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INTRODUCTION TO AIR CHEMISTRY

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Seattle, Washington

Here is a book on atmospheric chemistry specifically designed for those with no experience in the field — one that will introduce the reader to *successful* approaches to this important field. It covers air pollution, chemical cycles, and the chemical and physical behavior of trace atmospheric constituents. It uses the principles of both analytical chemistry and meteorology to cope with the most crucial problems of our atmospheric environment.

The first two chapters introduce the reader to air chemistry and outline the basic chemical and meteorological principles used throughout the book. The authors then discuss general methods of obtaining and evaluating air chemical data, emphasizing some of the analytical methods now available. Next, they deal with three main classes of chemical compounds (those containing sulfur, nitrogen, and carbon) which must be considered in the study of atmospheric trace constituents — covering significant atmospheric reactions, global budgets, and selected methods of analyzing these compounds. The final chapter describes the physical characteristics of aerosols.

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MICROCHEMICAL JOURNAL 18, 101–106 (1973)

Spectrophotometric Determination of Salicylamide In Complex Mixtures

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Received October 26, 1972

Salicylamide is widely used as an analgesic antipyretic and antirheumatic drug. It is generally administered in the form of tablets or capsules which contain several other compounds like phenacetin, caffeine, ascorbic acid, etc. During the past 20 yr, several methods for assaying salicylamide have become available. Some of the important methods are the following: gravimetric (*5*), bromatometric (*2, 3, 13, 18*), column chromatographic separation of salicylamide (*4, 6, 8*), separation of salicylamide by ion-exchange column chromatography and subsequent spectrophotometric determination (7) spectrophotometric determination (*1, 10, 16, 17, 19*), infrared spectroscopy (*12*), hydrolysis followed by photometric determination of the iron complex of the resulting salicylic acid (*3, 6*), colorimetric and photometric methods (*9, 11, 14*), etc. From among the photometric methods, the assay procedures recommended by Murai (*14*) and Masayoshi, Shigeo and Masou (*11*) are especially important.

In this paper we would like to report a new spectrophotometric method for the determination of minute amounts of salicylamide in multicomponent mixtures. Prior to this work it had been reported (*11, 14*) that the reaction between salicylamide and dimethyl-*p*-phenylenediamine in the presence of oxidizing agents led to the formation of a blue dye which gave a characteristic absorption maximum at 650 nm. We discovered that an isopropyl alcohol-water solution of salicylamide at pH 8 forms a blue colored indophenol derivative with *N*,2,6-trichloro-*p*-benzoquinoneimine. This dye was found to be stable enough for photometric determination. Figure 1 shows the absorption curve for the above dye at pH 8 using isopropyl alcohol-water as solvents.

The following mixtures were assayed by this method:

I. Salicylamide	37.70 g
Phenacetin	18.60 g
Caffeine	2.33 g

	Chloropheniramine maleate	0.25 g
	Ascorbic acid	16.15 g
	Ethylcellulose	0.91 g
	Cellulose prod.	11.90 g
	Starch	5.22 g
	Polyvinyl pyrrolidone	2.73 g
	Silicon dioxide	2.00 g
	Magnesium stearate	2.20 g
II.	Salicylamide	40.60 g
	Potassium-*p*-aminobenzoic acid	30.60 g
	Thiamine mononitrate	6.88 g
	Sodium ascorbate	7.70 g
	Cellulose prod.	7.08 g
	Silicon dioxide	1.38 g
	Hydrogenated vegetable oil	2.00 g
	Ethylcellulose	3.76 g
III.	Salicylamide	14.30 g
	Thyroid USP	13.40 g
	Starch	10.00 g
	Lactose	23.70 g
	Calcium phosphate dibasic	31.90 g
	Calcium stearate	1.00 g
	Silicon dioxide	0.50 g
	Water	0.74 g
IV.	Salicylamide	29.57 g
	Methapyrilene HCl	3.89 g
	Pyrilamine maleate	2.47 g
	Scopolamine aminoxide HBr	0.02 g
	Niacinamide	10.20 g
	Thiamine mononitrate	1.48 g
	Riboflavin	1.02 g
	Pyridoxine HCl	0.16 g
	Passion flower extr.	1.85 g
	Polyvinyl pyrrolidone	4.86 g
	Calcium phosphate dibasic	32.40 g
	Starch	4.36 g
	Lactose	0.54 g
	Magnesium stearate	4.57 g
	Stearic acid	1.31 g
	Silicon dioxide	0.43 g
	Talc USP	0.87 g

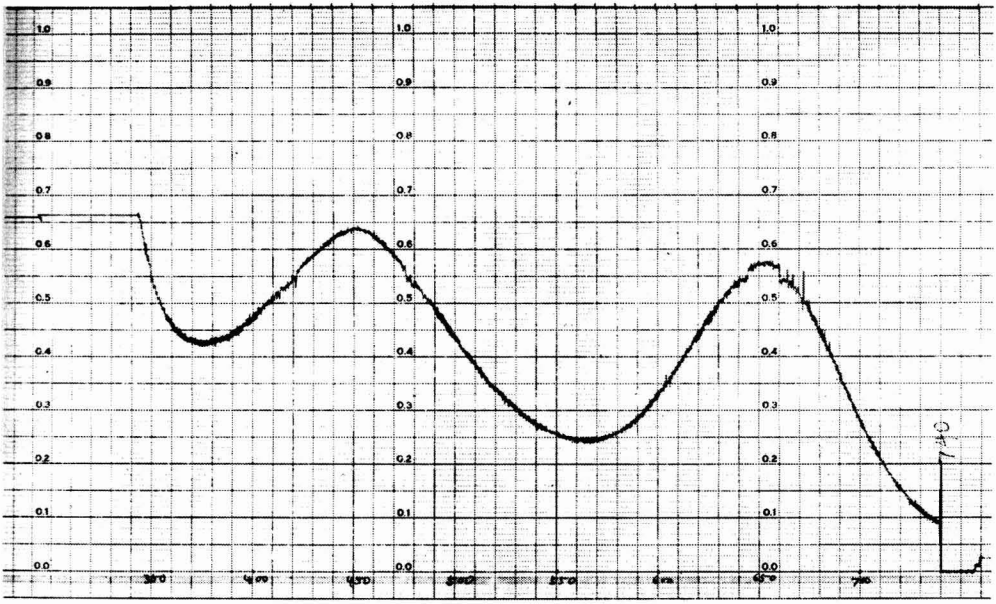

FIG. 1. Absorption spectrum of the indophenol derivative from salicylamide and *N*,2,6-trichloro-*p*-benzoquinoneimine.

