	Singlet	1	—OH (acid)
3.83	Singlet	1	—OH (alcohol)
3.55	Singlet	3	
~5.28	Triplet	2	—CH=CH—
~1.91	Multiplet	4	—CH_2—C=C—CH_2—
~2.20	Triplet	2	
~1.54	Multiplet	2	
~1.25	Singlet	24 alcohol 20 acid and ester	—CH_2—
~0.85	Triplet	3	—CH_3

TABLE 3
THE CHANGE OF CHEMICAL SHIFT (δ ppm) FOR THE HYDROXYL PROTON IN OLEYL ALCOHOL AND THE ACIDIC PROTON IN OLEIC ACID AFTER 14 DAYS OF OXIDATION AT 50 AND 60°C

Substance	The change of chemical shift (δ ppm) for the —OH proton		
	Ref. sample	Samp. oxid. at 50°C	Samp. oxid. at 60°C
Oleyl alcohol	3.83	4.20	4.27
Oleic acid	11.78	10.93	10.74

ficient, and with methyl oleate the decrease of pure compound in the oxidized samples was the least.

Table 2 shows the characteristics of the NMR spectra of oleyl alcohol, oleic acid, and methyl oleate, and ascribes individual bands to the proper proton groups in the molecules of the examined compounds.

After 14 days of oxidation one noticed the change of chemical shift for the acidic protons in oleic acid and those of hydroxyl group in oleyl alcohol. The numeral data are given in Table 3.

Assuming that all the solutions were of the identical concentration and the working temperature was constant, the above-stated changes of chemical shift for the singlets representing hydroxyl protons of alcohol and acid may be explained as follows: with forming of some new hydroxyl and carboxyl groups in the course of autoxidation; with forming of hydroperoxidic functional groups; with some changes of hydrogen-bond association involving the a/m groups.

TABLE 4
THE CHANGES OF THE RATIOS (a), (b), AND (c) OBSERVED AFTER 14 DAYS OF OXIDATION

Ratio	Oleyl alcohol			Oleic acid			Methyl oleate		
	Unoxid. samp.	Samp. oxid. at (°C) 50	60	Unoxid. samp.	Samp. oxid. at (°C) 50	60	Unoxid. samp.	Samp. oxid. at (°C) 50	60
a. $\frac{-OH}{-CH_3}$	0.33	0.34	0.37	0.31	0.35	0.37	nonexisting		
b. $\frac{-CH=CH-}{-CH_3}$	0.65	0.62	0.58	0.66	0.63	0.61	0.63	0.46	0.45
c. $\frac{-CH_2-C=C-CH_2-}{-CH_3}$	1.33	1.28	1.16	1.19	1.00	0.93	1.34	1.10	1.03

The quick exchange of these protons induces one singlet and its chemical shift is the weighted medium of all the components.

In the NMR spectra of the oxidized samples one noticed the change of absorption band intensities: —OH, —CH=CH— and —CH_2—C=C—CH_2—. In order to objectivate these changes the following integral ratios of energy absorption bands were calculated: —OH/—CH_3 (a); —CH=CH—/—CH_3 (b) and —CH_2—C=C—CH_2—/—CH_3 (c). This assumption was allowed that the integral value of triplet —CH_3, representing the terminal methyl group, is the reference value for the corresponding spectra, respectively. Table 4 shows the calculated values.

The integral ratio value of (a) increases significantly in the samples of alcohol and acid oxidized at higher temperature. It is a proof for the increase of the quantity of functional groups, which include hydroxyl.

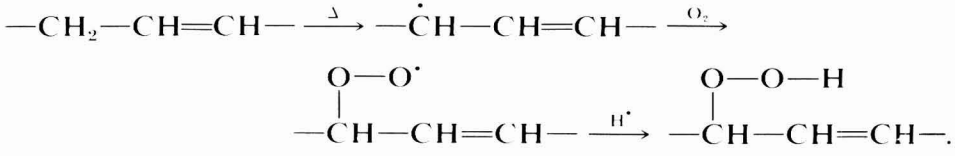
The diminution of the integral ratio value of (b) in the spectra of substances oxidized at both temperatures is effected with the decrease of the quantity of protons connected with these carbon atoms, which participate in double bonds.

The simultaneous diminution of the integral ratio value of (c) is evoked with the lowering number of protons connected with the alpha-carbon atoms, neighboring to those of double bonds.

DISCUSSION

The purpose of our work is to explain the differentiated course of autoxidation of alcohol, acid, and ester with the help of hydrogen-bond association, but first we must enumerate some preliminary assumptions.

According to the widely accepted scheme of autoxidation, we assume that this process starts at the alpha-carbon atoms, neighboring to those of double bonds, and tends to hydroperoxides with peroxidic radicals as intermediates.



This mechanism helps us to concentrate our attention on the factors leading to different levels of peroxidic products of autoxidation found in the alcohol, acid, and ester samples.

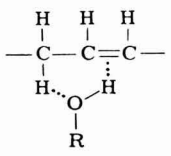
Besides we state that, from the point of view of capability to give hydrogen bonds, our substances may be ranged as follows:

$$\text{ester} < \text{alcohol} < \text{acid}.$$

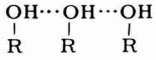
Last, we have to discuss the types of hydrogen bonds which probably exist in the unoxidized samples of our compounds.

Unoxidized methyl oleate lacks the sufficiently "acidic" hydrogen atom and, therefore, its aptitude to give hydrogen bonds is practically negligible.

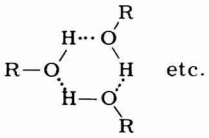
Unoxidized oleyl alcohol may give three types of hydrogen bonds: engaging π electrons of the double bond (a), chain associates (b), and cyclic associates with the different number of coordinated molecules (c):



(a)



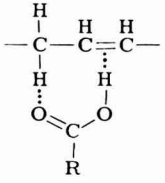
(b)



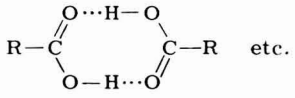
(c)

Type (a) represents the lowest level of hydrogen-bond energy, and type (c) the highest one (*6*).

Unoxidized oleic acid gives more frequently cyclic bonds. These bonds may occur: (a) involving π electrons of the double bonds, and (b) between the functional groups of acid (the higher bonding energy).



(a)



(b)

The rise in temperature (40, 50, and 60°C) causes the following changes: (a) acceleration of cumulating the peroxidic products of autoxidation; (b) acceleration of some other processes, which follow the autoxidation (the secondary reaction of oxypolymerization or oxidative splitting of carbon chain).

Another consequence is the change of association states in the discussed substances, which influences the whole course of autoxidation, as we shall try to show in the further part of these considerations. According to the physical meaning of the Van't Hoff's isobar formula, if we suppose that in the oxidized samples hydrogen bonds of different energies exist, those with the greater energy are more sensitive

to the temperature change and are broken more easily by the temperature rise.

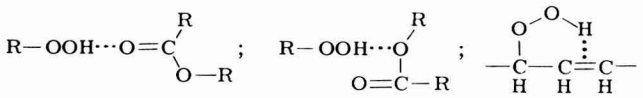
Now we must answer the question of why alcohol and ester oxidized with the comparatively high yields of peroxides, in contrast to acid with its low amounts of these products. Too, we must explain why the rise in temperature induces the higher yields of peroxides in the case of alcohol and ester, and the lower ones in the case of acid.

As mentioned before, ester, alcohol, and acid may be arranged in this way in conformity with their aptitude to form hydrogen bonds. The analogous arrangement is in regard to their efficiency in giving peroxidic products of autoxidation. In the case of ester these yields were the highest at all the temperatures, with alcohol and with acid lower and lowest, respectively. The conclusion is that the higher the energy of hydrogen bonds in the examined compounds, the lower are the yields of peroxidic products of autoxidation.

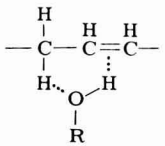
The NMR inquiries give some additional confirmation of the peroxide number measurements, as the lowering number of protons connected with the alpha-carbon atoms, neighboring to those of double bonds may be a proof for cumulation of the peroxidic products. In this way the changes of integral ratio value of $—CH_2—CH=CH—CH_2—/—CH_3$ are consistent with the peroxide number characteristics.

Assuming that with our compounds, the process of oxidation starts at the α-methylene groups, adjacent to double bonds, it seems obvious that the hydrogen-π-bond association plays the important role in the differentiation of the oxidation products. Now it ought to be mentioned, that at each temperature some equilibrium exists between the hydrogen-bond association through functional groups and this of the hydrogen atom-π-electrons type.

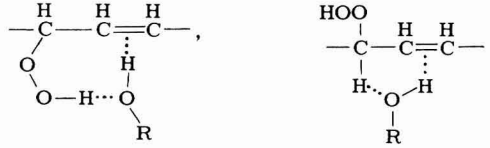
In the case of ester the hydroperoxidic products of oxidation may associate not only with the ester functional groups, but are capable to give intramolecular hydrogen bonds with the π-electrons:



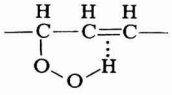
With alcohol the situation is more complex. Even in the unoxidized samples the possibility to associate through the π-electrons occurs:



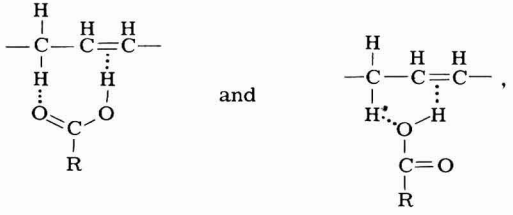
The above cyclic system induces the growth of reactivity of the second hydrogen atom, taking no part in the hydrogen bond. The further consequence is the appearance of the cyclic intermolecular systems engaging the newly formed hydroperoxides:



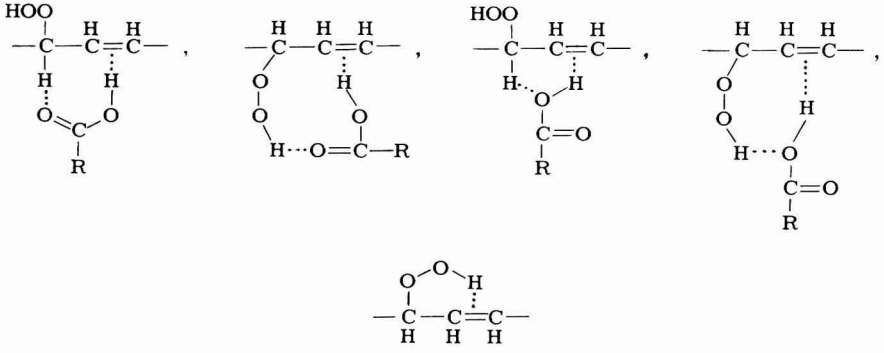
The hydrogen bonds extort some delocalization of the electron density in the range of presented systems, which causes their instability and enables their splitting accompanied with the destruction of hydroperoxide groups, or even of the whole carbon chains. Good reasons exist for the intramolecular bonds as well:



With pure oleic acid one expects the following structures:



which activate the α-hydrogen atoms. Then the occurrence of the ensuing formations is probable:



These structures seem not to be durable and one can expect their destruction with the simultaneous decay of hydroperoxides.

From the above-detailed deduction results, that both the hydrogen bond energies and the statistical reasons explain the strongest influence of association on the products of oxidation of oleic acid and the weakest one on those of methyl oleate. Considering the association in the range of π-bonds and newly formed hydroperoxidic groups, the condition specially promoting decay of hydroperoxides, one can find the explanation of the proportionally highest yields of these compounds in the case of ester and the lowest ones with acid.

Besides the distinct preponderance of the bonding energy and the probability of numerous combinations in the case of acidic associates seems to explain the phenomenon of the highest amounts of peroxidic products detected at the lowest temperature, and the lowest amounts at the highest one. The assumption that the rise of temperature accelerates the decay of the oxidation products, as well as their appearance, is fully grounded. Obviously, with oleic acid, the rise of temperature accelerates the decay of peroxidic products more efficiently than their cumulation.

SUMMARY

The autoxidation process of oleyl alcohol, oleic acid, and methyl oleate at 40, 50, and 60°C was considered with the special stress laid upon the mechanism of cumulating and destruction of peroxidic products. Interpretation of the observed differences was based on the hydrogen-bond association patterns.

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Uramyldiacetic Acid (UDA) As Absorptiometric Agent[1]

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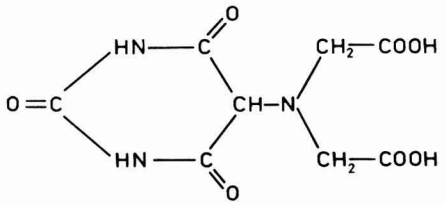
INTRODUCTION

Between the various derivatives of the iminodiacetic acid is uramyldiacetic acid:



UDA

also named 5-aminobarbituric-7, 7-diacetic acid (*2*), obtained from aminobarbituric acids. The reaction mixtures were often strongly red–violet colored owing to the formation of small amounts of murexide as a consequence of the oxidation by the air (*12*).

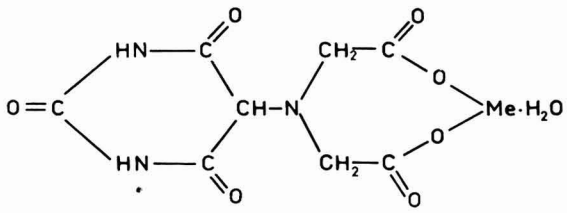
In 1946 Schwarzenbach *et al.* (*12*) noticed that UDA has the property of forming, even with sodium ions, water-soluble stable complexes. On the contrary, potassium is not complexed in great extent, while the lithium complex is more stable than the corresponding sodium complex. Of the chelons with only one nitrogen as a donor atom, the uramyldiacetic acid forms the strongest alkaline earth complexes, after the EDTA, however, which contains two nitrogen as donor atoms.

The same authors also calculated the value of the acidity constants by titration of the UDA with tetramethylammonium hydroxide. It was found that the two protons leave the molecule nearly at the same time, the second acidity constant being 8-fold greater than the first.

[1] Part LXXI in the series Analytical Applications of the Chelons.

The release of the first proton weakens the bonding of the second, so that the latter abandons the molecule at the same time as the former; the process is also affected by the fact that the I^- ion (formed as a consequence of the release of the first proton) suffers a change in its constitution. The remainder of the barbituric acid of the free UDA will be then in the diketo form without any proton upon the nitrogen.

To explain the constitution of the UDA chelates and its behavior as complexing agent, it is believed that only the iminodiacetate group of the molecule belongs to the coordination sphere of the metal,



while the ionogen enolate group of the barbituric radical works only as a strong electrostatic stabilizer because of the steric proximity. This conclusion was obtained on the basis of the behavior of 7-methyl uramyl 7, 7-dimethyluramyl, dibarbiturilamine, and uramil-7-mono acetic acid. All of them demonstrate no special affinity of the barbituric acid portion to react with the metals.

Hirving and Da Silva (*4*) found that the UDA forms a stable salt, U_3Ur, NaH_2Ur-H_2O being their acidity dissociation constants determined at various temperatures. The stability constants of the formed chelates between the Ur^{3-}anion and univalent cations were measured by potentiometric titration at 20, 27, 34, and 39°C, using tetramethylammonium hydroxide as titrant and tetramethylammonium nitrate to give anionic strength ($\mu = 0.1\ M$). The stability of the 1 : 1-formed complexes increases in the way K^+ Na^+ Li^+ Tl^+ H^+.

The metal chelates are stabilized by favorable changes (negative) which increase with the ionic radii, the entropy changes being negative and notable in the case of potassium and thallium.

The validity of this procedure for the precipitation of lead as its iodide after the masking of thallium (I) with EDTA was examined; the use of the UDA does not present any advantage. However, the EDTA does form with the majority of the divalent cations stronger complexes than the uramyldiacetic acid, but the situation is the other way around when dealing with the alkaline metals and beryllium whose chelate is quite stable ($\log K_{BeUr} = 10.36$).

The same authors (*5*) described the synthesis of the 1-methyl and 1, 3-dimethyl uramyl-*N*, *N*-diacetic acid; they calculated the acid dissociation constants of these compounds as well as the values of the stability constants at 20°C and $\mu = 0.1$ (tetramethyl ammonium nitrate) of the chelates formed with Li, Na, K, Tl, Be, Mg, Ca, Sr, and Ba. The results were compared with those obtained when using the uramyl diacetic acid and various poliaminecarboxilic acids.

Stein *et al.* (*15*) prepared from malonic esters and urea, several 1-alkyl-uramyl-7, 7-diacetic acids including the 1-octil-uramyl acid.

According to Buser (*1*) of the alkaline ions, only lithium and sodium form stable chelates with uramyldiacetic acid. He plotted the extension of the formation of the lithium and sodium chelate versus the pH. It is possible to achieve the separation of lithium and sodium from potassium by ion exchange at pH 9, and lithium from sodium at pH 6.5–7.5, the 90% being the lithium chelate and the 10% the sodium chelate.

Schwarzenbach (*10*) determined various metals quantitatively using the complexes between the 7, 7-uramyldiacetic acid, nitrilotriacetic acid, ethylendinitrilotetraacetic acid, and the alkaline earth, lithium, aluminium, rare earths, zinc, cadmium, mercury, lead, copper, iron, cobalt, nickel, and manganese. He also determined the water hardness by adding an amount of aminopolycarboxilic acid sufficient to complex the cadmium titration of a standard alkaline solution to the initial pH of the water (*13*).

Schwarzenbach, Kampitsch, and Steiner (*12*) studied the formation of the metallic complexes between the uramyldiacetic acid and several divalent ions. This chelating agent forms stable chelates of the type $(CaX)H$ or $(NaX)H_2$ with sodium, lithium, barium, strontium, calcium, and magnesium, but not with potassium. By titration with tetramethylammonium hydroxide in the presence of several metals the values of $pK_1 = 3.26$; $pK_2 = 2.86$, and $pK_3 = 10.44$ were obtained, the determination of thermodynamic stability of the complexes being possible. All the salts of the chelates are very water soluble but $(CaC_8\text{-}H_6O_7N_3)K$ might be insoluble by precipitation of ethanol. Similar salts of sodium do not solidify.

In 1948 Schwarzenbach and Biedermann (*11*) titrated metals with uramyldiacetic acid, ZH_3. The formation of a ZH^{2-} or Z^{3-} complex, freend H^+ ions, can be determined by alkaline titration. In this case the best results are obtained when the equivalent is 1, although for lanthanum and cerium it must be 2. The corresponding pH changes in the titration graph are the following, the ligand been as ZH^{2-} and Z^{3-}, respectively:

Metal	pH changes
Cu	5.1–6.1; 6.1
Ce	4.7–7.8; 6.5–8.5
Ca(a = 1 or 2)	4.7–9.0; 7.5–9.2
Co	4.8–8.1; 5.5–8.5
La	5.0–8.0; 6.5–8.5
Mg	5.3–8.1; 6.5–8.5
Mn	4.6–7.1; 6.5–8.5
Ni	4.6–8.0; 6.0–8.5
Zn	4.6–8.0; 6.0–8.5
Al(a = 1 or 2)	4.5–5.2–7.2; 4.5 and 6.5
(a = equivalent)	

Musil and Reimers (*7*) prepared from uramyl, $ClCH_2COOH$, and KOH the uramyldiacetic acid, accomplishing potentiometrically its titration with Et_4NOH in the presence of various amounts of magnesium, calcium, strontium, barium, lithium, and sodium the results being interpolated. In this way it is possible to extend the method to chelates with stability constants of 10^5. Two uramyldiacetic acid molecules react with one alkaline earth ion establishing the corresponding formula for the formed complex. The metallic ions of the alkaline earth group with higher atomic weight give weaker complexes. This question being discussed attending to the hydrated ionic radium. The uramyldiacetic acid can be used for simple determinations after pH changes. Thus, a procedure for the determination of magnesium and lithium is suggested.