EXPERIMENTAL METHODS

Reagents. All chemicals and reagents used in this work were either USP or NF grade. *N*,2,6-Trichloro-*p*-benzoquinoneimine was purchased from Eastman Kodak Co., and used without further purification.

Preparation of buffer solutions. (a) A solution of 16 g of ammonium chloride in water was added to a 100 ml measuring flask. After the addition of 16 ml of concd ammonium hydroxide (sp gr 0.910) to the flask, the mixture was diluted with water to 100 ml. (b) A 20% solution of sodium acetate in water was prepared separately.

N,2,6-Trichloro-p-benzoquinoneimine. A solution of 10 mg of *N*, 2,6-trichloro-*p*-benzoquinoneimine in 25 ml of isopropyl alcohol was prepared. This solution must be prepared freshly before use.

Salicylamide stock solution. A solution of 40 mg of salicylamide in about 150 ml of water was prepared. After adjusting the pH to 8.5–9.0, the solution was further diluted to 200 ml with water.

Salicylamide standard solution: The above stock solution (10 ml) was diluted to 100 ml with spectroscopy grade isopropyl alcohol.

Preparation of salicylamide mixture. The commercially available salicylamide mixtures (tablets) were ground into a fine powder and

dissolved in 150 ml of water. The pH of the solution was adjusted to 8.5–9.0. After stirring the solution for 20–30 min (using a magnetic stirrer) it was diluted to 200 ml with water. The above solution was filtered through a dry filter paper (Whatman No. 41) and the first 30 ml of the filtrate were discarded. The clear filtrate (10 ml) was diluted to 100 ml with spectroscopy grade isopropyl alcohol.

Estimation procedure. The salicylamide solution (5 ml) to be assayed was placed in a flask fitted with a ground glass stopper. To this solution were added 1 ml of the NH_4OH–NH_4Cl buffer and 1 ml of sodium acetate solution and finally 1 ml of the *N*,2,6-trichloro-*p*-benzoquinoneimine reagent solution. The mixture was shaken for about 10 sec. The standard salicylamide solution (5 ml) was treated with the buffer and the sodium acetate solution in the same way. After a few minutes both the solutions developed blue-green color. The color intensity was found to depend on the pH and the concentration and quality of the *N*,2,6,-trichloro-*p*-benzoquinoneimine reagent solution. The amount of salicylamide in each solution was determined using Beckman D.U. and Carey-14 uv spectrophotometers. The readings were taken at 650 nm using 1 cm cells and isopropyl alcohol as blank. The results are given in Table 1.

DISCUSSION

The assay results reported above indicate that the reaction between *N*,2,6-trichloro-*p*-benzoquinoneimine and salicylamide in isopropanol–water solution at pH 8.5–9.0 can be used to determine even minute amounts (0.1 mg) of salicylamide in the presence of several foreign materials such as phenacetin, caffeine, chlorpheniramine maleate, ascorbic acid, *p*-aminobenzoic acid, thyroid and several vitamins. One distinct advantage of using this method is that ascorbic acid, which is known to interfere with other colorimetric methods of estimating salicylamide, does not interfere with this method.

TABLE 1

ABSORPTION DATA OF SALICYLAMIDE-INDOPHENOL DERIVATIVE AT 650 nm

	Absorbance			
Mixture no.:	I	II	III	IV
Standard	0.340	0.580	0.225	0.520
Sample	0.330–0.350	0.570–0.590	0.222–0.227	0.510–0.530
Accuracy (%)	97–103	98–102	99–101	98–102

According to our initial observations this procedure is suitable for the determination of many phenol derivatives. Work on the determination of phenylephrine and 8-hyroxyquinoline is underway.

SUMMARY

This paper describes a photometric assay procedure for the determination of small amounts of salicylamide in the presence of a variety of foreign substances. This method is based on the formation of a blue colored indophenol derivative from the reaction of salicylamide with *N*,2,6-trichloro-*p*-benzoquinoneimine.

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Nitrogen Determination with a Modified Coleman Nitrogen Analyzer

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INTRODUCTION

The long duration of a microdetermination and the difficulties to obtain well trained microanalysts, have made it desirable, if not imperative, to change the classical micromethods of F. Pregl.

The Coleman analyzer is an apparatus without the difficulties mentioned above, since a determination takes only 10 min, and an unskilled analyst can perform determinations after a few hours of training. However, the basic principles of the Dumas determination remained unchanged. It is not surprising therefore that the previous difficulties have been observed with the Coleman instrument too.

The lack of oxidative possibilities can cause positive errors when hydrocarbons enter the nitrometer, and negative results if nitrogen-containing residues remain in the combustion tube (*9, 18, 23*). These difficulties have long been known, and different efforts have been made to improve the combustion. The most important are: Increasing the combustion temperature (*12*); moistening the sweeping gas (*25*) and use of more effective combustion catalysts (*26*). Users of the Coleman analyzer too are familiar with the failures of their apparatus and published a lot of improvements (*2–4, 6, 7, 13, 15, 16, 22, 24*).

From our side, we experienced that improvements were possible at several points in the instrument. In order to have at our disposal a fast, accurate and universal apparatus, we made the following modifications:

1. Improvements of the combustion system:
   Use of oxygen as combustion gas
   Use of a larger combustion tube
   Separation of the furnaces
   Heating of the long stainless steel tube connecting the combustion with the postheater tube.