Ryabchikov and Belyaeva (*8*) studied the reactions of $ThCl_4$ with sodium nitrilotriacetate (Na_2HX), sodium uramyldiacetate (Na_2HUr), and tetrasodium ethylendiamintetraacetate, cyclobutanediaminetetraacetate, cyclopentanediaminetetraacetate, and cyclohexanediaminetetraacetate, respectively, verifying on each case two determinations, one of then containing $ThCl_4$ and aminopolicarboxilate in a molar ratio of 1:1, 1:2, and 2:1. The whole system being titrated with NaOH. The other based on the titration of series of $ThCl_4$ solutions with aminopolicarboxilate solutions. In the first situation the minima conductivities when all the H^+ was neutralized by the OH^- were taken and in the second situation the maxima conductivities when all the H^+ was liberated as a consequence of the reaction was taken. The results showed that thorium reacts in a molar ratio of 1:2 at pH 8,2 with nitrilotriacetate and uramildiacetate to give $(NH_4)_2(ThX_2)\cdot 4\ H_2O$ and $(NH_4)_2(ThUr_2)\cdot 4\ H_2O$, respectively; with the other compounds, it reacts in a 1:1 molar ratio.

Heat and Clark (*3*) found that the following chelating agents increased the growth of the coleoptil sections wheat: 3-indolacetic acid (I), ethylenediaminetetraacetate (II), uramyldiacetic acid, antranilic acid, diacetic acid, nitrilotriacetic acid, iminodiacetic acid, 8-hydroxiquinolein, and sodium diethyldithiocarbamate. Only I or II inhibited the plant root growth. Noting a mutual antagonism between I and II upon the root growth, it was proposed that I acts upon the growth in the same way a chelating agent does, both forming the chelate compounds of complexes.

Laskorin *et al.* (*6*) summarized data and graphs for the chromatographic separations of barium and radium, aluminium and gallium, and zirconium and hafnium, using several ion-exchange resins with various chelating agents. The logarithm of the formation constants (log K) of the calcium, strontium, and barium complexes with numerous acids are the following:

Acid	log K
Citric	—, —, 2.54
Malonic	—, —, 1.36
Tartaric	1.80, 1.65, 1.62
Acetic	1.00, 0.97, 0.93
Malic	2.66, —, 2.19
Poliphosphoric	3.00, 2.80, 3.00
Iminodiacetic	3.41, 3.40, 1.67
Uramyl-N,N'-diacetic	8.77, 7.60, 6.80
Ethylenediaminetetraacetic	10.59, 8.63, 7.76
Propylenediaminetetraacetic	7.12, 5.18, 4.24
Nitrilotriacetic	6.41, 4.94, 4.82
1-2-Diaminecyclohexanetetraacetic	12.5, —, —

The log K values for the radium complexes with citric, malonic, and tartaric acids are 2.34, 0.95, and 1.24, respectively. Radium and barium are best separated by passing the solution (Ba 20 g/liter) and ethylenediaminetetraacetic acid (40 g/liter) at pH 6.5 through a series of columns filled with the cationic resin KU-2. Hafnium is best separated from zirconium when present in a solution with zirconium (20–30 g/liter), H_2SO_4 (0.65–0.75 F), and hafnium (0.7–1.0 ml/mole Zr) by passing the solution through the resin KU-2. Aluminium and gallium must be separated in a solution 3.7 F in HCl by passing the solution through an anionic resin AN-2F.

Semenov and Tregubenko (*14*) found the chelating agents to be suitable to eliminate various radioactive elements from animal tissues

owing to its capability to give soluble and stable compounds. In the way ytrium, cerium, and plutonium were eliminated as complexes by the urinary route. A comparative study based on the efficiency of the different chelating agents belonging to the aminocarboxyl and phosphate group indicated the existence of notorious numerous and quantitative differences among them. They demonstrated the efficiency of sodium uramyldiacetate and sodium ethylendiaminetetraacetate in the elimination of ytrium, while the hexametaphosphate was successful in the elimination of cerium and plutonium. Qualitative differences were found owing mainly to the fact that phosphates increase the accumulation of ytrium and plutonium in the soft parts of the organism but on the contrary as the aminocarboxylic chelating agents do, they reduce the accumulation of cerium in the skeleton. Thus, indicating the important role that the formation of the complexes plays together with their stability on the effectiveness of the chelating agents as well as the physicochemical situation of the metal in the blood and interstitial fluids. The reported results and the drawn conclusions show the possible role played by the natural biocomplex ones, such as amino acids, citric acid, phosphates, etc., in the mineral metabolism of the organism. Owing to its high toxicity, the good efficiency of the hexametaphosphates in connection with the cerium and plutonium elimination cannot be clinically exploited. Uramyldiacetic acid has been shown to be more effective than the EDTA usually employed in the heavy metal and poisonous radioiosotopes therapy.

Schwarzenbach (*9*) found that some aminopolycarboxylic acids as nitrilotriacetic acid, uramyldiacetic acid, and EDTA give stable complexes with alkaline earth cations, lithium, sodium, and various other ions; the formation of such complexes may be used for their determination either by titration at a fixed pH in the presence of a given indicator or in the presence of an indicator which gives a colored compound with the cation to be evaluated. Procedures for lithium, magnesium, calcium, strontium, barium, zinc, mercury, cadmium, copper, iron, nickel, manganese, and cerium has been given.

EXPERIMENTAL

In order to use the uramyldiacetic acid in the spectrophotometric determinations of colored ions, its behavior as chromogenic agent toward aqueous solutions of several metallic ions at various pH values was studied, taking notice of the color changes or enhancements.

The tests were carried out in Pyrex test tubes 15 × 1.5 cm. working at acidic, neutral, and alkaline pH.

To carry out the experiments the following procedure was used: 3 ml of the given solution were treated with 1 ml of 5% uramyldiacetic

acid solution, the necessary drops of 1 *F* HCl or NaOH solution to reach the wanted pH value and diluting with distilled water to final volume of 10 ml.

The following cations were investigated: copper (II), uranyl (VI), iron (III), iron (II), cobalt (II), vanadium (V), cerium (IV), chromium (III), nickel (II), manganese (II), lead (II), aluminium (III), mercury (II), and palladium (II). In all solutions the concentration in the respective cation being 0.01 *F*. The results are shown in Tables 1, 2, and 3.

The above results show that the uramyldiacetic acid reacts with several cations producing changes or enhancements of color thus indicating its potential usefulness as chromogenic reagent. Its reaction with Cu(II), UO_2(II), Fe(II), Fe(III), Co(II), Ce(IV), and Ni(II) are clear examples.

The chromogenic capability of the UDA is stronger in acid or neutral than in alkaline media, the enhancement of the original color of the cation solutions taking place in some cases (uranyl, cobalt, nickel); change of color in some others (iron, copper).

TABLE 1
UDA EFFECT UPON VARIOUS CATIONS SOLUTIONS[a]

Ion	Color		Notes
	Before	After[a]	
Cu(II)	Blue	Green	
UO_2(II)	Yellow	Strong yellow	
Fe(II)	Yellow	Brown–orange	
Fe(III)	Yellow	Brown–reddish	
Co(II)	Pink	Strong pink	
V(V)	Yellow	Blue	[b]
Ce(IV)	Yellow	Deep pink	[c]
Ce(III)	Colorless	Weak pink	
Cr(III)	Green	Violet–gray	
Ni(II)	Green	Increased green	
Mn(II)	Colorless	Weak pink	
Pb(II)	Colorless	White precipitate	
Al(III)	Colorless	Colorless	
Hg(II)	Colorless	Gray precipitate	[d]
Pd(II)	Brown	Brown	

[a] Concentration of metal 0.01 *F*; pH = 3.

[b] After the addition of UDA a green color appeared that soon changed to blue.

[c] At the beginning a turbidity appears, after which it changes to a brown color and at the end to a deep pink.

[d] A white precipitate is formed after the first treatment of the solution with UDA disappearing; when more UDA is added a pinkish clear solution is left.

TABLE 2
UDA EFFECT UPON VARIOUS CATIONS SOLUTIONS[a]

Ion	Color		Notes
	Before	After	
Cu(II)	Blue	Green	
UO_2(II)	Yellow	Deep yellow	
Fe(II)	Yellow	Brown–orange	
Fe(III)	Yellow	Brown–reddish	
Co(II)	Pink	Pink–violet	
V(V)	Yellow	Red–violet	
Ce(IV)	Yellow	Deep pink	
Ce(III)	Colorless	Weak pink	[b]
Cr(III)	Green	Turbidity	
Ni(II)	Green	Increased green	
Mn(II)	Colorless	Weak pink	[b]
Pb(II)	Colorless	White precipitate	
Al(III)	Colorless	White precipitate	
Hg(II)	Colorless	Gray precipitate	
Pd(II)	Brown	Brown–yellowish	

[a] Concentration of metal 0.01 *F*; pH = 7.
[b] All the weak-pink colors of the colorless solutions of cations after the addition of UDA come from the UDA solution because of its partial decomposition.

TABLE 3
UDA EFFECT UPON VARIOUS CATIONS SOLUTIONS[a]

Ion	Color		Notes
	Before	After	
Cu(II)	Blue	Green	
UO_2(II)	Yellow	Turbid yellow	
Fe(II)	Yellow	Weak brown	
Fe(III)	Yellow	Weak brown	
Co(II)	Pink	Pink–violet	
V(V)	Yellow	Violet	
Ce(IV)	Yellow	Deep pink	
Ce(III)	Colorless	Weak pink	[b]
Cr(III)	Green	Green–grayish	
Ni(II)	Green	Increased green	
Mn(II)	Colorless	Weak pink	[b]
Pb(II)	Colorless	White precipitate	
Al(III)	Colorless	White-precipitate	
Hg(II)	Colorless	Gray precipitate	
Pd(II)	Brown	Brown–yellowish	

[a] Concentration of metal 0.01 *F*; pH = 10.
[b] All the weak-pink colors of the colorless solutions of cations after the treatment with UDA come from the UDA solution because of its partial decomposition.

On adding UDA to the colorless solutions of some cations a weak-pink color owing to the own color of the UDA solution is developed.

The copper (II) solution suffers a color change from blue to green, this color being enhanced in alkaline medium.

The uranium (VI) solution originally yellow colored is increased in its color in neutral medium which weakens in alkaline medium and appears to have some turbidity.

The nickel solution green color increases slightly after treatment with UDA remaining unchanged in the pH intervals investigated.

The color of the complexes formed between UDA and copper(II), uranyl(II) and nickel(II) does change with time; however, it does not change when UDA reacts with other cations, e.g., cerium(IV) and vanadium(V).

UDA ABSORPTION SPECTRUM

First, a 5% aqueous solution of uramyldiacetic acid was prepared, the treatment with sodium hydroxide to dissolve it being necessary.

The uramyldiacetic acid when in contact with the air and light, suffers some decomposition giving a murexide derivative. That is the reason why its solutions in alkaline medium are slightly violet. In any case the alkaline solutions of the UDA are decomposed with time, with the corresponding variation of the titer, the color of the murexide affecting the changes of the indicators. Thus, to diminish this effect it is necessary to dissolve the compound in the minimum possible amount of sodium hydroxide in order to decrease the amount of color formed increasing then its stability.

The UDA absorption spectrum measurements were made in a Beckman model DU spectrophotometer using 10-mm light path quartz cells and distilled water as a blank. In Fig. 1 are shown the results obtained, a maximum at 520 nm being noticeable.

As the UDA gives owing to its oxidation small amounts of murexide, the spectra of the UDA solutions must show the superposition of the spectra of both compounds. In this way the murexide spectrum was run as shown in the same Fig. 1.

The murexide spectrum presents three maxima at 247, 325, and 520 nm, respectively, while the UDA spectrum has a maximum only at 520 nm.

Althought it seems as if both spectra do not coincide it is important to take into account that UDA uv spectrum masks the 247- and 325-nm murexide maximum, so only the 520-nm maximum of the latter will appear.

The UDA and murexide solutions were at pH 7 and the spectra were run in the interval 220–700 nm.

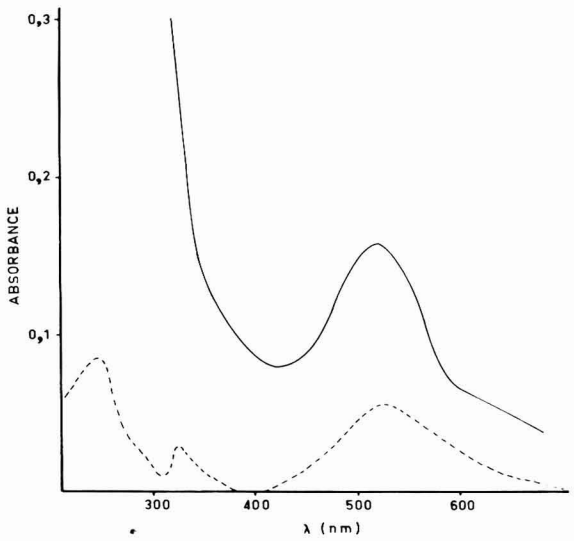


FIGURE 1.

Because of the strong absorbance of the UDA solutions in the uv range, the potential usefulness of this reagent for the spectrophotometric determination of colorless cations was considered.

Several experiments were carried out with lithium, potassium, magnesium, calcium, and cadmium solutions, the last one showing a considerable increase in the values of the absorbance in the uv range when treated with UDA solution.

SUMMARY

The analytical behavior of uramyldiacetic acid as potential chromogenic reagent for the spectrophotometric determination of trace materials is studied. As a result, interesting reactions with copper (II), uranium (VI), iron (II), iron (III), cobalt (II), cerium (II), and nickel (II) in neutral or acidic medium were observed. The absorption spectrum of UDA is established.

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Tris(2,2′-bipyridyl)iron(II)Tetraphenylborate as a Solid Analytical Reagent in the Spectrophotometric Determination of Ag(I), Tl(I), and Hg(II)

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INTRODUCTION

The solid analytical reagents or the englomerate salts as these have been termed earlier, are now being recognized as potent analytical reagents in the microanalysis of inorganic and organic constituents (*11*). These compounds result from the stoichiometric interactions of the low-charged (usually monovalent) bulky cations and anions of nearly equal dimensions. The predominant feature of such compounds is their unusually high aqueous stability and in many a case selective reactions with constituents of interest. Some recent applications of such reagents have been described in the earlier work of the authors and of others (*1, 3–6, 9, 10, 12, 14*). In the present study, are described the analytical uses of a new solid reagent prepared by the interaction of tris(2,2′-bipyridyl)iron(II) sulfate and sodium tetraphenylborate. The reagent has a high degree of aqueous stability. It reacts selectively with Ag(I), Tl(I), and Hg(II) cations to release the colored tris(2,2′-bipyridyl)iron(II) cation in solution. Based on these observations, a spectrophotometric method for the analysis of the three cations in microgram range is suggested.

MATERIALS AND METHODS

All the reagents used in this study were of analytical grade purity or better. Deionized distilled water was employed in the preparation of the stock and the standard solutions.

The Spectronic-70 Spectrophotometer of Bausch and Lomb Company having the flexibility of rectangular or cylindrical cell arrangement was employed in the absorption measurements.

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Preparation of Solid Reagent

The solid reagent was prepared by mixing equal volumes of the reagents tris(2,2′-bipyridyl)iron(II) sulfate (0.01 *M*) and sodium tetraphenylborate (0.02 *M*). The solutions were mixed very slowly in a near-neutral condition with a constant stirring on a magnetic stirrer. The resulting precipitate was allowed to remain in contact with the supernatant phase preferably overnight (12 hr). The aged tris(2,2′-bipyridyl)iron(II) tetraphenylborate reagent was filtered through Whatmann-42 and washed with a liberal supply of deionized distilled water. It was finally washed for a few times with anhydrous alcohol, vacuum dried at 60°C, and carefully stored in a dry atmosphere until further use.

Experimental Procedure

In the preliminary studies by batch equilibrium technique, it was established that this reagent has remarkable stability against aqueous decomposition. However, the solid combination selectively breaks up in the presence of Ag(I), Tl(I), and Hg(II) cations. In an interaction with each of the cations, the supernatant phase acquired a reddish color, which showed an absorption peak at 522 nm corresponding to the peak of absorption of the tris(2,2′-bipyridyl)iron(II) cation. In addition, the color intensity increased progressively with increasing concentration of a cation under study. Quantitative runs were made with 150 mg of the solid reagent to check for the linearity and the time of contact necessary to attain full color density. In spite of the necessary experimental precautions, the results were somewhat dispersed for a set of experiments at a specific concentration. Furthermore, more than 2 hr were required to complete the experiment.

The dynamic column operation technique was tried in turn to reduce the time of analysis and to increase the precision of the experimental results. Several modes were tried and finally satisfactory results were obtained with a mixture of the solid reagent and the asbestos (as an inert support). In the final analysis, an operating column of the solid reagent (1 g) mixed with asbestos (2 g) was set up in a glass tube of 3-cm diameter. The reagent mixture was held in place by a plug of glass wool in the constriction in the glass tube.

The prepared column was washed with deionized water till a constant blank value was obtained with a flow rate of 3 ml/min. The stock solutions of Ag(I), Tl(I), and Hg(II) in nitrate forms (0.01 *M*), were appropriately diluted to the required concentrations ranging from 0–80 ppm and a 50-ml volume of each was passed over the column at the same speed as the blank. The first 20–25 ml of the column effluents were discarded and afterward two 10-ml portions

were collected for the spectrophotometric analysis. In each case, the absorbance was measured at 522 nm. In addition to this, the effects of acidity and divers ions were also measured to establish the conditions necessary for a spectrophotometric procedure for these cations.

RESULTS AND DISCUSSION

This solid reagent, as mentioned earlier, has a certain degree of selectivity and sensitivity toward Ag(I), Tl(II), and Hg(II) cations. The analytical data on the three cations are shown in Tables 1, 2, and 3, respectively. Figure 1 shows that in each case, absorbance varies linearly in 5–50-ppm concentration range of the cations in near neutral conditions. In the case of Ag^+, the detection limit of 1 ppm was conveniently achieved while in the other two cases, it was 5 ppm.

The selectivity is shown by the fact that cations K^+, Rb^+, Cs^+, NH_4^+, Na^+, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, cause no change in the blank values even at a 500-ppm concentration. Likewise, the anions, NO_3^-, Cl^-, and SO_4^{2-} have no adverse effects. However, the anion Cl^- is intolerable in the medium on account of its direct reaction with Ag^+, Tl^+, and Hg^{2+} cations; hence, all anions having direct interaction with these cations would show some negative interferences.

The system can tolerate minor changes in the acidity or alkalinity. Increased acidity decomposes the tetraphenylborate anion, while the increased alkalinity tends to decolorize the reactive cation of the solid reagent. Nevertheless, the reagent functions satisfactorily in the pH range 3–10.

The analytical response of the reagent may be thought to result from ion displacement mechanism in the case of Ag(I) and Tl(I), since

TABLE 1
EXPERIMENTAL RESULTS FOR CALIBRATION CURVE FOR Ag^+ IN NEUTRAL CONDITION

Sample no.	Concn Ag^+ (ppm)	Number of determinations	Net average absorbance	Range	Standard deviation
1	0	6	.003	.001–.005	.002
2	1	5	.020	.019–.023	.001
3	2	6	.057	.053–.060	.003
4	5	6	.155	.152–.160	.003
5	10	6	.336	.332–.339	.003
6	20	6	.682	.670–.691	.009
7	25	6	.836	.829–.841	.004
8	30	6	1.038	1.037–1.043	.007
9	40	6	1.353	1.307–1.384	.013

TABLE 2
EXPERIMENTAL RESULTS FOR CALIBRATION CURVE FOR Tl^+ IN NEUTRAL CONDITION

Sample no.	Concn Tl^+ (ppm)	Number of determinations	Net average absorbance	Range	Standard deviation
1	0	6	.002	.001–.004	.002
2	5	6	.065	.062–.067	.001
3	10	6	.139	.134–.141	.003
4	20	6	.253	.250–.258	.003
5	25	6	.326	.324–.329	.002
6	40	5	.429	.418–.430	.002
7	50	6	.596	.592–.602	.004
8	80	6	.798	.794–.808	.005

TABLE 3
EXPERIMENTAL RESULTS FOR CALIBRATION CURVE FOR Hg^{2+} IN NEUTRAL CONDITION

Sample no.	Concn Hg^{2+} (ppm)	Number of determinations	Net average absorbance	Range	Standard deviation
1	0	6	.009	.005–.013	.006
2	5	6	.057	.052–.063	.005
3	10	6	.122	.115–.129	.006
4	25	6	.333	.324–.348	.009
5	30	6	.360	.348–.369	.008
6	40	5	.440	.434–.446	.005
7	50	6	.532	.518–.544	.010
8	80	5	.649	.631–.663	.015

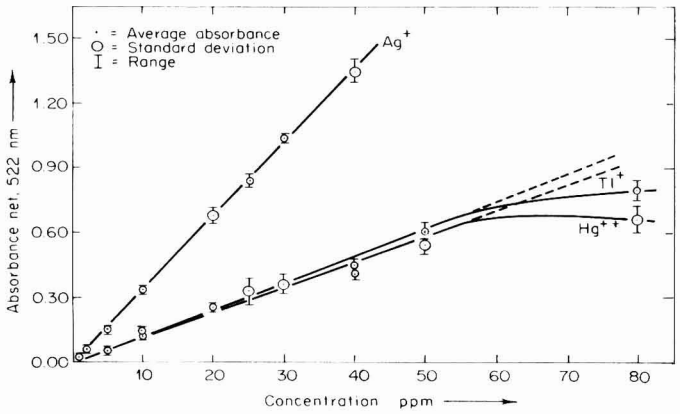

FIG. 1. Spectrophotometric response of the solid reagent as a function of Ag(I), Tl(I), and Hg(II) concentrations.

both of these form fairly insoluble tetraphenylborates (*8*, *13*). This may not be the case for Hg(II) reaction, since earlier reports indicate that mercury preferentially forms phenyl mercury with tetraphenylboron (*2*).

$$[(BPF)^{2+}(TPB^{-})_2]_{(s)} + 2M^{+}_{(aq)} \longrightarrow M(TPB)^{+}_{(s)} + (BPF)^{2+}_{(aq)}$$

$M^+ = Ag^+$ or Tl^+
BPF^{2+} = Reactive colored cation tris(2,2′-bipyridyl)iron(II)
TPB^- = tetraphenylborate anion

$$[(BPF)^{2+}(TPB)_2{}^{-}]_{(s)} + Hg^{2+} \longrightarrow \text{Phenyl mercury or } Hg(TPB)_2 + BPF^{2+}_{(aq)}$$

In any event, the colored reactive cation of the solid reagent is still released for the spectrophotometric determinations. This argument is substantiated by the fact that there is no shift in the absorption peak of the reactive ion (λ max 522 nm) in any case and hence the solid reagent essentially acts as an exchange site. The heavier alkali cations that also form insoluble tetraphenylborates do not interfere. Evidently their attraction for the tetraphenylborate ion is weaker than that of the reactive ion in the solid reagent. In other words, the solubility products of the alkali tetraphenylborates are higher than that of the solid reagent. This evidently is not the case for Ag(I) and Tl(I) tetraphenylborates which obviously are much more insoluble, such that Ag^+ and Tl^+ even in microgram range can combine with the anion part of the solid reagent.

The individual analytical applications of the tris(2,2′-dipyridyl) iron(II) cation and the tetraphenylborate anion are well-known in spectrophotometric, gravimetric, and titrimetric determinations (7). This study offers some new analytical uses of these two ions when locked together in a solid combination.

ACKNOWLEDGMENT

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SUMMARY

Analytical applications of a new solid reagent tris(2,2′-dipyridyl)iron(II) tetraphenylborate are described. The solid reagent selectively reacts with Ag(I), Tl(I), and Hg(II) cations to release the colored tris(2,2′-bipyridyl)iron(II) cation in solution, which is determined spectrophotometrically. The experimental data show that the Ag(I), Tl(I), and Hg(II) cations respond linearly in 5 to 50-ppm range.

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Rapid Determination of Trace Amounts of Selenium (IV), Nitrite, and Nitrate by High-Pressure Liquid Chromatography Using 2,3-Diaminonaphthalene[1]

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INTRODUCTION

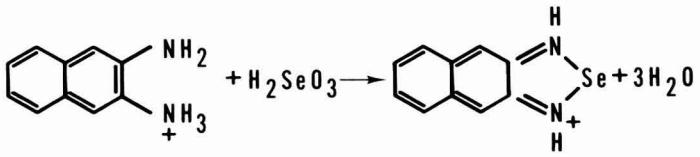
High-pressure liquid chromatography (HPLC) promises to become a versatile instrumental method for the quantitative identification of trace amounts of certain inorganic anions much as atomic absorption spectroscopy has become the most common instrumental method for trace inorganic cation analysis. Reported herein is the direct determination of selenite and nitrite and indirect nitrate analysis using an organic, anion reagent and HPLC. Fluorescent properties of the reaction products prompted the construction of an HPLC fluorometric detector to complement the ultraviolet absorbance detector.

The determination of Se (IV) is commonly performed by the reaction of the selenite ion with an aromatic *o*-diamine. Determination of trace quantities of Se (IV) are subject to interference from decomposition products of the aromatic *o*-diamine, excess aromatic *o*-diamine or sample constituents such as the nitrite ion, all of which possess or form products, with optical characteristics similar to the piazselenol, the desired reaction product. Variation of the HPLC stationary and mobile phases can result in optimized parameters which serve as a means to separate the components in an interfering matrix and permit the direct determination of selenite and/or nitrite ions.

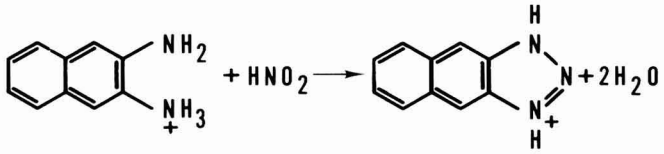
Among the aromatic *o*-diamine, piazselenol-forming reagents for selenium (IV), 2,3-diaminonaphthalene (DAN) is the most sensitive and is now the major choice for the fluorometric analysis of selenium (*9*). DAN was introduced as a reagent for the spectrophotometric and

[1] Paper presented at the International Symposium on Microchemical Techniques–1973, The Pennsylvania State University, University Park, Pennsylvania, U.S.A., August 19–24, 1973. This work was supported in part by funds received from the Research Council and the Graduate School of the University of Missouri–Kansas City.

fluorometric analysis of selenium (IV) by Cukor and Lott (*3*), who also investigated the kinetics and mechanism of the reaction between DAN and selenous acid to produce the fluorescent product naphtho-[2,3-*d*]-2-selena-1,3-diazole (DANSe) (*1*).



Recently, Wiersma used DAN as a spectrophotometric and fluorometric reagent for the analysis of nitrite ion based on the formation of 2,3-naphthotriazole (triazole) (*10*).



Previously, Wheeler *et al.* prepared triazole from DAN for use as a fluorometric reagent for silver (I) (*8*).

Sawicki used DAN for the fluorometric analysis of nitrate ion after reduction to nitrite (*5*).

Two techniques were developed to measure the concentration of DANSe and triazole by ultraviolet radiation absorbance or fluorescence intensity measurements.

In the direct procedure an aliquot of the treated aqueous sample is injected into the HPLC employing reverse-phase chromatography; the indirect procedure employs normal-phase chromatography by injecting an aliquot of an organic solvent extract of the treated aqueous sample into the HPLC.

Normal-phase liquid chromatography employs a polar stationary phase such as Durapak carbowax 400 whose surface functionality is $-(CH_2-OCH_2)_n-CH_2OH$, with a weakly polar mobile phase such as chloroform to achieve successful separations of the components of a chloroform-miscible sample; the lower polarity components elute first.

Reverse-phase liquid chromatography employs a nonpolar stationary phase such as Bondapak C_{18} whose surface functionality is $-(CH_2)_{17}CH_3$, with a polar mobile phase such as water or water/alcohol mixtures, to achieve successful separation of the components of a water-miscible sample; the higher polarity components elute first.

EXPERIMENTAL

Apparatus

Chromatograms were obtained with a Waters Associates model ALC 202 High-Pressure Liquid Chromatograph (HPLC) equipped with a 254/280 nm differential ultraviolet detector, and a Beckman 10 mV recorder; a Turner model 111 Filter Fluorometer (initial flow-cell accessory provided by Waters Associates) and a Sargent model SR 10 mV recorder.

Absorption and fluorescence spectra were obtained with a Bausch and Lomb model 505 recording spectrophotometer; fluorescence spectra were obtained using a fluorescence accessory (*11*) to prevent decomposition of the fluorescing species.

All pH readings were taken with a Corning Research Model 12 pH meter.

Separatory funnels were equipped with Teflon stopcocks.

Construction of Fluorometric Detector

Initial work was performed with a modified flow cell for a Turner Model 111 fluorometer loaned to us from the Development Laboratory of Waters Associates. However, as this detector was not commercially available, for continuing work we constructed a fluorometric flow cell from inexpensive components which has an effective volume of about 30 μliters.

The following supplies are needed.

Number	Description
2	No. 200-6-1 Swagelock reducing unions $\frac{1}{8}$ in. o.d. to $\frac{1}{16}$ in. o.d. with 10-μm snubbers (316).
2	Beckman No. 567026 gas chromatograph silicone rubber septa.
1	Quartz capillary tube 1.5–2 mm o.d., Bausch & Lomb Cat. No. 33-27/51.

Make a washer by cutting a 1/8-in. diameter disk from each septum with a no. 3 cork borer, then cut approximately a 1/16-in diameter hole in the center of each disk, using a 14-gauge hypodermic syringe needle, (preferably a cannula). Remove the 1/8-in. ferrules from the reducing unions and insert a washer into each large hex nut in place of the back ferrules. Carefully insert the quartz capillary through the holes of each hex nut washer to extend toward the union about 5/8 in. Replace the ferrules and firmly finger tighten the hex nuts to the reducing unions; check for tightness by attempting to pull the capillary from each union.

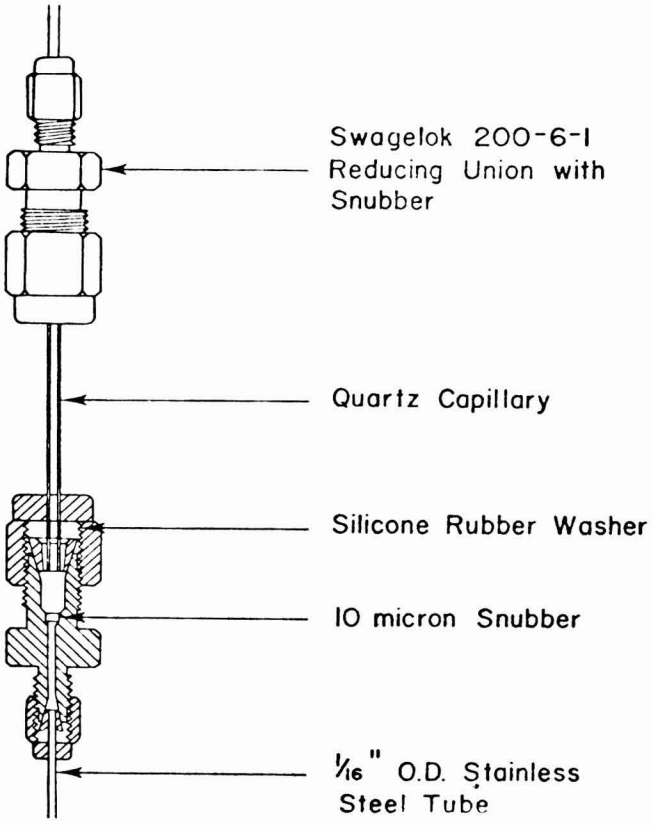


FIG. 1. Fluorometric flow cell.

This assembly (Fig. 1) is inserted into the cuvette holder of the conventional Turner Fluorometer door after the expansion pin which supports the cuvette has been gently tapped out with a small punch. The capillary positioning coincides very nearly to the exact optical center of the instrument without further adjustment.

Two small holes are drilled through the door near the ends of the 1/16-in. unions; appropriate connections are made with stainless-steel tubing to allow the mobile phase to flow directly from the column or from the ultraviolet absorption detector to the bottom of the flow cell, then to exit from the top to a waste reservoir.

Reagents

Standard selenium (IV) solution (1.00 mg per ml) was prepared weekly by dissolving 1.6280 g of reagent grade H_2SeO_3 in deionized water and diluting to 1 liter; all selenium (IV) solutions were stored in glass containers as prolonged contact with polyethylene resulted in reduction of selenite to elemental selenium.

Standard nitrite solution (1.00 mg per ml) was prepared daily by dissolving 1.500 g of reagent grade $NaNO_2$ in deionized water and diluting to 1 liter.

Standard nitrate solution (1.00 mg per ml) was prepared weekly by dissolving 1.630 g reagent grade KNO_3 in deionized water and diluting to 1 liter.

2,3-diaminonaphthalene (DAN) solution (J. T. Baker) was prepared weekly by dissolving 0.100 g of the reagent in 100 ml of 0.1 *N* HCl, stored at 5°C in the dark, and filtered by suction through a glass-fiber mat immediately before use.

HCl, 0.1 *N* was prepared by adding deionized water to 8.6 ml of concentrated hydrochloric acid to make 1 liter.

Extractions were made using 0.1 *N* HCl-saturated reagent grade chloroform and 1,2-dichloroethane.

Reduction of Nitrate to Nitrite

The simplest and most efficient chemical methods of reducing nitrate to nitrite are those of Sawicki (*5*), which uses hydrazine sulfate and copper sulfate in basic solution, and Strickland (*6*), which uses a cadmium–copper catalyst reduction column.

THE DETERMINATION OF SELENIUM (IV) AND NITRITE

Direct Procedure

Reverse-phase chromatography. Working-standard selenium (IV) solution (100 μg per ml) was prepared daily by diluting 10 ml of the standard selenium (IV) solution to 100 ml with 0.1 *N* HCl.

Working-standard nitrite solution (100 μg per ml) was prepared by diluting 10 ml of the standard nitrite solution to 100 ml with 0.1 *N* HCl, daily.

Working-standard nitrate solution (100 μg per ml) was prepared by diluting 10 ml of the standard nitrate solution to 100 ml with 0.1 *N* HCl, daily.

Selenium (IV) in a range from 0.5 to 10 μg per ml (0.5–10 ppm) and nitrite in a range from 0.1 to 1 μg per ml (0.1–1 ppm) can be determined by measurement of the height of the respective DANSe and triazole peaks from the chromatogram of an aliquot of the aqueous solution and DAN. To perform the analysis, pipet 100 ml of aqueous sample (filtered if containing suspended particles) into a 250-ml volumetric flask. Adjust to pH 1.0 with concentrated HCl or 10 *M* NaOH. Add 2.0 ml of DAN solution, stopper, mix thoroughly, and allow to stand out of direct sunlight at room temperature 4–5 min for nitrite analysis. For selenium (IV) analysis, wait 2 hr at room temperature or place solutions in a 50°C water bath for 15 min, then cool

rapidly to room temperature. Inject 20 μl of the aqueous solution into the HPLC using a 2-ft Bondapak C_{18} column and 1.25/2 by volume 95% ethanol to deionized water as the mobile phase, adjusted to 0.6 ml per minute flow rate (30% stroke).

Monitor the separation using a 254-nm differential ultraviolet radiation absorbance detector at 0.02 absorbance unit full-scale setting or the fluorometric detector at 1× slit setting and 7–51 primary and 3–69 secondary Corning glass filters to isolate the triazole peak; only the triazole fluoresces in aqueous solution (*4*).

Nitrate in a range of 0.1 to 1 μg per ml (0.1 to 1 ppm) can then be determined by subsequent treatment of the standards and samples by the most suitable of the previously cited methods for the reduction of nitrate to nitrite. After treatment of the solutions, inject a 20-μl aliquot into the HPLC and monitor the separation under the same conditions as for nitrite analysis. The difference in nitrite concentration (nitrite concentration after reduction minus nitrite concentration before reduction) multiplied by 1.348 (NO_3^-/NO_2^-) gives the nitrate concentration in the original solutions. If standards and samples are treated under the same conditions and a calibration curve constructed, the efficiency of reduction of nitrate to nitrite need not be known.

Linear calibration curves can be defined by adding 0.5- to 10.0-ml portions of the working selenium (IV) solution and 0.1- to 1.0-ml portions of the working nitrite and nitrate solutions to make 100 ml of 0.1 *N* HCl solution, then performing the direct procedure.

Extractive Procedure

Normal-phase chromatography. Working-standard selenium (IV) solution (1.0 μg per ml) was prepared daily by diluting 1.0 ml of the standard selenium (IV) solution to 1000 ml with 0.1 *N* HCl.

Working-standard nitrite solution (1.0 μg per ml) was prepared daily by diluting 1.0 ml of the standard nitrite solution to 1000 ml with 0.1 *N* HCl.

Working-standard nitrate solution (1.0 μg per ml) was prepared by diluting 1.0 ml of the standard nitrate solution to 1000 ml with 0.1 *N* HCl.

Selenium (IV) and nitrite in a range of 0.01–0.1 μg per ml (10–100 ppb) can be determined by measurement of the height of the DANSe and triazole peaks from the chromatogram of an aliquot of an organic solvent extract of the treated aqueous samples. To perform the analysis, pipet 100 ml of the aqueous sample into a 150-ml separatory funnel. Adjust to pH 1.0 with concentrated HCl or 10 *M* NaOH. Add 1.0 ml of DAN solution, mix thoroughly, and allow to stand out of direct sunlight at room temperature 4–5 min for nitrite analysis. Wait

2 hr for selenium (IV) analysis or place solutions in a 50°C water bath for 15 min. Cool rapidly, add 1.0 ml of chloroform for the extraction of DANSe, or 1,2-dichloroethane for the extraction of triazole, shake for 1 min and allow the phases to separate; leave the aqueous phase in the separatory funnels to prevent evaporation of chloroform. Draw off the 1,2-dichloroethane layer into a small sealable container, add 1 ml of absolute ethanol to it and mix (*6*). Inject a 20-μl aliquot of the organic solvent extract into the HPLC using a 2-ft Durapak carbowax 400 column and anhydrous chloroform as the mobile phase, adjusted to 0.5 ml per minute flow rate (20% stroke).