2. Improvements of the reduction system:
   Vertical placement of the new furnace
   Choice of a bigger tube with a more active filling.

3. Improvements of the end-point detection system and calculations:
   Removal of the back lash of the syringe
   Replacement of the O-ring in the syringe by a Kapseal.
   Use of a new syringe head with special valves
   Use of an Ellab thermometer
   Displacement and conditioning of the nitrometer
   Simplified calculations.

MATERIALS AND METHODS

Improvements of the Combustion System

In our carbon hydrogen determination (*19–21*) no difficulties have been encountered even with the so called "difficultly combustible substances." Thus it was obvious to submit the nitrogen analyzer to an equal combustion system.

The combustion in an oxygen atmosphere makes combustion tube filling in the vicinity of the *upper* furnace superfluous. This filling is largely responsible for fluctuations of the blank values.

Figure 1 shows the mechanical connection making it possible to burn in an oxygen atmosphere. The solenoid valve SV-3 is connected in such a way that it works with the program shown in Table 1.

During the first run without a temporary filling, we already found that the upper neoprene washer, holding the combustion tube, was exposed to an unusual high temperature. This resulted in high blank values and fast alteration of the washer. This trouble was avoided by mounting the upper combustion tube support 6 cm higher and using a 41 cm combustion tube instead of the original 35 cm one (Fig. 1B).

An extensive investigation was made regarding the different possibilities of combustion tube fillings. Cupric oxide supplied by Coleman, Carlo Erba and Merck; CO_3O_4 from Carlo Erba, $AgVO_3$ from Coleman and CeO_2 from Riedel de Haën were tried. A filling of CuO according to Pella (*17*) and CeO_2 used by Monar (*14*) gave the best satisfaction (Fig. 1B).

At this time we are using a gas cylinder as a source of highly pure oxygen (99.998%) although we started with an electrolytic cell. After a short time it seemed to us that the ozone delivered by the cell, although attractively high in concentration, was a disadvantage.

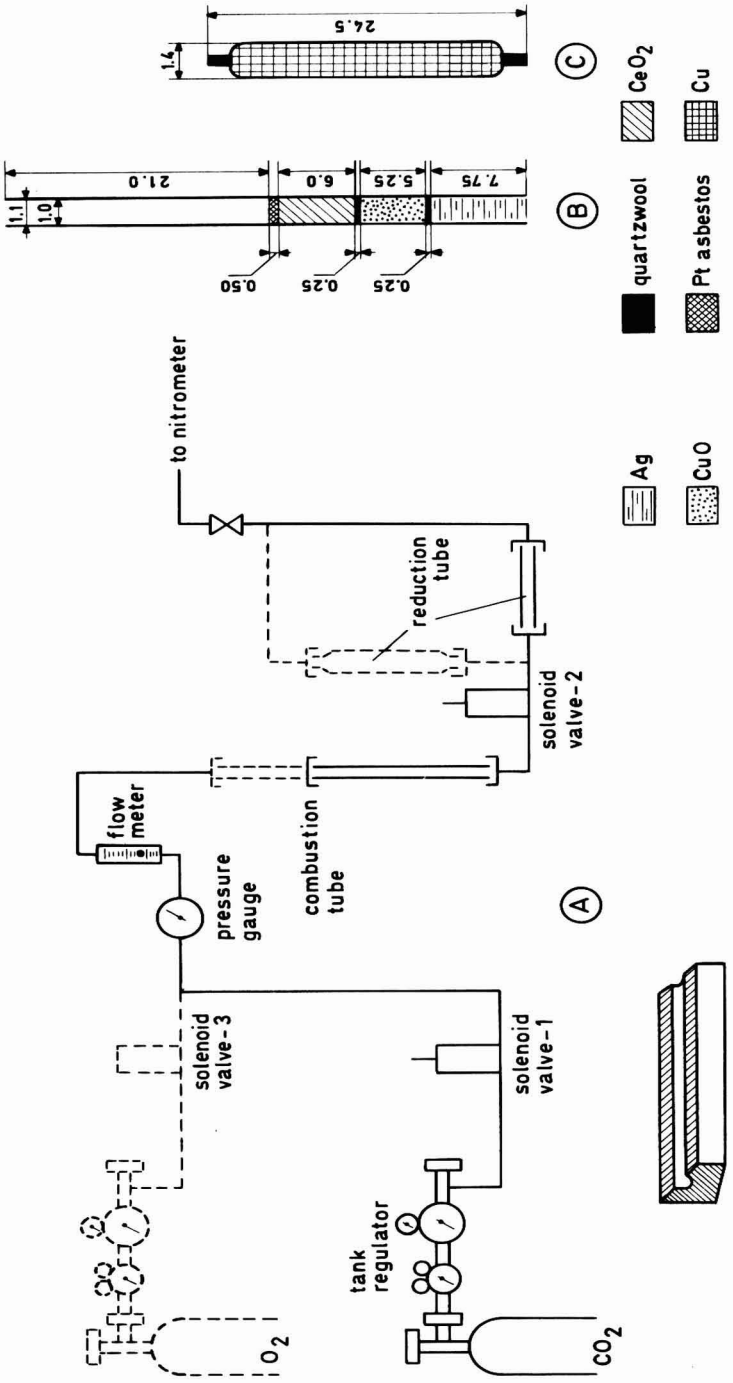

FIG. 1A. (——) Original apparatus; (– – –) modifications; (B) New combustion tube + filling (cm); (C) new reduction tube + filling (cm).