Monitor the separation as in the direct method, with a 254-nm differential ultraviolet radiation absorbance detector or a fluorometric detector with Corning 7–51 primary and 3–69 secondary filters for DANSe or Corning 7–60 primary and Wratten 47-B secondary filters for triazole; both the DANSe and triazole fluoresce in the organic solvents.

Linear calibration curves can be defined by adding 0.1- to 1.0-ml portions of the working-standard selenium (IV) solution and working-standard nitrite and nitrate solutions to make 100 ml of 0.1 *N* HCl, and performing the extractive procedure.

Nitrate in a range of 0.01–0.1 μg per ml (10–100 ppb) can be determined by reduction to nitrite by one of the previously cited methods then proceeding with the extractive nitrite analysis.

RESULTS AND DISCUSSION

Organic Extraction of DANSe and Triazole

Many water-immiscible organic solvents such as benzene, toluene, xylene, decalin, hexane, cyclohexane, chloroform, or carbon tetrachloride can be used to extract and concentrate DANSe from pH 1.0 aqueous solutions. All of these organic solvents are suitable for use with the fluorometric detector. However, use of the differential ultraviolet absorbance detector precludes extracting DANSe with an organic solvent which absorbs radiation at 254 nm. The organic solvents most suitable for the extraction and concentration of the triazole from pH 1.0 aqueous solutions were found to be 1,2-dichloroethane and 1,1,2,2,-tetrachloroethane, which will also extract DANSe.

Typical Chromatograms

Figure 2 presents typical reverse-phase differential ultraviolet and fluorescent HPLC chromatograms of 1 μg per ml nitrite and 1 μg per ml selenium (IV) at pH 1.0. A greater fraction of water in the mobile

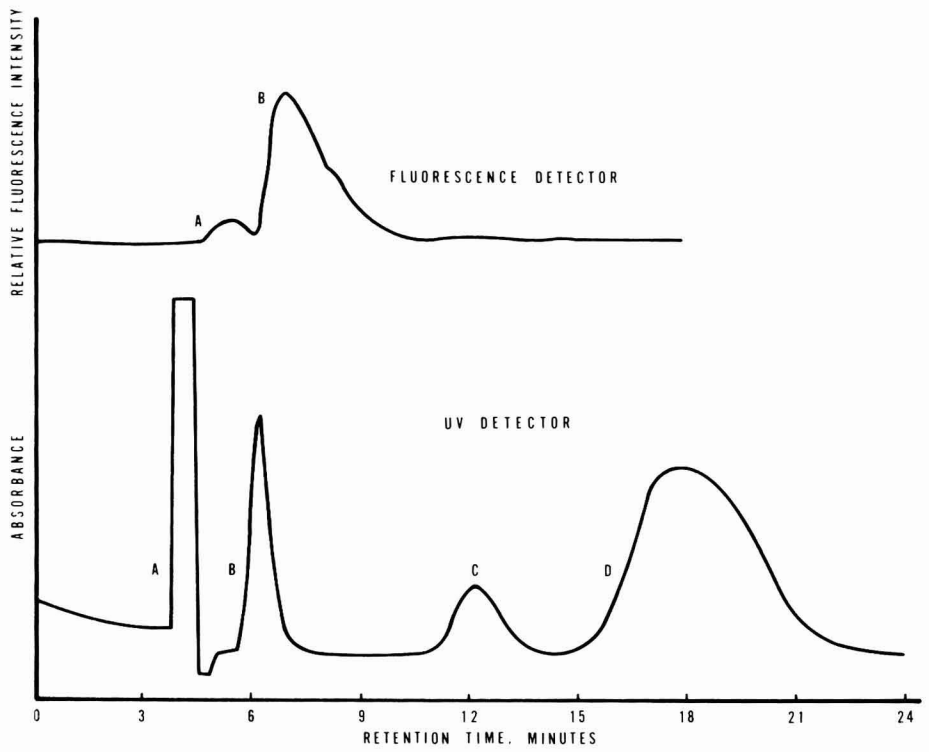


FIG. 2. HPLC chromatogram of pH 1.0 aqueous sample. A. Sample matrix; B. Triazole (1 mg/liter); C. DANSe (3 mg/liter); D. Excess DAN.

phase at the same flow rate resulted in longer retention times and considerable peak broadening; a lesser fraction of water resulted in shorter retention times and loss of resolution. Increased flow rate (pressure) with the specified ratio of ethanol and water in the mobile phase resulted in loss of resolution; decreased flow rate resulted in prohibitively long retention times and peak broadening.

Figure 3 shows the differential ultraviolet monitor, reverse-phase chromatogram of the reaction mixture of Fig. 2 raised to pH 12 by addition of sodium hydroxide pellets. At pH 12, the excess $DANH^+$, converted to the free amine, has a retention time greater than that of the triazole and less than that of the DANSe, which are unaffected. Analyses may be made more rapid by raising the solutions to pH 12 by adding five NaOH pellets immediately before taking an aliquot for injection into the HPLC.

Typical normal-phase differential ultraviolet and fluorescent HPLC chromatograms of a dichloroethane extract of 50 ng per ml (50 ppb) nitrite and chloroform extract of 50 ng per ml (50 ppb) of selenium (IV) are shown in Figs. 4 and 5.

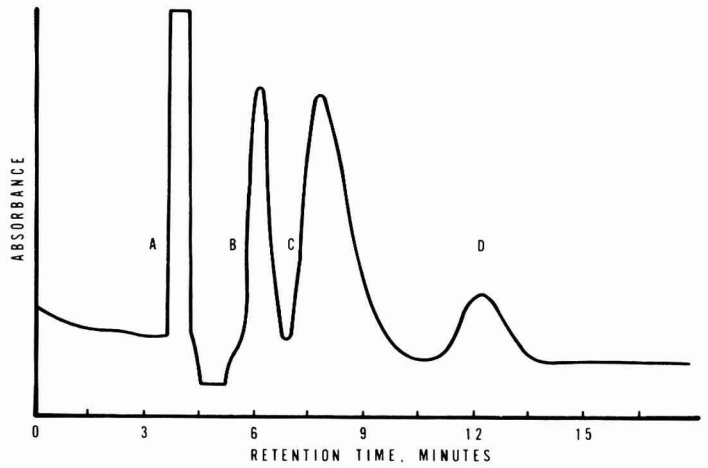


FIG. 3. HPLC chromatogram of pH 12 aqueous sample (Differential uv detector—254 nm). A. Sample matrix; B. triazole (1 mg/liter); C. excess DANH; D. DANSe (3 mg/liter).

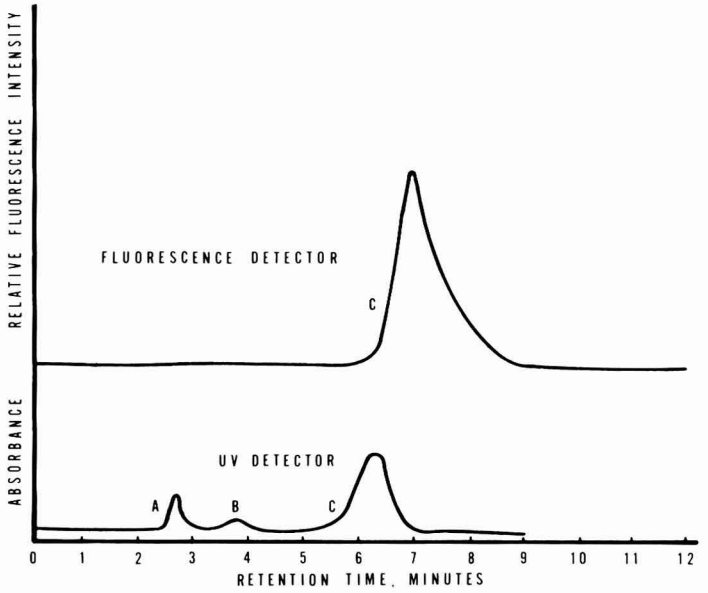


FIG. 4. HPLC chromatogram of 1,2-dichloroethane extract of pH 1.0 aqueous 50 ppb NO_2^- sample. A. Excess DAN; B. sample matrix; C. triazole.

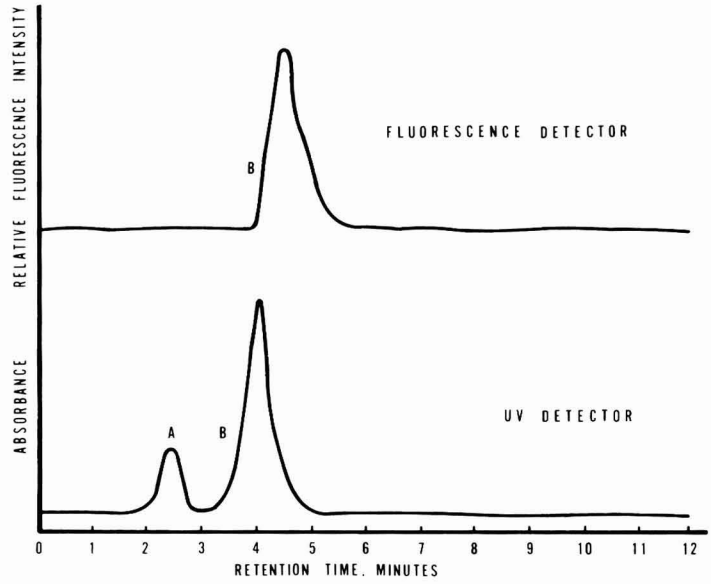


FIG. 5. HPLC chromatogram of chloroform extract of pH 1.0 aqueous 50 ppb Se(IV) sample. A. Septum bleed; B. DANSe.

Specified flow rates (percentage stroke) and solvent mixture ratios are not stringent requirements, as they may be easily varied to suit the need of separating an interference contained in a particular sample matrix.

Interference Studies

The selectivity of DAN for selenium (IV) and nitrite was studied by not employing masking agents and observing the effect of foreign ions on their respective determination of nitrite and selenium (IV) ions in aqueous solutions. Table 1 presents results of the presence of common ions on the determination of 5 ppm selenium (IV): negative deviations occurred in the presence of reducing agents such as Sn(II) which reduces Se(IV) to elemental selenium, high concentrations of oxidizing agents such as Fe(III), Sn(IV), and Ce(IV) which oxidize DAN before complete reaction may occur, Pd(II) which reacts with DANSe to form a complex precipitate (*2*), and Cu(II) and Ni(II) which appear to decompose DANSe. Positive deviations were not observed.

Table 2 presents results of the presence of common ions on the determination of 1 ppm nitrite ion: negative deviations occurred in the presence of reducing agents such as Sn(II) and sulfide which reduce nitrite to ammonium, oxidizing agents such as Fe(III) and Ce(IV)

TABLE 1
EFFECT OF FOREIGN IONS IN THE ULTRAVIOLET DETECTION OF 5 ppm Se(IV)

Ion[a]	Se(IV) found (ppm)
Al(III)	5.20
As(III)	5.16
Ba(II)	5.03
Bi(III)	5.15
Ca(II)	5.05
Cd(II)	4.95
Ce(IV)	5.01
Co(II)	5.00
Cr(III)	4.87
Cu(I)	5.01
Cu(II)	4.95[b]
Fe(II)	5.04
Fe(III)	0.71
Hg(II)	4.97
Mg(II)	4.90
Mn(II)	4.98
Ni(II)	5.12[b]
Pb(II)	5.00
Pd(II)	0
Sb(III)	4.90
Sn(II)	0
Sn(IV)	0.28
Te(IV)	5.05
Zn(II)	5.04
ClO_4^-	5.11
CN^-	5.03
$C_2O_4^=$	4.96
PO_4^{3-}	5.01
$SO_4^=$	5.00

[a] One millimole of cation present as either the sulfate, chloride, nitrate, or oxide; 1 mmole of the anion present as either the sodium or potassium salt.

[b] Decreases to 0 on standing a few hours.

which oxidize DAN before complete reaction may occur, and Cu(II) which appears to decompose the triazole. Positive deviations were not observed.

The reagent DAN is probably synthesized by the reaction of ammonia (with heat and pressure) and 2,3-dihydroxynaphthalene (DHN) yielding a reaction mixture of starting material (DHN), 2-amino-3-hydroxynaphthalene (AHN) and DAN, from which DAN is isolated by recrystallization.

TABLE 2
EFFECT OF FOREIGN IONS ON 1 ppm NO_2^- ANALYSIS

Ion[a]	NO_2^- found (ppm)	
	Ultraviolet	Fluorometric
Al(III)	1.06	1.10
As(III)	1.05	1.02
Ba(II)	1.00	0.96
Bi(III)	1.07	1.05
Ca(II)	1.00	1.00
Cd(II)	1.06	1.05
Ce(IV)	0.40	0.34
Co(II)	1.05	1.03
Cr(III)	1.05	1.00
Cu(I)	1.00	1.00
Cu(II)	0.86	0.80
Fe(II)	1.06	1.00
Fe(III)	1.00	0.95
Hg(II)	1.03	0.93
Mg(II)	1.05	1.00
Mn(II)	1.09	1.05
Ni(II)	1.13	1.08
Pb(II)	1.00	1.01
Pd(II)	0.10	0.12
Sb(III)	1.16	0.85
Se(IV)	1.00	1.00
Sn(II)	0.82	0.76
Sn(IV)	1.15	1.04
Te(IV)	1.18	1.08
Zn(II)	1.12	1.02
ClO_4^-	1.03	1.01
CN^-	1.07	1.05
PO_4^{3-}	1.15	1.07
$S^=$	0.36	0.31

[a] One millimole of cation present as either the sulfate, chloride, nitrate, or oxide; 1 mmole of the anion present as either the sodium or potassium salt.

For the assay of DAN, 20-μl aliquots of individual chloroform solutions (1 μg/ml) of pure DAN, AHN, and DHN were injected into the HPLC using the normal-phase conditions; retention times were monitored with the 254-nm differential ultraviolet absorbance detector. Relative retention times were as follows: DAN, 1.0 min; AHN, 1.8 min; DHN, 2.1 min.

One peak was observed for the DAN reagent signifying a purity of 99% plus, and an absence of ultraviolet-absorbing impurities. The analysis was confirmed by nonaqueous potentiometric titrations.

Elimination of Interferences

Oxidizing interferences are eliminated either by addition of DAN in excess of that oxidized or addition of CN^-, oxalate (*7*), or EDTA (*2*) to complex the ions; interfering reducing agents, Pd(II), Cu(II), and Ni(II) can also be complexed. Care must be taken when choosing masking agents, as some, such as EDTA, absorb radiation at 254/280 nm and cover the DANSe and triazole peaks; CN^- or pyrophosphate may be used successfully with the differential ultraviolet radiation absorbance detector. If the fluorometric detector is used exclusively, any masking agent which does not fluoresce may be used, EDTA being the most suitable.

Error Analysis

The results of two calibration curves (by each method) constructed 1 mo apart showed excellent reproducibility of the slope of the lines. The linear relationship, between ultraviolet radiation absorbance/fluorescence intensity and concentration over each range permits the use of a standard solution at one concentration level.

Based on the results of replicate standards, the relative percentage standard deviation for the determinations of 50 ppb Se(IV) and 1 ppm NO_2^- ions by differential ultraviolet radiation absorbance measurements is 0.61% and 2.8%, respectively, and for the determination of 50 ppb Se(IV) and 1 ppm NO_2^- by fluorescence intensity measurements is 1.24% and 4.3%, respectively. Table 7, summarizes the concentration ranges for each of the determined substances and analytical procedure.

Advantages

The combination of the reagent's specificity for selenium (IV) and nitrite, speed of analysis, diversity of concentration range and isolation techniques are major advantages of this method. A sample can be prepared and a separation achieved relatively rapidly; if the concentration of selenium (IV) or nitrite is not detectable by the direct procedure, the samples can be extracted with an organic solvent and a normal-phase chromatogram monitored.

The sample size, when using a limited quantity of material such as saliva or other biological fluids, may be less than 1 ml if a smaller volume of a more concentrated DAN reagent solution is employed, since only a 20-μl or smaller aliquot is needed for analysis by the HPLC.

Selenium (IV) and nitrite can be determined simultaneously if desired; after selenium (IV) and nitrite analysis, samples can be treated as described and nitrate, by nitrite difference, may be determined.

TABLE 3
RESULTS OF DIRECT SELENIUM(IV) ANALYSES

Sample[a]	Se(IV) added to make	Se(IV) found	Average Se(IV) content
Asphaltic concrete plant discharge	0 ppm	0 ppm	None
	1.0	0.90	
	3.0	2.80	
Auto bumper plating discharge	0 ppm	0 ppm	0.12 ppm
	1.0	1.15	
	3.0	3.20	
Lithographer discharge	0 ppm	0.55 ppm	0.63 ppm
	1.0	1.75	
	3.0	3.60	

[a] Environmental Protection Agency analyzed samples containing approximately 500 mg/liter dissolved solids and possessing specific conductances of approximately 2000 μmhos/cm.

ANALYSIS OF ACTUAL SAMPLES

The aqueous industrial discharge samples reported in Tables 3 and 4 were merely filtered by suction before procedural work up. Water-insoluble solid samples such as boullion and soil, Table 5, were extracted with 100 ml of 0.1 *N* HCl and filtered by suction before procedural work up; water-soluble samples such as sodium or potassium nitrate were dissolved in 0.1 *N* HCl to make 100 ml. Table 6 presents the analysis for NO_3^- in two different types of samples. Aqueous samples and neutral aqueous extracts were adjusted to pH

TABLE 4
RESULTS OF DIRECT NITRITE ANALYSES

Sample[a]	NO_2^- added	NO_2^- found	Average NO_2^- content
Plant effluent (industrial)	0 ppm	0.10 ppm	0.23 ppm
	0.30	0.60	
	0.50	0.79	
Meat packing plant discharge	0 ppm	3.25 ppm	3.28 ppm
	0.30	3.50	
	0.50	3.90	
Auto bumper plating discharge	0 ppm	0 ppm	None
	0.30	0.29	
	0.50	0.52	

[a] Environmental Protection Agency analyzed samples containing approximately 500 mg/liter dissolved solids and possessing specific conductances of approximately 2000 μmhos/cm.

TABLE 5
RESULTS OF DIRECT NITRITE ANALYSES

Sample	NO_2^- added to make	NO_2^- found (ppm)		Average NO_2^- content
		Ultraviolet	Fluorescence	
Boullion cube	0 ppm	0 ppm	0 ppm	None
(5.0-g sample)	0.50	0.53	0.54	
	1.00	1.07	1.10	
$NaNO_3$ U.S.P.[a]	0 ppm	11.15 ppm	11.20 ppm	11.12 ppm
(10.0-g sample)	0.50	11.67	11.69	
	1.00	12.20	12.22	
KNO_3 Reagent[b]	0 ppm	0 ppm	0 ppm	None
(10.0-g sample)	0.50	0.53	0.55	
	1.00	1.05	1.09	
Soil extract[c]	0 ppm	0 ppm	0 ppm	None
(10.0-g sample)	1.00	1.05	1.03	
	3.00	3.11	3.05	

[a] Labeled 0.001% NO_2^-.
[b] Labeled $<0.001\%$ NO_2^-.
[c] Unfertilized loam.

TABLE 6
NITRATE ANALYSES

Sample	NO_3^- added to make	NO_3^- found	Average NO_3^- content
River water[a]	0 ppb	0 ppb	None
	50	51	
	100	106	
Soil[b]	0 ppm	15.5 ppm	15.9 ppm
	1.0	17.0	
	5.0	21.3	

[a] Blue River water collected near Crete, Nebraska.
[b] Fertilized loam.

TABLE 7
CONCENTRATION RANGES

Substance	Concentration range	
	Direct procedure	Indirect procedure
Se(IV)	0.5–10 μg/ml (ppm)	10–100 ηg/ml (ppb)
NO_2^-	0.1–1	10–100
NO_3^-	0.1–1	10–100

10.5 with 10 *M* NaOH solution after addition of copper sulfate catalyst. Then hydrazine reagent was added and the samples placed in 65°C water bath for 30 min, as described by Sawicki (*5*). The samples were then analyzed for NO_2^- by the appropriate method depending on the concentration range of the NO_3^-.

SUMMARY

Selenite, nitrite, and nitrate ions have been determined spectrophotometrically and fluorometrically using the reagent 2,3-diaminonaphthalene and high-pressure liquid chromatography. A fluorometric detector was constructed for the HPLC systems. The developed procedures were applied to the analysis of water and biological materials.

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Computers in Titrimetry[1]

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Although titrimetry is a classical analytical technique, it has retained much of its importance and popularity. This is largely because it still competes successfully with modern techniques with respect to accuracy and precision. Moreover, titrimetric analyses are often rapid, easy to automate, and can be used to determine an analyte within a large concentration range. The number of possible titrimetric procedures has been considerably increased by the recent development of ion-selective electrodes (*28*).

Computers have found application both in titration theory and in the automation of titration procedures. They have been used to simulate titration procedures in order to determine the optimum experimental conditions for a particular titration procedure and the most accurate and precise method of evaluating the titration data. Lately, there has been a tendency to make evaluation calculations more elaborate in order to increase both accuracy and precision, and, in some cases, to eliminate time-consuming chemical pretreatment of the samples. This has led to the development of computer-orientated titrators capable of collecting titration data and rendering them in a form (e.g., punched tape) suitable for subsequent computer processing (passive titrators) and, more recently, of computer-processed titrators (active titrators).

The aim of this paper is to discuss the advantages which in general may be gained by the use of computers in titrimetric analysis. No attempt will be made to cover all the known applications and for more detailed information the reader is referred to the articles cited. Also, since emphasis is placed on analytically useful procedures, titration procedures for the determination of stability constants, etc. will not be dealt with. Those procedures discussed are mainly photometric or potentiometric.

[1] Paper presented at the International Symposium on Microchemical Techniques—1973, The Pennsylvania State University, University Park, Pennsylvania, August 19–24, 1973.

COMPUTER CALCULATIONS IN TITRIMETRY

Computer programs

One of the major problems in equilibrium calculations is the computation of free concentrations from known total concentrations and equilibrium constants. Although the combination of mass balance conditions and equations for the relevant constants must always provide sufficient information to compute all the free concentrations in all types of titrations (*11*), a systematic use of these equations is seldom made (*10, 13*). The reason for this is primarily numerical since the solution normally involves terms of the third degree or higher. Even the simplest problems, such as the calculation of the pH value obtained when a weak monoprotonic acid is dissolved in water, results in a third-degree equation if the concentrations of all the species, including OH^-, are taken into consideration. Consequently, much attention has been given, both in basic training and research, to the problem of making correct approximations in order to reduce the arithmetical difficulties. To this end, graphical representations, such as logarithmic distribution diagrams, are invaluable (*10, 13*).

From the analytical point of view, the most useful approximate method for the treatment of ionic equilibria is that of conditional constants. This approach is by no means restricted to complexometric and acid-base titrations but can be used for any type of titration in which the concentration of one or more components (e.g., $[H^+]$) can be regarded as being constant (*31*).

A stringent use of mass balance conditions is, however, now possible owing to the availability of digital computers and general computer programs which can deal with all types of ionic equilibria (*9, 18, 30*). During their execution these programs compare the known total concentrations of all components, including protons, with the corresponding total concentrations computed from known stability constants and a given set of free concentrations. The free concentrations are then adjusted until all the computed total concentrations agree to within a specified difference, e.g., 0.1%, with the known total concentrations. One of the greatest problems in the construction of such programs is that free concentrations can attain very small numerical values, e.g., $[Hg^{2+}] = 10^{-20} M$. On the other hand, all the free concentrations must, of course, be positive. The general computer programs available differ only in the strategy according to which the free concentrations are adjusted in order to obtain the most rapid convergence. In general, the newest programs solve the problem more elegantly while those which have been tested for a long time at different laboratories are more likely to work reliably.

Simulation of titration curves

In analytical titrations two parameters are normally measured. One of these is always the total amount of titrant added, either volumetrically or coulometrically. The other parameter, which is more flexible, is a more or less selective measurement of the free concentration of one of the species present in the solution, e.g., $[H^+]$ in a protolytic titration, or the concentration of a metal–indicator complex in a spectrophotometric complexometric titration. In order to simulate the titration curve it is thus necessary to calculate the free concentrations of all species at different total concentrations of titrant. Apart from determining which reactions dominate in the different parts of the titration curve (*12*), the general computer program can be used to calculate the pH value at the equivalence point of, for example, a redox titration and the dependence of the form of its titration curve on the total proton content (*1*, *2*). In connection with titrations exploiting ion-selective electrodes as sensors, computer calculations have been used, for example, to simulate the precipitation titration of fluoride with lanthanum at different total proton and buffer concentrations (*3*, *4*) and to estimate the interference of different ions with the ion-exchange electrode used as sensor in the complexometric titration of calcium (*19*). For photometric titrations it is possible to calculate the absorbance at the equivalence point taking the mass balance conditions for the indicator into consideration (*12*).

Objections have been raised against computer-simulated titration curves to the effect that the relevance of the calculation depends entirely on the relevance of the equilibrium constants used. This is indeed true, but the argument is, of course, equally valid for manual calculations. Obviously, a sensible choice of equilibrium constants, with respect to the composition of the ionic medium used, is vital. If a computer is employed, it is, however, possible to test a large number of different values for a specific uncertain constant and thus estimate the degree of uncertainty in the calculated titration curve due to this parameter (*16*). The effect of variations in ionic strength on activity factors could also be included in the computer program were a generally valid arithmetic expression for this correction available.

Evaluation methods

From computer calculations it is possible to determine the optimum evaluation method for a particular titration with respect to accuracy, precision, and time required for the evaluation. This is achieved by applying a number of potential evaluation methods to a computer-simulated titration curve. In this way it is easily seen, for example,

whether or not the point of maximum slope of a potentiometric titration curve coincides with the equivalence point. For a photometric titration the ratio between the proton and the metal indicator complex be calculated (*5*).

Perhaps even more important is the actual evaluation of the experimental titration data. Suitable computer programs not only reduce the time required but permit a more complicated arithmetical treatment of a larger number of experimental data. The inclusion of all, or nearly all, the experimental data in the evaluation normally yields a higher precision, while a greater accuracy is often obtained through a more complicated arithmetical treatment, since many of the approximations inherent in the simpler evaluation methods are no longer necessary.

Evaluation methods which exploit as many of the experimental data as possible must be based on the mass balance and equilibrium constant equations, i.e., on the equations which govern the form of the titration curve. These methods often involve the conversion of the titration data into a linear form, after which the equivalence point is determined by means of straight-line regression (*6, 15, 20, 23, 24, 29*). In potentiometric titrations such linear plots are often referred to as Gran plots (*15*). In the derivation of Gran plots, in their simplest form, it is assumed that the titration reactions are quantitative in all parts of the titration curve (*12*). This is normally true at the beginning of a titration, but competing side reactions often become important near the equivalence point (*17*). If experimental data in this region of the titration curve are exploited in the linear regression, a systematic error will be obtained. The magnitude of the systematic error can be estimated by applying the particular linear plot to a computer-simulated titration curve (*16*).

That experimental data close to the equivalence point ought not to be exploited in the evaluation is particularly serious for potentiometric titrations, since the maximum change in emf signal per unit titrant increment always occurs in this region. This means that, if systematic errors are to be avoided, an important part of the experimental information must be discarded, resulting in decreased precision. If, however, a theoretical titration curve has been calculated, the nature of the competing side reactions is known and the Gran plot can be modified to include all the titration data without any loss of accuracy (*16, 26*).

In photometric indicator titrations the problem of competing side reactions close to the equivalence point may be avoided by choosing the experimental conditions so that the indicator transition occurs at a

part of the titration curve where the titration reaction dominates (*23*). Obviously, a computer-simulated titration curve in this case facilitates the determination of the optimum experimental conditions (*19*).

COMPUTER-ORIENTATED TITRATORS

In classical automatic titrators the reagent is added continuously at a constant speed, and the change in sensor signal is registered continuously. Although it is, of course, possible to use the same principle in a computer-orientated titrator, it has been found advantageous to add the titrant in increments and to register an equilibrium reading between each addition (*24, 25*). This method is nececessary if a linear evaluation is to be used.

There are two main types of computer-orientated titrators. One type operates in the passive mode ("off-line"), the computer being used solely for the evaluation of the experimental data collected by the titrator. The other type operates in the active mode ("on-line"), the computer processing the performance of the titration procedure, collecting the data, and evaluating the results.

Passive computer systems

Although a large number of different off-line titrators have been designed, many of them have never been described in detail in easily available journals (*21, 24*). Inevitably, most of them have several features in common and a block-scheme for a typical titrator is shown in Fig. 1. Increments of titrant are most often added by means of automatic syringe burets, pneumatic pipets, or peristaltic pumps. Coulometric generation is, in general, preferable when small amounts of

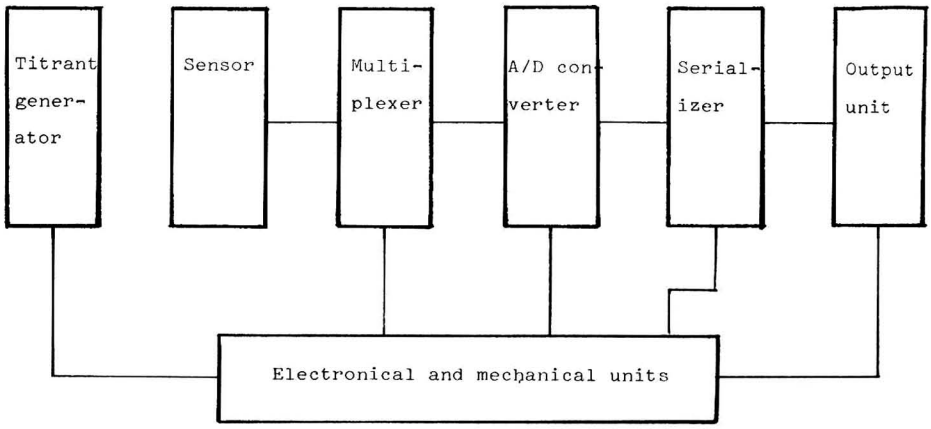

FIG. 1. Major parts of an off-line titrator.

reagents are to be generated accurately, e. g., in the titration of dilute samples.

All the sensors frequently used in titrations can be made to deliver analog voltage signals in the range 0–10 V. Some sensors, such as certain ion-selective electrodes, have signal impedances of the order of magnitude of several megohm. Unless the A/D converter has a very high input impedance (10^{10}–10^{13} Ω), it is necessary to include an amplifier step after the sensor. Normally this is an opertional amplifier coupled as a voltage follower (current amplifier). A multiplexer is used if measurements are required on more than one sensor. Most multiplexers can operate only in a sequential mode, i. e., the different sensors are always measured in the same order. A large number of different types of A/D converters are now available. Since titrations are, however, characterized by a relatively slow flow of data (10 – 100 readings/s), the signal differing little from one titration point to the next, an integrating digital voltmeter, equipped with a suitable prefilter, is often the best choice.

Most modern A/D converters can be equipped with a binary electronic parallel output, usually based on some BCD code. Since the output units used in off-line titrators can only print the A/D representation digit by digit, it is necessary to include a parallel to series converter (serializer) between the A/D and the output unit.

The output from passive titrators is usually in the form of punched tape. As in almost all other computer-orientated scientific equipment, the ASR 33 teletype is the most popular tape puncher. To a relatively low cost this equipment can be made to print the experimental data both on punched tape and on paper, thus facilitating an immediate inspection of the data. In addition, the teletype keyboard is equipped with both letters and numerals so that it is possible for the operator to add additional instructions or messages to be used in the computer treatment of the experimental data. The main drawbacks of the ASR 33 are the limited printing speed (max 10 characters/second), that it is by no means suitable for use 24 hr/day, and that it operates in ASCII code, which cannot be recognized by all computers. This last means that the evaluation program must have been a subprogram which can translate ASCII code into the relevant computer input code.

The electromechanical titrator unit can differ considerably in sophistication in different titrators. In its simplest form it consists of a programmable clock and a number of relays. During the execution of the titration the titrator starts by triggering the titrant generator to cause it to deliver a programmable amount of reagent. It then waits a programmable duration of time after which it prints the A/D signal and triggers the titrant generator again. Using digital circultary and

mechanical devices such as step-motors and thumb-wheels, the titrator program can, however, be made much more eleborate (*14*). The titrator can, for example, be designed so as to operate the wavelength adjustment of a spectrophotometer or a sample changer.

Off-line titrators facilitate the automation of a great number of different types of titration procedures. Apart from the gain in time and labor, one also profits in accuracy and precision, provided that the evaluation programs make optimum use of the experimental data. Computer simulation of titration curves can here be used to optimize the performance of an automatic titrator.

The precision of an off-line titrator is ultimately limited by the precision of the titrant generator. For a volumetric application this limit is of the order of magnitude of 0.01–0.005% of a full buret. This value can, however, be obtained only if the temperature of the titrant is either carefully controlled or if the titrant temperature is measured for each sample and correction made for density variations. In practice, the precision of the sensor(s) often sets a limit on the precision of the titration results, i. e., the precision can be increased by the registration of a larger number of independent experimental data. Normally photometric titrations yield results of higher precision than potentiometric titrations.

Although off-line titrators are now available commercially (*27*), they have still not come into widespread usage. This is probably because many analytical laboratories do not have immediate access to computer facilities. Since, however, most modern computer centers can now offer terminal facilities in the different chemical laboratories this problem should soon be overcome. As an alternative to a commercial titrator the analytical chemist can, of course, purchase the different pieces of equipment separately, and thus obtain an apparatus which is perhaps more suited to his particular need. The minimum price for the equipment shown in Fig. 1 is, at present, approximately $5.000.

Active titrators

Although passive titrators are sufficient for many analytical titration procedures, active titrators have several advantages and, owing to the continual decrease in price of minicomputers, it would seem likely that active titrators will surpass passive titrators in popularity (*7, 23*).

In its simplest form an active titrator differs from the passive titrator of Fig. 1 in that the electronical unit and the serializer are replaced by a CPU unit and a core memory. The CPU unit and core memory may be a minicomputer or part of a larger computer capable of processing several analytical instruments on a time-sharing basis.

The main advantage of an active titrator over a passive titrator is that the former can, on the basis of collected data, process the titration procedure during its execution (*19*). Other advantages are that the final results are obtained almost immediately after the end of the titration, that the titrator can be programmed to wait for a stable signal between titrant increments and that loss in precision due to high signal/noise ratios from sensors can be reduced by signal averaging. Active titrators can, moreover, be programmed to operate two burets, one containing titrant and the other analyte, thus permitting scanning back and forth over a particular region of the titration curve (*23*).

There are no commerical active titrators and it is doubtful whether such titrators will be available commercially in the near future. It is, of course, possible to purchase the separate parts. When so doing, perhaps the most important choice is that of computer language level. Since, even if several titration procedures are carried out simultaneously, data are delivered at a slow rate on computer time scale, interpretative languages such as FOCAL or BASIC can be used and have the advantage that they are extremely easy to learn. This is, of course, important if titration procedures are to be changed frequently. These simple languages can, however, not be used if data are delivered at a speed greater than 5 kHz, and another disadvantage is that the compiler always occupies part of the core memory. Recent software development has facilitated the simultaneous operation of several titration procedures on a scale close to real time (*8*).

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Ultramicrodetermination of Nitrogen in Organic Compounds

I. Application of Interpolation in the Sealed Tube Method

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The sealed-tube method invented by Hozumi and Kirsten (*1*, *2*, *4*) is of great use for the determination of the absolute quantity of nitrogen in a sample; only small amounts of reagents are required, and the combustion in the sealed tube at high temperature is complete in 90 min.

However, obtaining satisfactory analytical results with this method is accompanied by a real difficulty in accurately replacing the nitrogen column in the capillary tubes with mercury, which has a high surface tension.

We have found that reliable measurement of the nitrogen volume in the capillary tube can be easily and precisely made by interpolation in the region of the meniscus.

DESCRIPTION OF METHOD

Apparatus

The apparatus presently used in the author's laboratory is shown in Figs. 1 and 2. The combustion tube is of Heatron-P or Miracron-PH-3 glass. The tube is swept with oxygen and sealed off as described in the original papers (*1*, *2*, *4*). The burner (Fig. 1,b) uses coal gas and oxygen and gives a uniform height of flame. The oxygen generator is fitted up to be convenient for routine of the tube use (see Fig. 2). During the continuous sweeping of the tube with 7 ml/min of pure oxygen, a 1.7-A current is put through the 700-ml electrolysis cell, which is cooled by water jacket. When not in use, a small electrolytic current of about 0.03-A should be continuously passed through the cell by day and a 0.002-A current from a dry cell overnight. The pressure at the anode side during the sweeping procedure is balanced by a mercury seal on the cathode. When the cell is not in use the pressure on the anode side is normally reduced because of the decrease in oxygen flow rate; this results in a fall in the level of liquid in the cathode

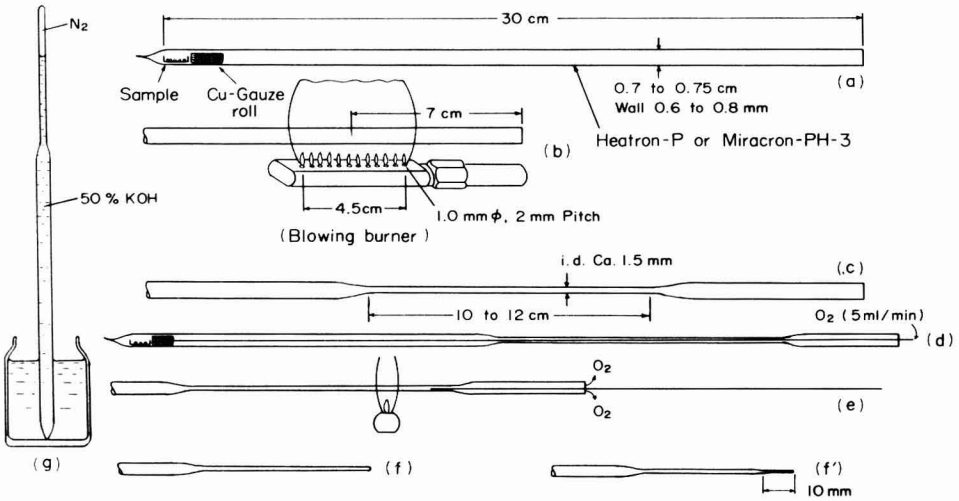

FIG. 1. Sweeping and sealing of combustion tube: (f) capillary for normal analysis, (f') capillary for measuring blank value.

compartment and interruption of the generation of oxygen. To prevent this the pressure is raised on the anode side by choking the oxygen flow with a capillary tube fixed on the end of the stainless-steel tube.