TABLE 1

Program	Gas flow (ml/min)				Analyst's pursuit	Approx time schedule
	Oxygen		Carbon dioxyde			
	In postheater	To atmosphere, through	In nitrometer	To atmosphere, through		min sec
Stand by	Nil	≈ 10-SV-3	Nil	≈ 300 SV-2		
Start-purge	Nil	≈ 10-SV-3	Nil	≈ 300 SV-2	Calculate	1 30
Preheat first half	Nil	≈ 10-SV-3	Nil	≈ 10 SV-1	and	1 00
Preheat second half; first combustion	≈ 5	Nil	Nil	≈ 10 SV-1	weigh	2 15
Final combustion; sweep + 1 min extra	Nil	≈ 10-SV-3	≈ 30	Nil		4 45
Stand by	Nil	≈ 10-SV-3	Nil	300 SV-2	Read volume + temp + pressure; reset counter and meniscus; change comb. boats	± 1 30
Total time						12

Ozone strongly attacks the rubber connections in the solenoid valves and even the neoprene washers. It was furthermore impossible to obtain the prescribed 5 psi pressure.

The premature degradation of some types of compounds leads to erroneous results as described in the manual. We found that the Coleman cooling blocks were not always effective, and besides, it is a clumsy and time-consuming procedure. A much easier solution can be found in removing a part of the sidewall of the instrument and lowering the lower furnace 2.5 cm. As a result, the furnaces stay 7.5 cm away from each other during preheat, making premature combustion very rare. This arrangement allows adjustment of the subsequent temperatures without burning the washers or decomposing the substances too soon.

	Temp in stand by	Max temp during comb.
Upper furnace	850	950
Lower furnace	850	950
Post heater	560	560

The rather long stainless steel tube connecting the postheater with the combustion tube is electrically heated at about 100°C in order to prevent water condensation (*21*).

Improvements of the Reduction System

Using the larger volume of oxygen for complete combustion we needed a bigger postheater tube with more effective tube filling. The different kinds of copper supplied by Coleman, Heraeus, Merck and Carlo Erba are either too fine or too coarse for our purposes. Making copper from CuC_2O_4 after Kainz and Zidek (*10*) takes a lot of time but produced a good quality metal. Ultimately the Baker's Copper Grit catalogue No. 1980 (*1*) gives the best satisfaction. We have been informed recently that Baker dropped the Copper Grit No. 1980 from the new catalogue (1972).

It was found that channeling occurs in the bigger postheater (Fig. 1C) when placed horizontally. Therefore, it was put in a vertical position as follows: The front tube support was mounted 25 cm above the rear support which was turned 90°. A Heraeus combustion furnace placed around the postheater tube, connected with a 130 V supply and variable transformer provided the heating at 560°C.

This larger postheater tube causes a retention of the combustion gases and therefore the nitrogen did not reach the nitrometer in the preset sweeping time. For this reason 1 min extra sweeping was

needed. This could easily be done by using the auxiliary timer. In order to sweep with the furnaces retracted—this means in the sweep period instead of in the final combustion—the commanding microswitch needed readjustment.

Improvements of the Volumetric Measuring System and in Calculating the Results

The nitrometer with magnetic stirrer is the most specific part of the Coleman instrument. Indeed, this idea makes it possible to absorb the carbon dioxide very fast, and to complete a determination in about 10 min (*8*). Yet, at this point too, a lot of improvements were possible:

First of all our technician was able to remove the back lash of the syringe piston.

Afterwards the replacement of the O-ring in the syringe by a Kapseal (*11*) (Fig. 2) increased the reproducibility of the zero setting considerably.

It appears from the literature that there are different possibilities to calculate a nitrogen result. The calculation prescribed by Coleman is rather intricate, and the corrections to be made are very large (1.5 μl for each 0.1°C temperature difference between start and end of the determination). This adversely affects the accuracy and precision.

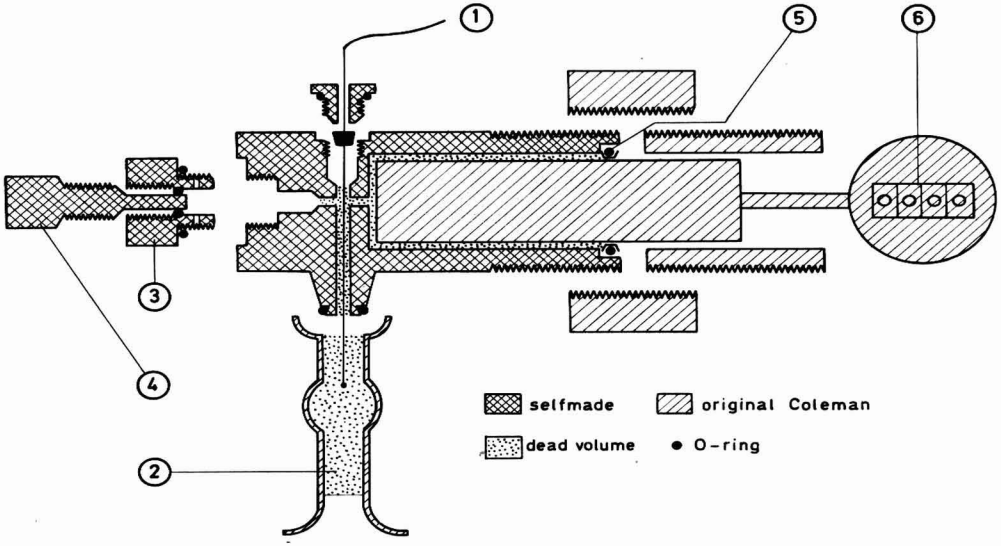

FIG. 2. (1) Ellab thermometer; (2) nitrometer; (3) vent valve; (4) fine adjustment valve; (5) Kapseal; (6) 4 digit counter.

The large temperature correction arises from the unnecessary large dead volume (about 4 ml) in the syringe head (Fig. 2). Therefore, we constructed a new head with a gas chamber as small as possible (1.0 ml). To minimize temperature differences as much as possible, the nitrometer, stirrer and syringe were placed away from the influence of the furnaces, about 20 cm further than in the original apparatus.

As a further improvement, the temperature of the nitrogen is measured in the gas chamber of the nitrometer, not outside, as in the Coleman apparatus. This was achieved by placing an Ellab (5) thermometer in the nitrogen atmosphere of the nitrometer. With this arrangement, we determined blank values during 2 days, and noticed that the temperature of the gas in the nitrometer followed the temperature movements of the laboratory air. The exothermic reaction of the sweeping gas CO_2 and the potassium hydroxide causes a temperature difference of about 1.0°C, between the gas in the nitrometer and the air in the laboratory (Fig. 3).