With normal use, water is added to the cell at 6-mo intervals. The capillary is sealed off as shown in Fig. 1,e.

Combustion furnace. We recommend the use of a potter's cylinder in which eight combustion tubes are placed in a circle concentric with the center of the furnace; combustion tubes can be simply put in and taken out from the furnace.

Procedure

The sample is burned in a sealed tube filled with pure oxygen and containing a small amount of copper wire gauze, and the nitrogen is collected above 50% potassium hydroxide as described in the original procedures (*2*, *4*).

The capillary is cut off at a point about 20 mm below the meniscus and placed horizontally on a comparator.

The length, L, between the sealed end of capillary and lowest point of the meniscus is measured to 0.001 mm with the comparator (see Fig. 3, a).

The temperature is read with a thermometer placed in the vicinity of the tube at the time when the length of capillary is measured, and the barmetric pressure is also read.

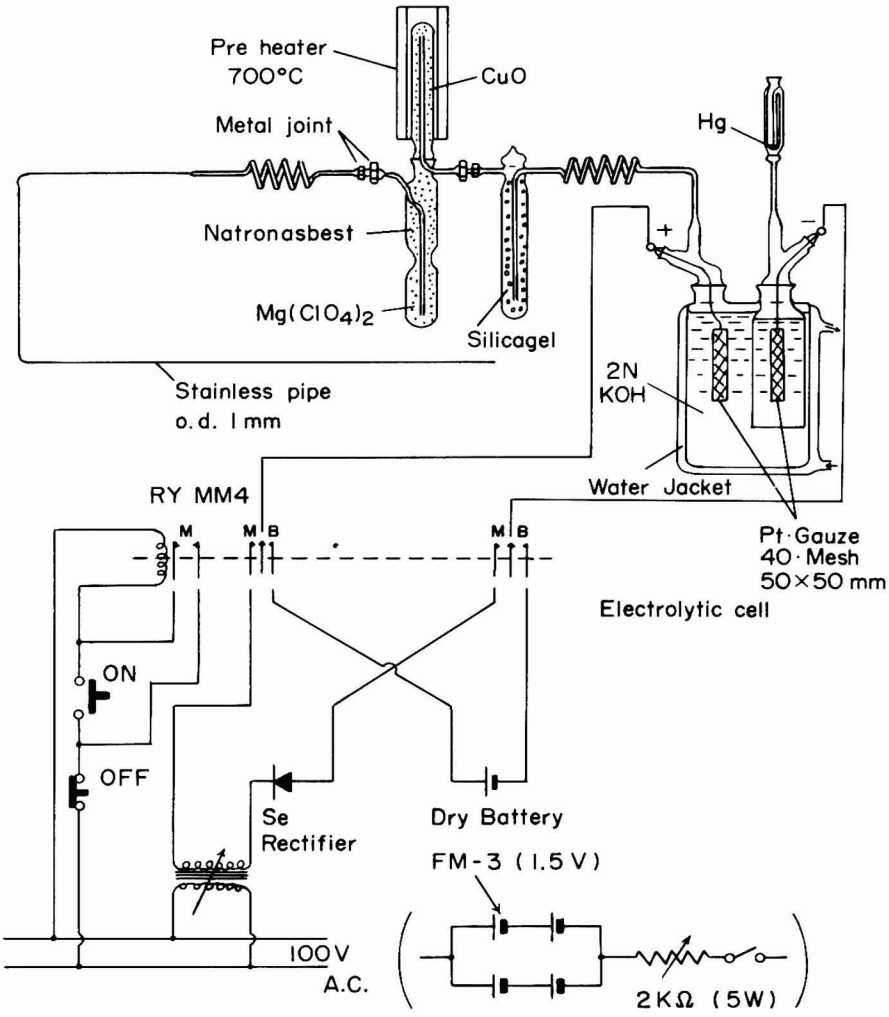

FIG. 2. Apparatus for supplying electrolytic oxygen: (RY) relay, (M) make conjunction, (B) break conjunction.

A horizontal mark about 1 mm wide is made with Magik inks on the glass capillary at the position of the meniscus. The tube is then carefully washed with water and acetone making sure that the mark is not erased. It is then dried and weighed.

Mercury is introduced by syringe up to the vicinity first of the inside then of the outside of the ink mark, and the lengths, L_1 and L_2 are measured, respectively, as above (see Fig. 3, b). The weight of the tube is measured in each case.

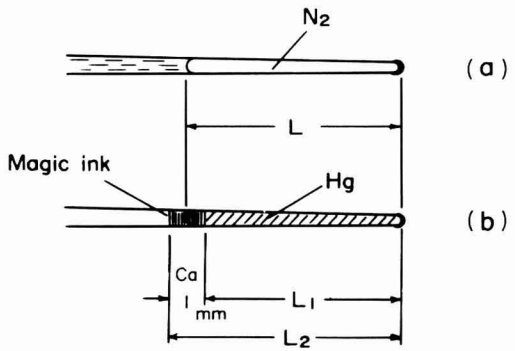

FIG. 3. Measurement of gaseous nitrogen by interpolation: (a) collected nitrogen, (b) nitrogen column has been replaced by a mercury column of roughly the same length.

Calculation

The weight of mercury corresponding to the length L can be accurately determined by interpolation from the weights of mercury in lengths L_1 and L_2. The nitrogen volume is obtained in microliters by dividing the weight of mercury, in milligrams, by the density of mercury.

From this volume of nitrogen collected is subtracted a correction for the vapor pressure of water over the potassium hydroxide solution (*3*, *5*, *6*), and a correction necessary for temperature correction of barometer reading (*6*), and the blank value.

DISCUSSION

It is important that the relationship between the length of mercury column and its weight is linear within the horizontal mark of about 1 mm wide on the glass capillary.

This was confirmed to be so, as shown in Fig. 4. The origin represents the weight and length of the shorter mercury column with each capillary tube. So, in drawing out the capillary a wide variation in internal diameter is permissible (see Fig. 4), allowing the job to be done by semiskilled workers.

When the blank value is measured, the capillary should be again drawn out to a finer capillary, with a length of about 20 mm and an inside diameter of about 0.2 mm, which is then sealed off and used for measuring the blank value (see Fig. 1, f'). The blank value is accurately measured by interpolation as above, and is obtained using a pure organic compound which contains no nitrogen (see Table 1).

Table 2 shows the results of analyses on several organic compounds by the original method. They indicate the occasional difficulty

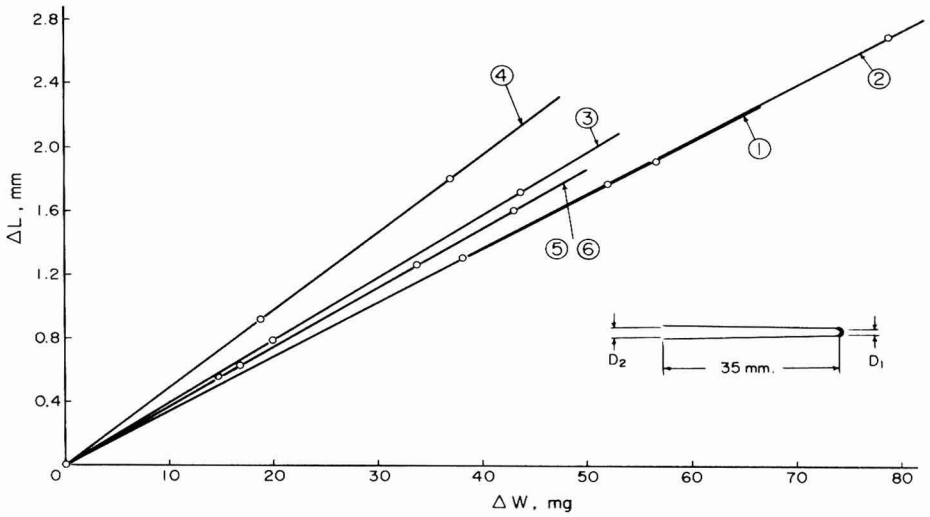

FIG. 4. Relationship between weight of mercury column and its length: (ΔW) differences in weight between shortest and longer mercury columns, (ΔL) differences in length between shortest and longer mercury columns, (1) $d_1 = 0.25$ mm, (2) $d_2 = 0.08$ mm, (3) $d_3 = 0.32$ mm ($D_2 < D_1$), (4) $d_4 = 0.08$ mm, (5) $d_5 = 0.7$ mm, (6) $d_6 = 0.01$ mm ($D_2 < D_1$), where d is differences of internal diameter between D_2 and D_1.

of replacing precisely the nitrogen column in the capillary tube with mercury.

Table 3 shows the results of analyses on several organic compounds using interpolation. The results are satisfactory at the decimilligram level.

TABLE 1
BLANK VALUE[a]

Substance (with ca. 300 μg)	Wt of mercury (mg)	Temp (°C)	Blank (μl)
Sucrose	2.386	18.0	0.176
	1.256	18.0	0.093
Benzoic acid	2.362	16.4	0.174
	1.798	18.3	0.133
Cholesterol	2.321	17.3	0.171
	1.159	17.5	0.086
Naphtalene	1.572	17.5	0.116
	1.814	17.5	0.134
Mean			0.135

[a] Sweep time: 7 min; flow of oxygen: 5 ml/min.

TABLE 2

RESULTS WITH SEVERAL ORGANIC COMPOUNDS BY THE ORIGINAL METHOD

Sample	Sample (mg)	Hg wt (mg)	N_2 vol (μl)	Temp (°C)	Barom. press. (mm Hg)	Found (%)	Error[a] (%)
Sulphathiazole	0.3119	599.56	44.29	22.7	765.6	16.53	+0.07
	0.3707	699.40	51.66	22.7	765.6	16.23	−0.23
	0.3899	742.38	54.83	22.9	766.0	16.35	−0.11
	0.3430	656.14	48.46	22.9	766.3	16.43	−0.03
Acetanilide	0.3685	448.49	33.12	20.8	767.6	10.56	+0.20
	0.3379	383.66	28.33	21.0	767.0	9.82	−0.54[b]
	0.3462	414.93	30.64	21.0	767.0	10.37	+0.01
	0.3053	380.37	28.09	21.7	770.5	10.81	+0.45[b]
Thio-urea	0.2830	1230.88	90.92	22.9	766.5	37.39	+0.59[b]
	0.2743	1163.17	85.91	22.9	766.5	36.46	−0.34
	0.3055	1312.27	96.93	22.9	766.3	36.88	+0.08
	0.3560	1520.22	112.29	22.9	766.3	36.67	−0.13

[a] Standard deviation of error = 0.315%.

[b] Abnormal value.

TABLE 3

RESULTS WITH SEVERAL ORGANIC COMPOUNDS USING INTERPOLATION

Sample	Sample (mg)	Hg wt (mg)	N_2 vol (μl)	Temp (°C)	Barom. press. (mm Hg)	Found (%)	Error[a] (%)
Phenacetine	0.3870	342.03	25.30	13.7	769.7	7.89	+0.07
	0.4189	368.02	27.22	17.0	765.4	7.70	−0.12
4-Methyl-5-β-chloro-ethylthiazole picrate	0.4448	728.53	53.89	14.0	769.7	14.63	+0.29
	0.3587	587.23	43.43	17.0	765.4	14.35	+0.01
Picric acid	0.2967	609.22	45.06	13.2	771.4	18.39	+0.05
	0.3467	726.41	53.23	13.2	771.4	18.77	+0.43
Licorine	0.2598	140.71	10.41	13.2	771.4	4.85	−0.20
	0.3386	187.92	13.90	13.2	771.4	4.97	−0.08
Antipyrine	0.3953	666.91	49.33	14.0	769.5	15.04	+0.16
	0.3858	660.04	48.82	15.2	769.2	15.18	+0.30
3-Chlorophenothi-azine	0.2961	201.39	14.90	16.0	769.2	6.01	+0.02
	0.3701	253.95	18.78	16.0	769.2	6.07	+0.08
$C_7H_{11}ON_4I$	0.4099	888.87	65.75	15.0	769.7	19.26	+0.21
	0.2850	615.32	45.51	15.0	769.7	19.18	+0.03
$C_8H_{13}ON_2I$	0.3699	409.89	30.32	16.0	769.5	9.81	−0.19
4-Acetoamido-2-bromopyridine	0.3103	428.50	31.69	15.0	761.3	12.12	+0.10
Thio-urea	0.2698	1142.5	84.51	17.2	767.4	37.21	+0.41
$C_6H_6ON_4$	0.3333	1422.2	105.19	17.9	766.7	37.37	+0.05
Acetyl urea	0.3810	1225.2	90.62	20.0	760.8	27.75	+0.31
$C_7H_7N_4CIS$	0.2678	815.78	60.34	20.0	760.8	26.29	+0.19
$C_6H_8N_3Cl$	0.2565	777.25	57.49	17.5	766.3	26.55	−0.11

[a] Standard deviation of error = 0.197%.

This can be smoothly achieved by making application of the interpolation in the reliable measurement of nitrogen volume in the capillary tube.

SUMMARY

A method is described for the decimilligram determination of nitrogen in organic compounds based on measuring by interpolation the volume of nitrogen collected in a capillary tube. The blank value is determined indentically.

ACKNOWLEDGMENT

The author is indebted to Tomo Takaoka and to Yasuko Yokoyama for their skilled technical assistance.

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MICROCHEMICAL JOURNAL 19, 423–428 (1974)

Ultramicrodetermination of Nitrogen in Organic Compounds

II. A New Simple Nitrometer for the Sealed Tube Method

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The sealed-tube method of K. Hozumi and W. J. Kirsten (*1–3*) for decimilligram determination of nitrogen has from the start been considered very promising, but until recently various difficulties in its operation have prevented its widespread use.

Continued interest in this method has been due to the fact that it is simple and required no special apparatus, and that complete combustion is assured.

In an attempt to develop the method to be as practical in use as the ordinary Dumas micromethod, the author has designed a new simple nitrometer for use in ultramicrodetermination. Unlike presently used methods for measuring nitrogen produced by the sealed-tube method, the new method does not involve weighing replaced mercury (*1, 2*), or expanding collected nitrogen and titration with micropiston burette (*3*). It is direct and simple in use and has been found to give completely satisfactory results.

DESCRIPTION OF METHOD

Apparatus

The nitrometer for the sealed-tube method is illustrated in Fig. 1. It consists essentially of two parts, a calibrated measuring tube and a receiving chamber. The measuring tube is a glass capillary tube of about 1.0 mm internal diameter. It is about 25 cm long, with a capacity of about 0.20 ml, and is marked off in 0.001-ml graduations numbered as shown. Mitsui's zero mark (*4, 5*) is about 15 mm below the upper nozzle-type valve, B, which has been described previously (*6*). The bottom of the capillary tube widens out into a stem which is joined to near one end of the receiving chamber, E, a 10-mm-diam tube of 85 mm over-all length. At this same junction a short glass side arm F is joined, at right angles to the other tubes. This is connected by a Tygon tube to a water reservoir, R. The end of the receiving chamber nearest the junction of the tubes is blocked with a self-

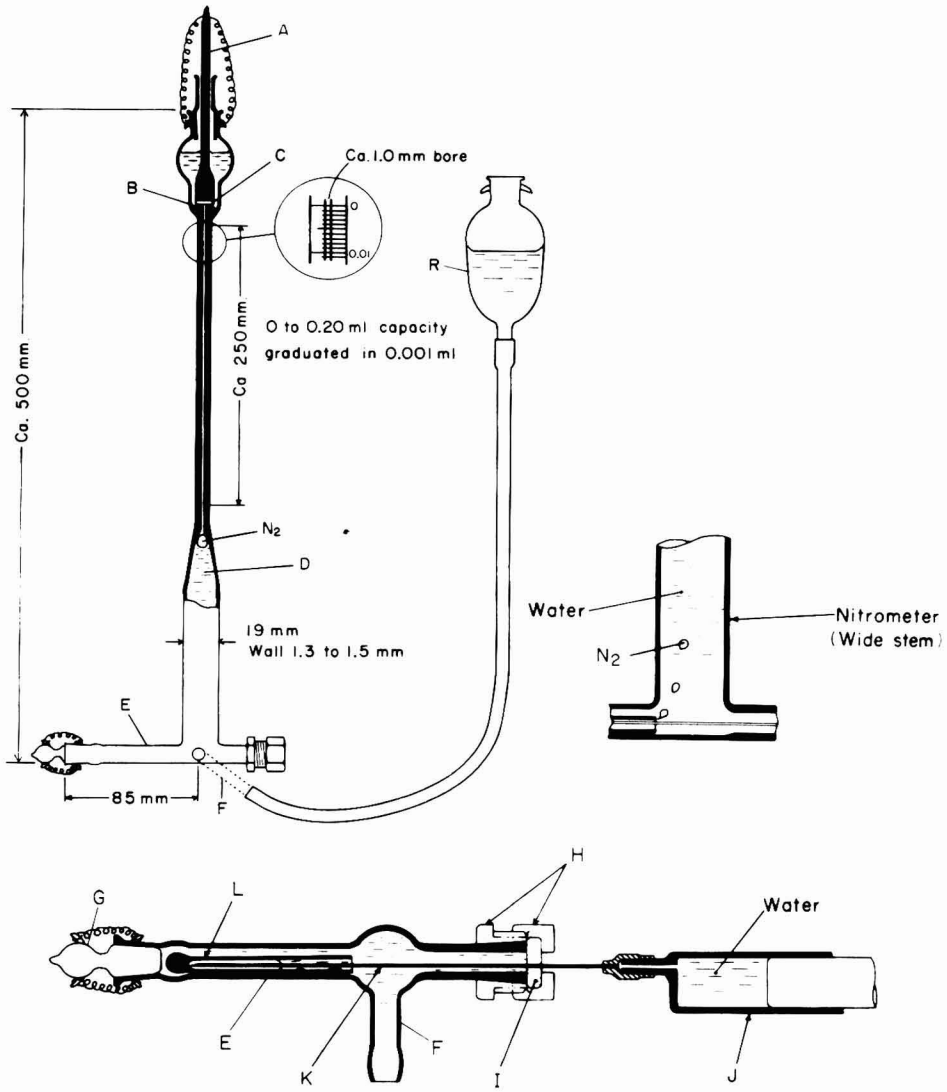

FIG. 1. Illustration of apparatus. A, plunger; B, nozzle; C, rubber sleeve; D, water; E, receiving chamber; F, side arm (connected with Tygon tubing to reservoir R); G, stopper; H, stuffing-box joint; I, rubber cap; J, hypodermic syringe; K, injection needle; L, capillary tube (containing nitrogen collected above KOH solution).

sealing rubber cap, I, held on by a metal stuffing-box-type joint, H, the other end is stopped with a 10/18 ground glass stopper, G.

Procedure

The combustion tube is broken under alkali solution to contain the nitrogen over a column of alkali solution, as described previously

(*1,2*), then the capillary of the combustion tube is cut about 20 mm below the meniscus, and placed in the horizontal receiving chamber with its open end facing the rubber cap (see Fig. 1). With plunger A raised, the nitrometer is now filled with water by raising reservoir, R. Until the water level is half-way up the nitrometer tube the stoppers G and H are left loose so that they are flushed with water and any air bubbles trapped in the receiving chamber and stoppers are swept out. They are then fixed tightly and filling of the nitrometer is completed. The water used should have been left to stand overnight to each absorption equilibrium with the air. Plunger A is then lowered and held with spring, the reservoir bulb is lowered, and the nitrogen in the capillary tube is pushed out by water injected through a syringe introduced through the rubber cap. The syringe, J, is a 10-ml hypodermic syringe with a 0.8 × 125 mm injection needle, K. Care should, of course, be taken to remove any air bubbles from the needle before it is injected through the rubber cap. Slight movement of the needle while water is being injected will help displacement of the nitrogen which rises in bubble to the top of the wide stem of the measuring tube. Next the upper meniscus of the nitrogen is adjusted to coincide with the zero mark of the measuring tube by raising the plunger and the reservoir. The nitrogen volume is read after 5 min. A blank determination is carried out as described in the previous paper.[1] We have found a blank value of 0.15 μl in our case. The determination is repeated using a number of tubes.

Calculation

The percentage of nitrogen present in the sample is calculated using the formula:

$$\% \text{ nitrogen} = \frac{V \cdot F}{S} \times 100,\ V = f[V' - (C_1 + C_2)]$$

where

F: weight of 1 ml of nitrogen under the analytical conditions, S: weight of the sample, μg, f: factor from Table 1, V: volume of nitrogen read, μl, C_1: blank, C_2: calibration correction of the nitrometer.

DISCUSSION

A correction of 0.69% for adhesion of water to the inner wall of the nitrometer was found necessary (see data in Table 2), and the drainage time is 5 min. This correction factor changes as repeated

[1] See Ultramicrodetermination of nitrogen in organic compound. I.

TABLE 1

"*f*" FACTOR, INCLUDING: (1) CORRECTION FOR ADHESION OF DILUTE POTASSIUM HYDROXIDE SOLUTION[a], (2) TEMPERATURE CORRECTION OF BAROMETER READING, (3) CORRECTION FOR VAPOR PRESSURE OF WATER

	Temp at (mm Hg):				Temp at (mm Hg):		
f	750	760	770	*f*	750	760	770
0.979[b]	9.7	9.9	10.1	0.957	25.0	25.2	25.5
0.978	10.8	10.9	11.1	0.956	25.5	25.7	25.9
0.977	11.7	11.9	12.1	0.955	26.0	26.2	26.4
0.976	12.6	12.8	13.0	0.954	26.4	26.7	26.9
0.975	13.5	13.7	13.9	0.953	26.9	27.1	27.3
0.974	14.3	14.5	14.7	0.952	27.3	27.5	27.8
0.973	15.1	15.3	15.5	0.951	27.7	28.0	28.2
0.972	15.9	16.1	16.3	0.950	28.2	28.5	28.7
0.971	16.6	16.9	17.1	0.949	28.6	28.9	29.1
0.970	17.4	17.6	17.8	0.948	29.0	29.3	29.5
0.969	18.1	18.3	18.5	0.947	29.4	29.7	29.9
0.968	18.7	18.9	19.2	0.946	29.8	30.1	30.3
0.967	19.4	19.6	19.8	0.945	30.2	30.5	30.7
0.966	20.0	20.2	20.4	0.944	30.6	30.8	31.1
0.965	20.6	20.8	21.1	0.943	31.0	31.2	31.4
0.964	21.3	21.5	21.7	0.942	31.3	31.6	31.8
0.963	21.8	22.1	22.3	0.941	31.7	31.9	32.2
0.962	22.4	22.6	22.9	0.940	32.0	32.3	32.5
0.961	23.0	23.2	23.4	0.939	32.1	32.6	32.9
0.960	23.5	23.7	23.9	0.938	32.7	33.0	33.2
0.959	24.0	24.2	24.5	0.937	33.1	33.3	33.6
0.958	24.5	24.7	25.0	0.936	33.4	33.7	33.9
	25.0	25.2	25.5		34.0	34.3	34.5

[a] 0.7% from Table 2.

[b] For example, $f = 0.979$ corresponds to the temperature range 9.7 ~ 10.8°C at 750mm Hg, 9.9 ~ 10.9°C at 760mm Hg, and 10.1 ~ 11.1°C at 770mm Hg.

measurements are made with the same water and the concentration of potassium hydroxide increases. The volume of 50% KOH contained in the 20-mm length of capillary is about 50 μl, and the total volume of water in the nitrometer is about 70 ml; this means that after six or seven separate determinations the concentration in the water becomes about 0.5% and the correction factor is then increased to 0.72% (Table 2). Hence, a correction of 0.7% in the volume read should be made. This value depends on the ratio of the area of the inner surface of the capillary to its volume, and also on the nature of the glass surface. Accordingly, this value should be measured for each nitrometer used.

TABLE 2
CORRECTION FOR ADHESION OF DISTILLED WATER AND DILUTE ALKALI SOLUTION[a]

Time (min)		Vol (μl)	Temp (°C)
Dry	0	188.2	22.1
Wet	1	190.2	22.2
	2	189.9	22.2
	3	189.7	22.2
	4	189.6	22.2
	5	189.5	22.2
	.	.	.
	.	.	.
	.	.	.
	10	189.4	22.1

[a] $[(189.5 - 188.2)/189.5] \times 100 = 0.69\%$; 0.5% KOH soln: $0.72\% \div 0.7\%$.

TABLE 3
RESULTS OBTAINED WITH SEVERAL ORGANIC COMPOUNDS USING THE NEW NITROMETER

Sample	Sample (μg)	N_2 vol (μl)	Temp (°C)	Barom. press. (mm Hg)	Found (%)	Error[a] (%)
3-Chloro-phenothiazine	282.3	14.2	21.9	767.9	5.87	−0.12
	336.8	17.5	21.9	767.9	6.07	+0.08
	282.1	14.9	22.0	767.9	6.17	+0.18
Acetoanilide	337.7	30.3	22.4	762.9	10.36	0
	359.3	32.1	22.3	763.0	10.33	−0.03
	415.4	37.9	22.2	763.3	10.57	+0.21
	299.3	27.3	22.3	763.4	10.54	+0.18
Nicotinic acid	327.6	31.6	20.3	769.3	11.34	−0.04
	311.7	29.5	20.3	768.9	11.10	−0.28
	293.3	27.8	20.3	768.9	11.14	−0.24
5-Methyl-3-sulfanylamide isoxazole acetate	342.7	42.2	20.8	763.6	14.36	+0.13
	347.1	42.7	19.0	761.5	14.41	+0.18
Antipyrine	401.5	51.5	21.3	763.6	14.95	+0.07
Sulphathiazole	376.0	52.8	21.9	768.0	16.36	−0.10
	313.6	43.9	21.9	768.0	16.32	−0.14
	367.1	51.7	22.3	768.0	16.38	−0.08
	326.1	46.3	21.8	768.0	16.53	+0.07
Picric acid	328.3	51.9	19.6	761.8	18.50	+0.16
2,5-Dimethyl-4NH-6 chlorpyrimidine	391.6	90.6	20.5	759.2	26.87	+0.21
Thio urea	303.5	96.4	23.0	757.0	36.47	−0.33

[a] Standard deviation of error = 0.168%.

Table 3 shows the results of analyses on suitable standard compounds using the new nitrometer.

Ultramicrodetermination of nitrogen using this nitrometer is so rapid and convenient that one operator can easily carry out 16 complete analyses in a 8-hr working day in our laboratory.

This nitrometer can also be used as general volumeter for a gas determined by other methods.

It may be considered that the error which arises in measurement by a Dumas' nitrometer, due to solution of the glass by the concd alkali, is almost completely eliminated in our nitrometer in which only water is used.

SUMMARY

A new simple nitrometer has been developed for use in the ultramicrodetermination of nitrogen by the sealed-tube method. The nitrometer consists of a water-filled receiving chamber in which the capillary containing collected nitrogen is placed, and a vertical graduated measuring tube. Nitrogen in the capillary is displaced into the measuring tube by water injected through a hypodermic syringe introduced through a rubber sealing cap at the end of the receiving chamber.

ACKNOWLEDGMENT

The author is indebted to Tomo Takaoka and Yasuko Yokoyama for their skilled technical assistance.

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MICROCHEMICAL JOURNAL 19, 429–438 (1974)

Ultramicrodetermination of Nitrogen in Organic Compounds

III. Rise in Pressure of a Gas in a Nitrometer Due to the Capillary Phenomenon

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To obtain accurate and reliable results in the determination of nitrogen by the sealed-tube method, precise measurement of the gaseous nitrogen resulting from the sealed-tube combustion is essential.

A new nitrometer recently developed by us[1] has been found unsatisfactory due to its tendency to give low results with samples of high nitrogen content, and we have suggested (*5*) that although the effect of capillary action on the alkali solution can be disregarded in an ordinary micronitrometer, the effect is too big to be ignored in an ultramicronitrometer (*5*).

In the determination of nitrogen in decimilligram samples by the Dumas procedure using a Kuck nitrometer it has been suggested (*2*) that reading of the gas volume at atmospheric pressure can be facilitated by equipping the nitrometer with an external parallel tube of identical internal bore to the nitrometer tube, the final reading being taken after setting the two adjacent menisci to the same height. On the other hand, Kirsten has said (*1*) that it is best to measure the nitrogen volume without correction, then to apply a calculated average relative deviation which contains corrections for all systematic errors involved in the method.

We have found that the actual rise in pressure of the gas in a nitrometer due to capillary action agrees with results calculated by a theoretical formula, and we have shown that analytical results corrected accordingly agree well with expectations.

THE THEORETICAL FORMULA

The theory of capillarity was first developed by Lord Rayleigh (*4*) and Samuel Sugden (*6*). We are concerned here only with the rise in pressure of the gas in an ultramicronitrometer.

[1] See Ultramicrodetermination of nitrogen in organic compounds. II.

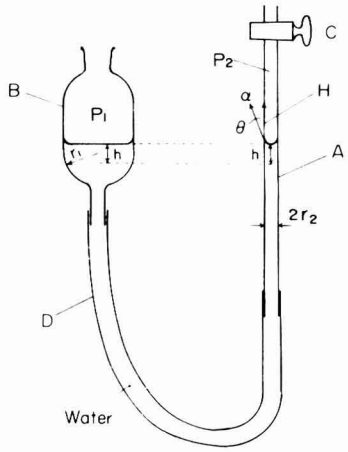

FIG. 1. Illustration of levelling operation: (A) graduated stem of nitrometer, (B) levelling bulb, (C) stopcock, (D) rubber tubing.

The basic operation of this principle is shown in Fig. 1. When stopcock C is opened, the level of the water in the capillary is drawn up higher than the level of water in the levelling bulb by the action of the surface tension of water.

The drawing force, H, on the nitrometer side can be expressed in terms of surface tension, α:

$$H = -\frac{2\pi r_2 \alpha \cos\theta}{\pi {r_2}^2} = -\frac{2\alpha \cos\theta}{r_2}. \tag{1}$$

A similar expression is obtained for the levelling bulb side:

$$H' = -\frac{2\alpha \cos\theta}{r_1}, \tag{2}$$

where the direction of the acceleration due to gravity is taken as the plus direction and the α value for water is 72.75 dyne/cm at 20°C from table (*3*).

Stopcock C is now closed, and the levelling bulb is raised until both menisci are at the same level. The atmospheric pressure, P_1 is not equal to the pressure P_2 in the nitrometer, and considering the pressure at a distance h below each meniscus we can write

$$P_1 + \rho g h - \frac{2\alpha \cos\theta}{r_1} = \rho g\left(h + \frac{r_2}{3}\right) + P_2 - \frac{2\alpha \cos\theta}{r_2}, \tag{3}$$

where r_1 = inside radius of the levelling bulb; r_2 = inside radius of the

graduated stem; θ = contact angle of water; ρ = density of water; g = acceleration due to gravity.

The meniscus in a tube of 1 mm diameter or less has a hemispherical profile, so the term $\rho g r_2/3$ has been added on the right side of Eq. (3). The contact angle of extensive organic solvent and water is equal to zero. We can hence express the pressure rise in the nitrometer tube, ΔP, as follows:

$$\Delta P = P_2 - P_1 = 2\alpha \left(\frac{1}{r_2} - \frac{1}{r_1}\right) - \rho g \frac{r_2}{3}. \tag{4}$$

EXPERIMENTAL

Observation of capillary action. As it is difficult to observe capillary action in a short, sealed capillary tube, we used a tube connected to a vessel of relatively large volume, as shown in Fig. 2. We compared the height, h_A, of a water column produced in this closed system to the height h_B produced in the tube after it had been severed from the vessel (at the dotted line d in the figure). The capillary was heated in chromic acid mixture then boiled in water and dried in vacuum before use. The height of the column was measured with a cathetometer to 0.01 mm in a constant-temperature room. Height h_A is nor-

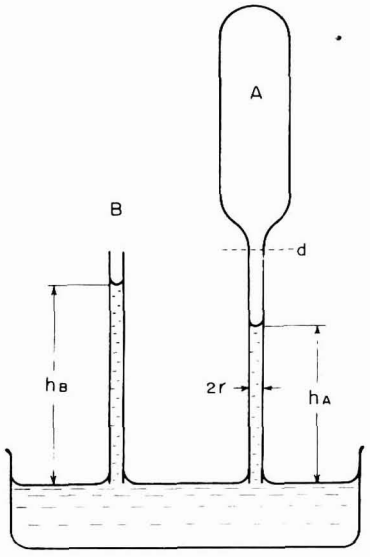

FIG. 2. Observation of capillary phenomenon: (A) vessel, (B) capillary cut off at the dotted line (d) from the vessel (A).

TABLE 1
COMPARISON OF THE HEIGHT OF WATER COLUMNS PRODUCED BY CAPILLARY ACTION IN CLOSED AND OPEN CAPILLARY TUBES

Tube no.	Vol of A (ml)	Inside radius of capillary, r (mm)	Height of water column			Difference, $h_B \sim h_C$ (mm)
			h_A (mm)	h_B (mm)	h_C (mm)	
1	103.91	0.406	32.20	35.40	36.56	1.16
2	51.28	0.386	29.70	38.50	38.47	0.03
3	32.14	0.419	24.00	35.50	35.38	0.12

mally less than h_B because of the pressure rise in vessel A. Further, h_A decreases with decreasing volume A. Height H_B agreed to within 1 mm with the height h_C calculated from the term $h + r/3$ $(=2\alpha/\rho g r)$, for capillary action (see data in Table 1).

Table 2 shows a comparison of the rise in pressure produced by the capillary action in tubes of differing bore. ΔP_A is the rise in pressure caused by compression of volume A by the water column h_A, plus the rise in pressure $\rho g h_A$ produced by the levelling procedure (see Fig. 2 and Table 1). ΔP_B is the pressure rise $\rho g h_B$ produced by the levelling procedure. The calculated values, ΔP_C, are in fair agreement with the experimental values, ΔP_B, and the experimental values ΔP_A are somewhat less than the other two (see data in Table 2). It is confirmed that the rise in pressure of a gas in a nitrometer tube having a bore of about 1 mm or less, produced in the levelling procedure by capillary action, is not negligible.

Procedure for determination. The nitrometer is designed as shown in Fig. 3. It is essentially of three parts, a calibrated measuring tube (M), an external parallel tube of the same internal bore (E), and a receiving chamber (R). In our apparatus the internal bores of M and E are 0.901 mm and 0.993 mm, respectively. Nitrogen is collected in the measuring tube as described previously[1] and its volume is read

TABLE 2
RISES IN PRESSURE IN CLOSED CAPILLARY TUBES OF DIFFERENT BORES

Tube no.	Pressure raising (mm Hg)			Difference (mm Hg)	
	ΔP_A	ΔP_B	ΔP_C	$\Delta P_A \sim \Delta P_C$	$\Delta P_B \sim \Delta P_C$
1	2.48	2.59	2.68	0.20	0.09
2	2.38	2.82	2.82	0.44	0
3	2.07	2.60	2.59	0.52	0.01

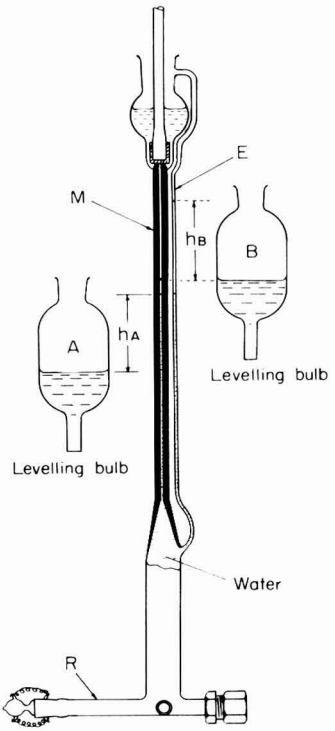

FIG. 3. Illustration of nitrometer: (A) level A, (B) level B, (M) calibrated measuring tube, (E) external parallel tube, (R) receiving chamber.

after 5 min. The levelling bulb is first adjusted to position A (see Fig. 3) at which the menisci in the two capillary tubes are at the same level. The volume nitrogen, V_A, is now the volume at atmospheric pressure. V_A and the height of the water column h_A are measured, then the levelling bulb is raised to position B at which the level of water in the bulb coincides with that in the measuring tube. The new nitrogen volume V_B and the difference in height between the two capillaries h_B are now measured, V_B being the volume at a pressure of atmospheric pressure plus the pressure rise due to capillarity.

Calculation of nitrogen content was made using both volumes, V_A and V_B, and the results were compared.

DISCUSSION OF RESULTS

A series of results of analyses carried out by the procedure described above are given in Table 3. The results calculated from V_A are in fair agreement with those from V_B.

Table 4 shows a comparison of capillary phenomenon on a cali-

TABLE 3

DECIMILLIGRAM DETERMINATION OF NITROGEN IN SUITABLE ORGANIC COMPOUNDS[a]

Sample	Wt of sample (μg)	Temp (°C)	Vol of nitrogen (μl)	Press. (mm Hg)	N_2 (%) Found[b]	Calcd	Dev
2.5-Dimethyl-4-amino-6-chlor pyrimidine	346.4	29.1	V_A,[c] 81.4	759.6	26.58	26.66	−0.08
			V_B,[c] 81.3	762.0	26.62		−0.04
	322.2	29.1	V_A, 76.5	759.5	26.85		0.19
			V_B, 76.3	761.9	26.86		0.20
5-Methyl-7-hydroxy-1.2.4-triazolo[2.3-*a*] pyrimidine	345.9	28.7	V_A, 114.8	757.7	37.49	37.32	0.17
			V_B, 114.4	760.1	37.44		0.12
	295.9	28.8	V_A, 97.6	757.5	37.26		−0.06
			V_B, 97.2	759.9	37.21		−0.11
	332.8	28.8	V_A, 110.4	757.3	37.46		0.14
			V_B, 110.2	759.7	37.44		0.12
Sulfathiazole	317.7	28.8	V_A, 46.3	755.0	16.38	16.46	−0.08
			V_B, 46.2	757.4	16.39		−0.07
	353.2	28.7	V_A, 51.5	755.0	16.42		−0.04
			V_B, 51.5	757.4	16.43		−0.03
Vitamin B_1 dipicrate	295.6	28.8	V_A, 50.7	755.0	19.29	19.36	−0.07
			V_B, 50.6	757.4	19.31		−0.05
	286.4	28.3	V_A, 48.7	759.9	19.24		−0.12
			V_B, 48.6	762.3	19.25		−0.11

[a] The formulae were established by other analyses, carried out in routine work.