A second establishment of our experiment was the fact that there was a good correlation between the temperature movements and the

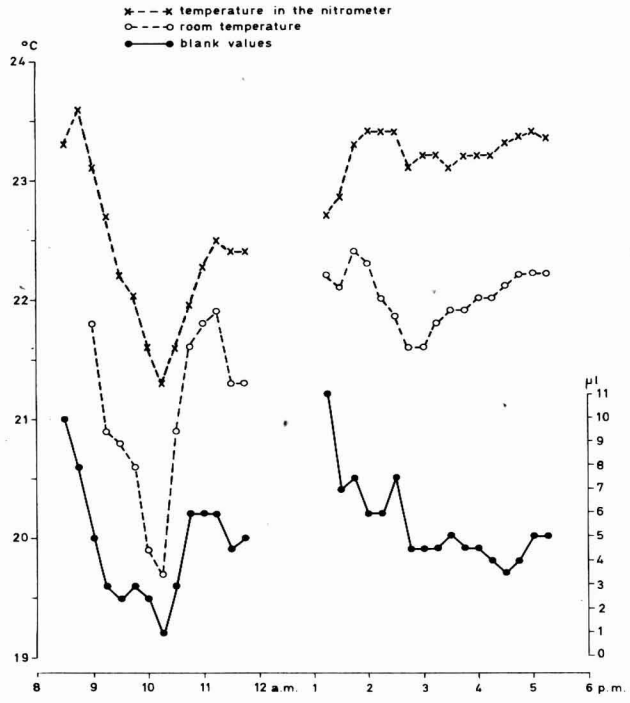

FIGURE 3

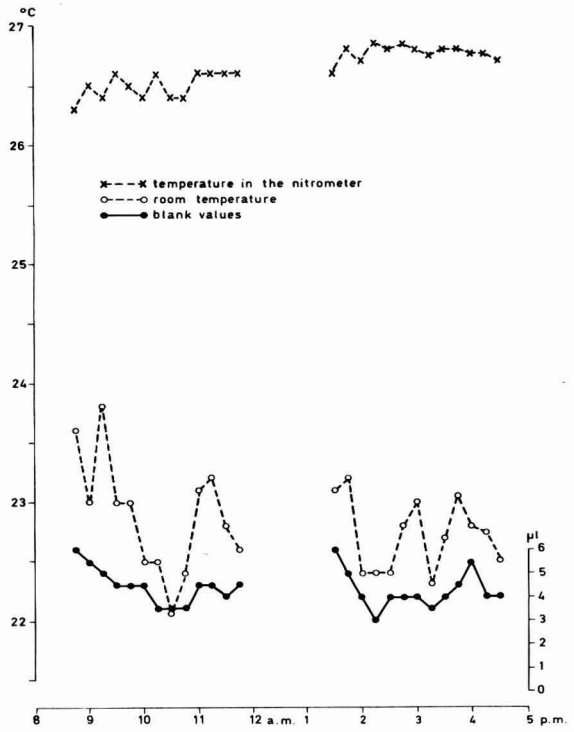

FIGURE 4

blanks. It was obvious that only reproducible blanks were found when the room temperature was quite stable.

Because there was no possibility to improve our air-conditioning system we tried to stabilize the environment of the nitrometer and more precisely the nitrometer part where the nitrogen is measured. Mounting a cage around the nitrometer, the stirrer and the syringe did not give satisfaction. But when stabilized air of approximately 26°C was blown into the cage pointed at the calibration mark of the nitrometer, we achieved good results (Fig. 4).

The blanks produced using this conditioning system give satisfaction, because they were stable and fully independent of the temperature changes in the laboratory. This makes temperature corrections superfluous.

Next to temperature, pressure too plays an important role in converting a gas volume to a gas weight. Here too we wanted to prevent any correction if possible. Replacement of the Coleman syringe vent valve by a selfmade valve [Fig. 2(3,4)] resulted in equal pressures in the nitrometer and in the laboratory. The latter can be read easier

from a barograph, checked regularly against an accurate mercury barometer.

Some other improvements worth mentioning are:

The installation of a buzzer indicating the end of the combustion cycle.

The use of a black background behind the nitrometer together with a spotlight to make readings of the meniscus easier. This bulb may light only during adjustment of the meniscus, because of the heat evolved.

The introduction of a brass rod in which the combustion tube is placed, for faster cooling, during the volume reading and readjustment of the syringe (Fig. 1A).

The New Working Procedure

We weigh our samples on a Sartorius Model 4125 electrobalance. The balance surpasses the classical microbalances in speed, precision and simplicity of manipulation for samples not exceeding 10 mg. We generally try to get between 300 and 400 μl nitrogen, although 1500 μl still gives results within the generally accepted $\pm 0.3\%$ limits.

During the sweep period the temperature of the KOH rises due to the exothermic reaction with CO_2. Therefore it is practical to sweep the apparatus every morning until the temperature of the KOH has risen about $\pm 1°C$ (sweeping 5 min at 50 ml/min. is sufficient). Then we combust an unweighed melamine sample and during this run the first melamine standard is weighed. When the buzzer announces the end of the combustion cycle, the 4 digit counter is turned to zero, with the syringe vent valve open (Fig. 2). KOH is added or taken away until the meniscus is adjusted to the calibration mark. Then the vent valve is closed pressing down the meniscus. With the fine adjustment valve [Fig. 2(4)] the meniscus is readjusted and the sample can be placed in the meanwhile cooled, combustion tube. The first determination is started and the analyst has ample time to weigh the next sample before the buzzer rings (approximately 10 min).

At that time, nitrogen volume and temperature are noted together with the barometric pressure in the laboratory. The next combustion is started after readjusting the meniscus and the counter.