[b] See the calculation of "Ultramicrodetermination of nitrogen in organic compounds. II".

[c] Standard deviation of error calculated from V_A: 0.123%. Standard deviation of error calculated from V_B: 0.113%.

TABLE 4

A COMPARISON OF CAPILLARY PHENOMENON IN A CALIBRATED MEASURING TUBE AND A EXTERNAL PARALLEL TUBE[a]

Expt	h_A mm	h_A mm Hg	h_B mm	h_B mm Hg	$h_A \sim h_B$ (mm Hg)
1	29.5	2.2	24.0	1.8	0.4
2	30.0	2.2	26.5	2.0	0.2
3	27.5	2.0	26.0	1.9	0.1
4	28.0	2.1	27.0	2.0	0.1
5	27.0	2.0	25.5	1.9	0.1
6	28.0	2.1	26.5	2.0	0.1
7	28.0	2.1	27.0	2.0	0.1
8	26.0	1.9	25.5	1.9	0
9	27.5	2.0	26.5	2.0	0
Mean	27.9 mm	2.1 mm Hg	26.1 mm	1.9 mm Hg	0.12 mm Hg
σ	1.21 mm	0.087 mm Hg	0.53 mm	0.071 mm Hg	0.12 mm Hg

[a] h_A: Value from calibrated measuring tube (ΔP value from Eq. (4), at 20°C: 2.3 mm Hg). h_B: Value from external parallel tube (ΔP value from Eq. (4), at 20°C: 2.1 mm Hg).

TABLE 5
DECIMILLIGRAM DETERMINATION OF NITROGEN IN A SERIES OF SEVERAL ORGANIC COMPOUNDS[a]

Expt	Sample	Wt of sample (μg)	Temp (°C)	Vol of nitrogen (μl)	Press. (mm Hg)	Found (%)	Error[b] (%)	Uncorrection Found (%)	Uncorrection Error (%)	Difference (%)
1	Acetanilide	364.4	24.4	V_A 32.4	765.7	10.27	−0.09	10.24	−0.12	−0.02
				V_B 32.3	767.7	10.26	−0.10			
2		301.1	24.5	V_A 27.2	765.7	10.46	+0.10	10.39	+0.03	−0.03
				V_B 27.0	767.7	10.42	+0.06			
3	Antipyrine	363.0	24.3	V_A 47.1	765.8	15.02	+0.14	14.93	+0.05	−0.04
				V_B 46.8	767.8	14.97	+0.09			
4		323.7	24.3	V_A 41.6	765.7	14.85	−0.03	14.82	−0.06	−0.04
				V_B 41.5	767.7	14.86	−0.02			
5	Sulfathiazole	317.7	28.8	V_A 46.2	755.0	16.32	−0.14	16.28	−0.18	−0.05
				V_B 46.1	757.0	16.33	−0.13			
6		263.5	24.7	V_A 37.6	763.9	16.48	+0.02	16.44	−0.02	−0.06
				V_B 37.6	765.9	16.50	+0.04			
7	Picric acid	377.7	24.3	V_A 59.5	765.6	18.23	−0.11	18.20	−0.14	−0.04
				V_B 59.4	767.6	18.24	−0.10			
8		319.3	23.9	V_A 50.7	764.8	18.37	+0.03	18.30	−0.04	−0.05
				V_B 50.5	766.8	18.35	+0.01			
9	Vitamin B_1 dipicrate	295.6	28.8	V_A 50.6	755.1	19.22	−0.14	19.19	−0.17	−0.05
				V_B 50.5	757.1	19.24	−0.12			
10		286.4	28.3	V_A 48.6	759.9	19.21	−0.15	19.17	−0.19	−0.05
				V_B 48.5	761.9	19.22	−0.14			
11	5-Methyl-6-chloro-7-methylmercapto-*s*-triazolo[2.3-*a*]pyrimidine	313.5	23.9	V_A 71.1	767.6	26.36	+0.26	26.29	+0.19	−0.06
				V_B 70.9	769.6	26.35	+0.25			
12		349.9	24.3	V_A 79.6	765.6	26.31	+0.21	26.21	+0.11	−0.07
				V_B 79.3	767.6	26.28	+0.18			
13	2.5-Dimethyl-4-amino-6-chlor pyrimidine	346.4	29.1	V_A 81.3	759.6	26.52	−0.14	26.49	−0.17	−0.07
				V_B 81.2	761.6	26.56	−0.10			
14		322.2	29.1	V_A 76.3	759.5	26.79	+0.13	26.72	+0.06	−0.07
				V_B 76.2	761.5	26.79	+0.13			
15	Thiourea	322.8	26.5	V_A 103.8	768.3	37.06	+0.26	36.92	+0.12	−0.10
				V_B 103.5	770.3	37.02				
16		291.6	26.8	V_A 94.9	759.1	37.00	+0.20	37.92	+0.12	−0.10
				V_B 94.7	761.1	37.02	+0.22			
17	5-Methyl-7-hydroxy-1.2.4-triazolo[2.3-*a*]-pyrimidine	332.8	28.8	V_A 110.2	757.3	37.28	+0.04	37.22	−0.10	−0.10
				V_B 110.0	759.3	37.32	0			
18		352.9	26.8	V_A 115.2	761.4	37.21	−0.11	37.12	−0.20	−0.10
				V_B 115.0	763.4	37.22	−0.10			
19		267.3	26.5	V_A 86.6	768.3	37.33	+0.01	37.24	−0.08	−0.11
				V_B 86.4	770.3	37.35	+0.03			
20		345.9	28.6	V_A 114.5	757.7	37.40	+0.08	37.27	−0.05	−0.10
				V_B 114.2	759.7	37.37	+0.05			
21	4-Aminopyrimidine	330.1	23.4	V_A 125.3	769.5	44.29	+0.10	44.19	0	−0.11
				V_B 125.0	771.5	44.30	+0.11			
22		320.9	23.8	V_A 122.0	768.3	44.18	−0.01	43.97	−0.22	−0.11
				V_B 121.5	770.3	44.08	−0.11			
23	3-Amino-4-allyl-4H-*s*-triazole	295.7	24.1	V_A 115.3	768.3	45.29	+0.16	45.07	−0.06	−0.11
				V_B 114.7	770.3	45.18	+0.05			
24		328.0	24.1	V_A 127.6	768.0	45.18	+0.05	45.05	−0.08	−0.11
				V_B 127.2	770.0	45.16	+0.03			
25	4.6-Diaminopyrimidine	230.8	23.7	V_A 100.2	769.5	50.66	−0.22	50.47	−0.41	−0.13
				V_B 99.8	771.5	50.60	−0.28			
26		347.1	23.6	V_A 152.6	762.1	50.76	−0.12	50.63	−0.25	−0.13
				V_B 152.2	764.1	50.76	−0.12			
27	Dicyanodiamide	227.6	24.2	V_A 131.1	767.8	66.90	+0.26	66.75	+0.11	−0.18
				V_B 130.8	769.8	66.93	+0.29			
28		242.2	24.1	V_A 139.3	767.7	66.81	+0.17	66.58	−0.06	−0.18
				V_B 138.8	769.7	66.76	+0.12			

[a] Bore of measuring tube: 0.99 mm.

[b] Standard deviation of error calculated from V_A: 0.143%; standard deviation of error calculated from V_B: 0.139%.

brated measuring tube and a external parallel tube. The observed heights agree with the values calculated by Eq. (4).

It was found that the results obtained by author's method, which considers the rise in pressure produced by capillarity, agreed very closely with those obtained by external parallel tube method (*2*). Hence, using three nitrometers having three different capillary tube bores, we determined the nitrogen in a series of compounds containing 10–70% nitrogen. Tables 5 and 7 show the results using the

TABLE 6
DECIMILLIGRAM DETERMINATION OF NITROGEN IN A SERIES OF SEVERAL ORGANIC COMPOUNDS[a]

Expt	Sample	Wt of sample (μg)	Temp (°C)	Vol of nitrogen (μl)	Press. (mm Hg)	Found (%)	Error[b] (%)	Uncorrection Found (%)	Uncorrection Error (%)	Difference (%)
1	Acetanilide	329.5*	23.9	28.7	771.4	10.17	−0.19	10.13	−0.23	−0.04
2		340.5	24.0	29.7	771.4	10.17	−0.19	10.15	−0.21	−0.02
3	Antipyrine	341.1	23.9	42.8	771.9	14.65	−0.23	14.59	−0.29	−0.06
4		317.0	23.8	40.0	771.1	14.70	−0.18	14.64	−0.24	−0.06
5	Sulfathiazole	321.3	24.8	45.0	768.4	16.23	−0.23	16.17	−0.29	−0.06
6		385.8	22.8	53.8	772.0	16.35	−0.11	16.27	−0.19	−0.08
7	Picric acid	336.8	24.2	52.1	771.4	18.06	−0.28	17.99	−0.35	−0.07
8		294.2	22.9	45.8	771.8	18.29	−0.05	18.17	−0.17	−0.12
9	Vitamin B_1 dipicrate	309.1	24.1	51.2	768.6	19.26	−0.10	19.19	−0.17	−0.07
10		301.2	24.1	49.9	768.4	19.23	−0.13	19.18	−0.18	−0.05
11	5-Methyl-6-chloro-7-methylmercapto-*s*-triazolo[2.3-*a*]pyrimidine	343.1	24.3	77.7	771.4	26.38	+0.28	26.30	+0.20	−0.08
12		320.8	22.9	71.6	771.8	26.15	+0.05	26.05	−0.05	−0.10
13	2.5-Dimethyl-4-amino-6-chlor pyrimidine	306.2	24.3	69.9	771.3	26.58	−0.08	26.47	−0.19	−0.11
14		340.9	24.4	78.3	771.3	26.75	+0.09	26.63	−0.03	−0.12
15	Thiourea	327.4	24.1	103.6	771.6	36.96	+0.16	36.80	+0	−0.16
16		379.5	24.1	120.1	771.6	36.94	+0.14	36.81	+0.01	−0.13
17	5-Methyl-7-hydroxy-1.2.4-triazolo[2.3-*a*]pyrimidine	316.0	24.0	101.2	771.2	37.36	+0.04	37.20	−0.12	−0.16
18		321.7	24.2	103.3	771.3	37.46	+0.14	37.30	−0.02	−0.16
19	5-Methyl-7-hydroxy-1.2.4-triazolo[2.3-*a*]-pyrimidine	329.4	23.5	105.5	773.0	37.49	+0.17	37.36	+0.04	−0.13
20		325.3	22.9	104.1	770.8	37.46	+0.14	37.30	−0.02	−0.16
21	4-Aminopyrimidine	283.4	23.4	107.2	769.7	44.12	−0.07	43.95	−0.24	−0.17
22		301.8	23.6	114.5	768.7	44.22	+0.03	44.04	−0.15	−0.18
23	3-Amino-4-allyl-4H-*s*-triazole	317.9	23.2	123.5	770.6	45.44	+0.31	45.27	+0.14	−0.17
24		299.8	23.3	116.5	770.6	45.38	+0.25	45.22	+0.09	−0.16
25	4.6-Diaminopyrimidine	301.2	24.1	131.8	770.5	51.03	+0.15	50.83	−0.05	−0.20
26		260.7	24.2	113.4	770.5	50.71	−0.17	50.51	−0.37	−0.20
27	Dicyanodiamide	206.4	23.5	118.5	769.2	66.87	+0.23	66.61	−0.03	−0.26
28		208.1	23.6	119.6	769.0	66.97	+0.33	66.70	+0.06	−0.27

[a] Bore of measuring tube: 0.73 mm.
[b] Standard deviation of error: 0.183%.

TABLE 7

DECIMILLIGRAM DETERMINATION OF NITROGEN IN A SERIES OF SEVERAL ORGANIC COMPOUNDS[a]

Expt	Sample	Wt of sample (μg)	Temp (°C)	Vol of nitrogen (μl)	Press. (mm Hg)	Found (%)	Error[b] (%)	Uncorrection Found (%)	Uncorrection Error (%)	Difference (%)
1	Acetanilide	309.4	23.9	V_A 27.5	764.7	10.30	−0.06	10.23	−0.13	−0.08
				V_B 27.4	770.1	10.31	−0.05			
2		323.0	23.9	V_A 28.5	764.6	10.21	−0.15	10.14	−0.22	−0.08
				V_B 28.3	770.0	10.22	−0.14			
3	Antipyrine	337.2	24.0	V_A 43.2	765.0	14.81	−0.07	14.72	−0.16	−0.09
				V_B 42.9	769.5	14.81	−0.07			
4		336.0	23.8	V_A 43.0	765.0	14.82	−0.06	14.72	−0.16	−0.09
				V_B 42.7	769.5	14.81	−0.07			
5	Sulfathiazole	265.6	25.2	V_A 37.9	765.0	16.48	+0.02	16.31	−0.15	−0.11
				V_B 37.6	770.4	16.42	−0.04			
6		365.3	26.6	V_A 53.0	763.9	16.62	+0.16	16.50	+0.04	−0.11
				V_B 52.6	769.3	16.61	+0.15			
7	Picric acid	296.2	23.9	V_A 46.4	765.1	18.11	−0.23	18.00	−0.34	−0.12
				V_B 46.1	769.6	18.12	−0.22			
8		305.3	23.8	V_A 48.0	765.0	18.19	−0.15	18.08	−0.26	−0.12
				V_B 47.7	769.5	18.20	−0.14			
9	Vitamin B_1 dipicrate	271.6	24.5	V_A 45.2	765.4	19.21	−0.15	19.09	−0.27	−0.13
				V_B 44.9	769.9	19.22	−0.14			
10		348.6	24.5	V_A 58.2	765.3	19.29	−0.07	19.17	−0.19	−0.12
				V_B 57.8	769.8	19.29	−0.07			
11	5-Methyl-6-chloro-7-methylmercapto-*s*-triazolo[2.3-*a*]pyrimidine	324.7	23.5	V_A 73.0	765.0	26.05	−0.05	25.91	−0.19	−0.17
				V_B 72.6	769.5	26.08	−0.02			
12		270.9	23.7	V_A 61.2	764.9	26.20	+0.10	26.08	−0.02	−0.14
				V_B 61.0	769.4	26.22	+0.12			
13	2.5-Dimethyl-4-amino-6-chlor pyrimidine	292.9	25.7	V_A 67.8	763.1	26.59	−0.07	26.48	−0.18	−0.21
				V_B 67.5	768.5	26.69	+0.03			
14		354.6	25.5	V_A 82.4	762.3	26.66	0	26.55	−0.11	−0.14
				V_B 82.1	766.8	26.69	+0.03			
15	Thiourea	203.7	23.4	V_A 64.8	765.1	36.89	+0.09	36.68	−0.12	−0.24
				V_B 64.5	769.6	36.92	+0.12			
16		202.4	23.0	V_A 64.0	767.5	36.84	+0.04	36.63	−0.17	−0.19
				V_B 63.6	772.0	36.82	+0.02			
17	5-Methyl-7-hydroxy-1.2.4-triazolo[2.3-*a*]pyrimidine	267.6	25.4	V_A 86.5	763.6	37.17	−0.15	36.99	−0.33	−0.18
				V_B 86.1	768.1	37.17	−0.15			
18		206.2	23.1	V_A 66.4	767.1	37.47	+0.15	37.25	−0.07	−0.24
				V_B 66.0	771.6	37.49	+0.17			
19		189.2	23.4	V_A 60.9	767.9	37.47	+0.15	37.21	−0.11	−0.27
				V_B 60.5	773.3	37.48	+0.14			
20		190.6	23.4	V_A 61.4	767.9	37.49	+0.17	37.24	−0.08	−0.26
				V_B 61.0	773.3	37.50	+0.18			
21	4-Aminopyrimidine	185.2	23.4	V_A 70.9	766.6	44.46	+0.27	44.22	+0.03	−0.23
				V_B 70.5	771.1	44.45	+0.26			
22		187.8	23.3	V_A 71.4	766.6	44.20	+0.01	43.96	−0.23	−0.23
				V_B 71.1	771.1	44.19	0			
23	3-Amino-4-allyl-4H-*s*-triazole	172.9	24.0	V_A 67.4	765.0	45.09	−0.04	44.84	−0.29	−0.29
				V_B 67.0	769.5	45.13	0			
24		190.6	23.5	V_A 73.7	766.1	44.90	−0.23	44.67	−0.46	−0.29
				V_B 73.4	770.6	44.96	−0.17			
25	4.6-Diaminopyrimidine	151.4	23.7	V_A 66.2	765.0	50.68	−0.20	50.39	−0.49	−0.33
				V_B 65.8	769.4	50.72	−0.16			
26		136.9	24.2	V_A 59.9	765.2	50.67	−0.21	50.34	−0.54	−0.33
				V_B 59.6	769.7	50.67	−0.21			
27	Dicyanodiamide	121.6	23.3	V_A 69.5	765.1	66.29	−0.35	65.92	−0.72	−0.44
				V_B 69.2	769.6	66.36	−0.28			
28		226.1	23.5	V_A 130.3	764.4	66.79	+0.15	66.43	−0.21	−0.45
				V_B 129.6	768.9	66.88	+0.24			

[a] Bore of measuring tube: 0.48 mm.

[b] Standard deviation of error calculated from V_A: 0.151%; standard deviation of error calculated from V_B: 0.146%.

nitrometer illustrated in Fig. 3; Table 6 gives the results using the nitrometer we described previously[1], taking into account the rise in pressure produced by capillary phenomenon.

It was found that as the inner diameter of graduated stem decreases, there is an increasing tendency for low results to be obtained if the capillary effect is neglected. This tendency also increases with increasing nitrogen content.

SUMMARY

In using a nitrometer having a measuring tube of less than 1 mm bore for the determination of nitrogen in decimilligram samples, the question of rise in pressure due to capillarity becomes important. The present work has shown that application of an equation to calculate the rise in pressure gives results which are accurate and reliable.

ACKNOWLEDGMENT

The author is indebted to Tomo Takaoka and Yasuko Yokoyama for their skilled technical assistance.

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ERRATUM

Volume 19, No. 1 (1974), in the article, "The Microdetermination of Molecular Weight by Vapor Pressure Equilibrium," by H. SWIFT, pp. 18–25:

Page 21, *Calculation,* line 1 should read: Use the formula $M_u = M_k W_u S_k / W_k S_u$. . . .

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2. Pease, D. C., "Histological Techniques for Electron Microscopy," 274 pp. Academic Press, New York, 1960.
3. Stern, K. C., Electrophoresis and ionophoresis. *In* "Physical Techniques in Biological Research" (G. Oster, ed.), Vol. 2, pp. 61–121. Academic Press, New York, 1956.

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