The new program is represented in Table 1. Every week the combustion tube is changed. The nitrometer is filled every morning with fresh caustic and replaced every week by a clean one. In order to keep the correction as constant as possible, we sweep the train overnight with a reduced CO_2 stream of 5 ml/min.

RESULTS AND DISCUSSION

The results are calculated in the most simple way. After three melamine analyses the mean deviation from the true volume is calculated, and used as the correction for the following analyses

$$\frac{\mu\text{l read} - \text{correction } \mu\text{l} \times F_p^{t^0} \times 100}{\mu\text{g sample}} = \quad \% \text{ N}.$$

The factor F is the weight of 1 μl nitrogen at temperature t^0 and pressure p noted. Since there are no rapid temperature changes in our conditioned cage we do not need temperature correction for the nitrogen volume. The pressure read on the barograph adjusted for our altitude makes temperature corrections needed in mercury barometers superfluous too. The calculation is therefore very simple.

From experience we learned that the volume correction does not change considerably so that once the equipment is adjusted only a few standard determinations suffice.

Although we lengthened the analysis time using the auxiliary timer we could make more analyses a day than before, because we only need two or three standard determinations to calculate the correction for the one combustion tube we use. Working without a movable combustion tube filling gives also a considerable gain in time. First of all: results are more accurate, reducing repeats, and no CuO regeneration is to be carried out.

In Table 2 the results of two series of 3 hr consecutive nitrogen determinations are represented. The samples were selected from over 40 commercially available microchemical standards from which the following had been eliminated: products not containing nitrogen, products which cannot be dried to constant weight at 50°C or higher, products found to be impure in a thin-layer or gas chromatographic assay.

The standard deviation from the calculated nitrogen content for the group of 12 standards is calculated as follows:

$$\text{standard deviation } \sigma = \left[\frac{\Sigma(\chi_s - \mu_s)^2}{n-1}\right]^{1/2},$$

where χ_s = found corrected volume of nitrogen for each one of the 12 standards;

μ_s = calculated volume of nitrogen for the same amount of standard substance;

$n - 1$ = number of analyses minus one = 23.

Thus, according to Table 2, the standard deviation for nitrogen is 1.29

TABLE 2

Name	% N theor	Δ μl N		Δ % N corrected	
Melamine	66.64	+4.8	+5.7	−0.22	−0.06
		+6.0	+6.4	0.00	+0.07
		+6.8	+6.7	+0.13	+0.13
		After 6 μl correction ($\chi_s - \mu_s$)			
Atropine	4.84	+1.4	−0.9	+0.04	−0.01
8-Hydroxyquinoline	9.65	+0.1	+0.8	0.00	+0.01
Trifluoroacetanilide	7.41	+2.4	+0.6	+0.08	+0.01
Phenacetin	7.82	0.0	−1.2	0.00	−0.02
Acetanilide	10.36	−1.1	−0.1	−0.04	0.00
p-Nitroaniline	20.28	−1.0	−0.8	−0.04	−0.05
Cyclohexanone 2,4-dinitrophenyl hydrazone	20.14	+0.6	−1.0	+0.04	−0.08
m-Dinitrobenzene	16.67	−0.5	+1.2	−0.01	+0.05
1-Cl-2,4-dinitrobenzene	13.83	+2.3	+0.3	+0.06	−0.01
Sulfanilic acid	8.09	0.0	+1.3	0.00	+0.03
S-Benzylthiuronium chloride	13.82	+1.4	−1.7	+0.04	−0.07
Sulfamic acid	14.43	+2.8	−1.7	+0.11	−0.08
σ		1.29		0.05	
2σ		2.58		0.10	

μl and the 2σ-confidence limit is 2.58 μl. This corresponds to a 2σ confidence limit of less than 0.10% N for the whole group of standard substances.

Table 2 makes it clear that the improved method is accurate and precise. Yet, in our point of view the largest advantage cannot be recognized from Table 2. It is the fact that up to now (after 2 yr of use) we have not found a single sample that needs a modification of the described combustion technique to give good results. In the past, using the normal Coleman technique, several difficulties had been encountered, which could not always be solved even by adding oxidizers.

SUMMARY

A very fast, universal, precise and accurate micro-nitrogen method is described. Using a Coleman nitrogen analyzer with a modified combustion and reduction system and an improved end-point detection method, a 2σ-confidence limit of 0.1% N is reached. These improvements together with the use of an electromicrobalance and a new calculating system make the attendance much easier.

ACKNOWLEDGMENTS

The authors thank Dr. P. Janssen for his permission to publish this work. They likewise thank Mr. L. De Proost and Mr. A. Boermans for their indispensable technical help.

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Growing Single Crystals in Gel Matrices

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In 1896, Liesegang, a German chemist, poured a solution of gelatine containing $K_2Cr_2O_7$ on a glass plate. When it had set, he placed a drop of $AgNO_3$ solution in the center. Instead of the expected mass precipitation, he observed well-defined concentric rings of crystals separated by clear spaces (*1*). A completely satisfactory explanation of this phenomenon of periodic precipitation, now known as "Liesegang rings" has never been advanced. It has been used to explain such natural occurrences as the annual growth rings in trees, stripes in animal fur and bird feathers, rings of ice around a broken icicle, etc., but never with entire success or total acceptance. Many papers have been published on the subject and Stern (*2*) has collected 786 of them (from 1855 to 1965), indexing them by author as well as by application.

The use of a gel matrix (*3*) for growing single crystals is an adaptation of this principle. The most satisfactory matrix is sodium metasilicate, but commercial water glass, agar, gelatine, or almost any other gel can be used. A strict control of temperature is usually unnecessary and reproducible results can be obtained at ambient temperatures. Gels that are too dense yield poor crystals and gels that are not dense enough take too long to set and are unstable. A specific gravity of about 1.02 is the lowest practical limit with $Na_2Si_2O_3$. When this is used, prolonged exposure to the atmosphere should be avoided to prevent absorption of CO_2.

EXPERIMENTAL METHODS

LIESEGANG RINGS

About 200 mg $K_2Cr_2O_7$ was dissolved in about 20 ml of hot 10% felatine, poured into a petri dish and allowed to set. Then a crystal of

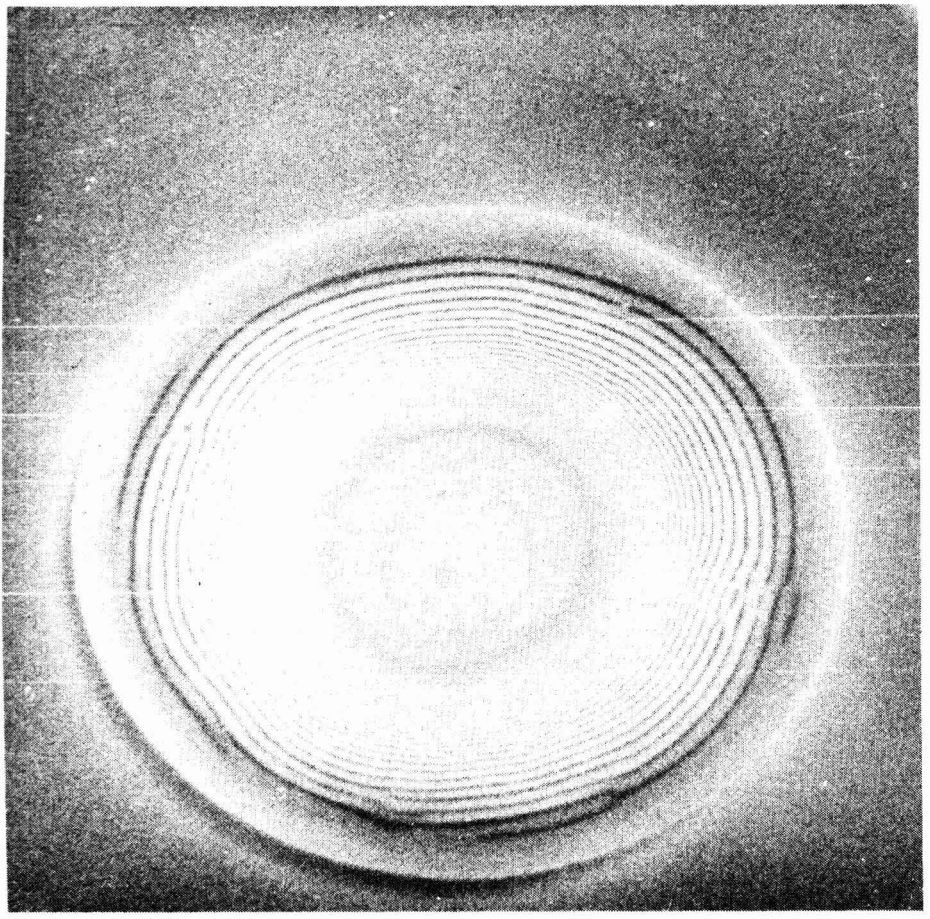

FIG. 1. Liesegang rings formed with $K_2Cr_2O_7$ and $AgNO_3$.

$AgNO_3$ was dropped in the center of the dish. Immediately, a ring formed which was followed by a series of concentric rings that continued to form until all the available Ag and/or Cr_2O_7 ions were exhausted. The dark, bluish rings against the yellow chromate background made an impressive and colorful demonstration of the Liesegang ring phenomenon (Fig. 1). The same procedure using oxalic acid in place of $K_2Cr_2O_7$ produced rings similar but not as extensive with Co, Ca, and Cu but not with Ag ions (Fig. 2). With tartaric acid, the Liesegang ring phenomenon did not occur with any of these ions (Fig. 3). These differences might be due to the more complex structure of the organic acids.

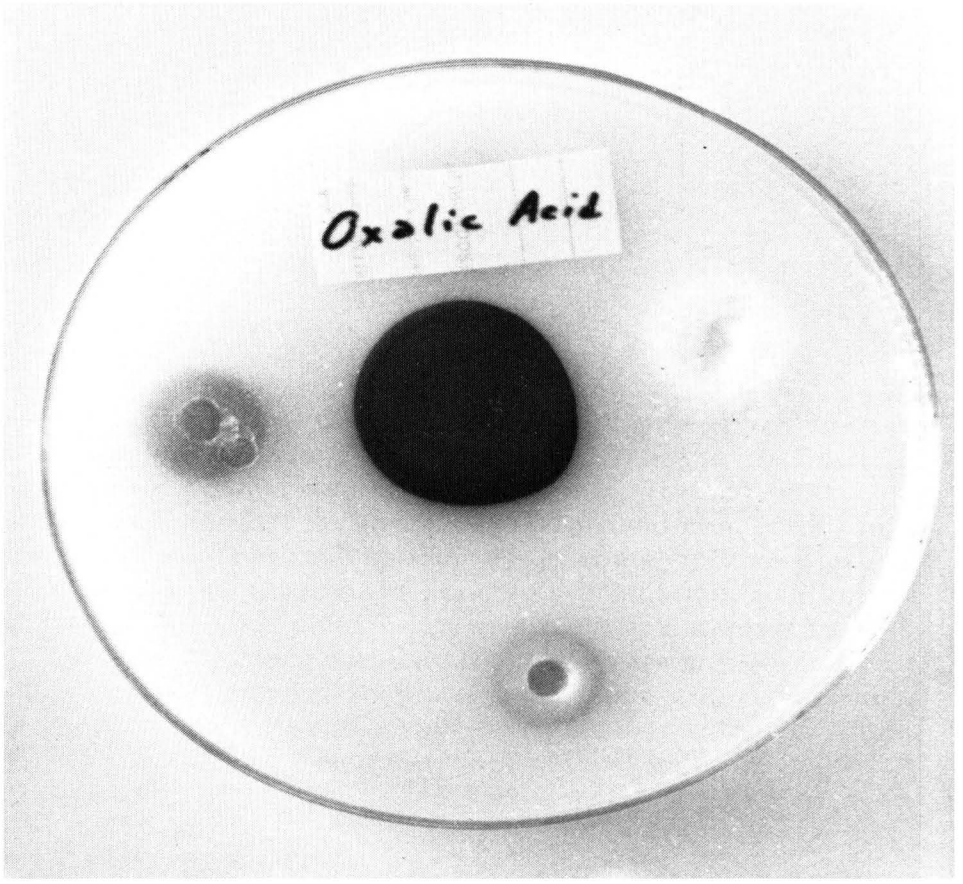

FIG. 2. Liesegang rings with oxalic acid: (left) orange rings with Co ion; (right) white rings with Ca ion; (bottom) blue-green rings with Cu ion. (center) no rings with Ag ion.

CRYSTAL GROWTH IN GELS

A solution of 50 g Na_2SiO_3 (Certified ACS) in 200 ml distilled H_2O was prepared and divided in half. In one half, 7 g oxalic acid were dissolved and in the other, 20 g tartaric acid. Each solution was distributed into four vials of a convenient size and allowed to set. Solutions of approximately 0.1 *M* of $CACl_2$, $CuCl_2$, $CoSO_4$, and $NiSO_4$ were also prepared. When the gels had set, a convenient amount of the cation solution was floated on top of the gel, taking care not to disturb the surface. The vials were kept at room temperature, and in due time, crystals grew (Fig. 4). Crystals were fished out with a platinum loop and photomicrographs were taken by the method previously described (*4*). (Figs. 5–9).

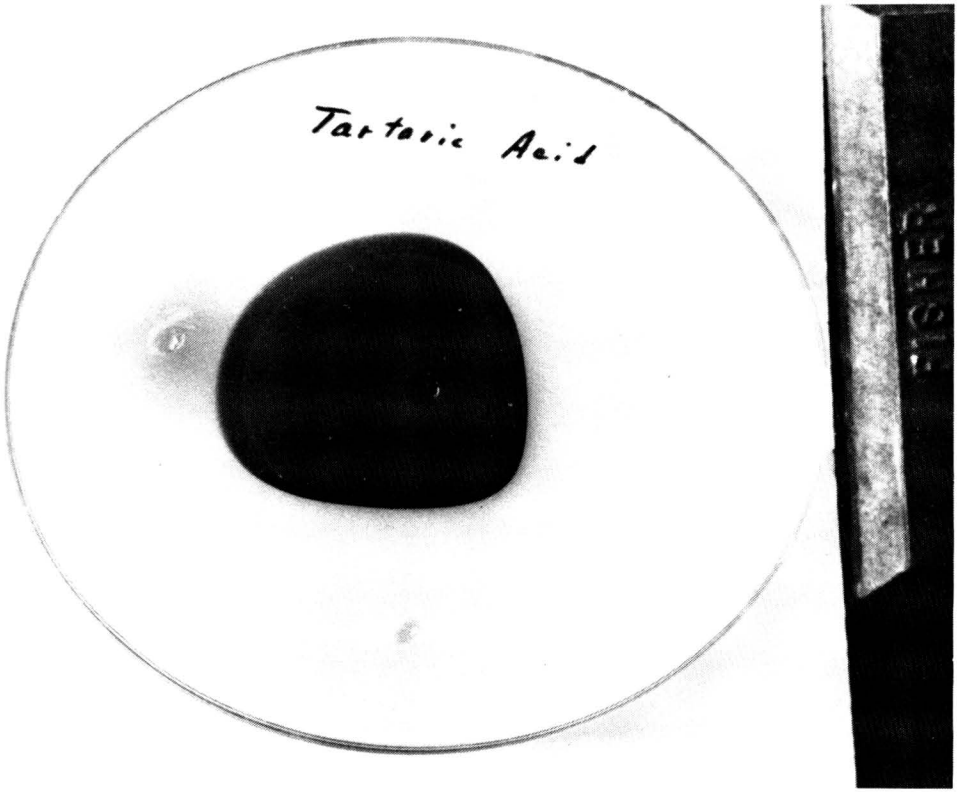

FIG. 3. Same as Plate II with tartaric acid instead of oxalic acid. There was some diffusion but no rings were formed with any of the ions.

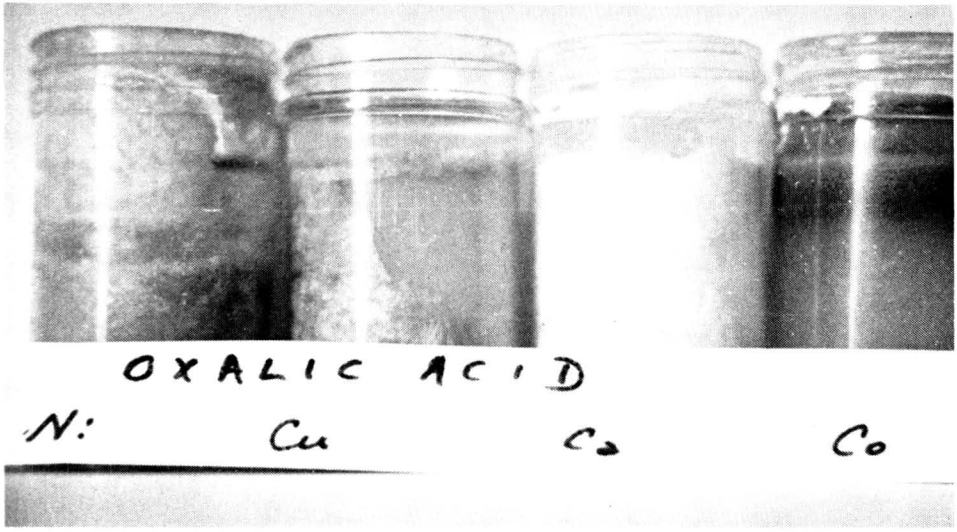

FIG. 4. Vials containing Na_2SiO_3 gels with ion solutions floated on top.

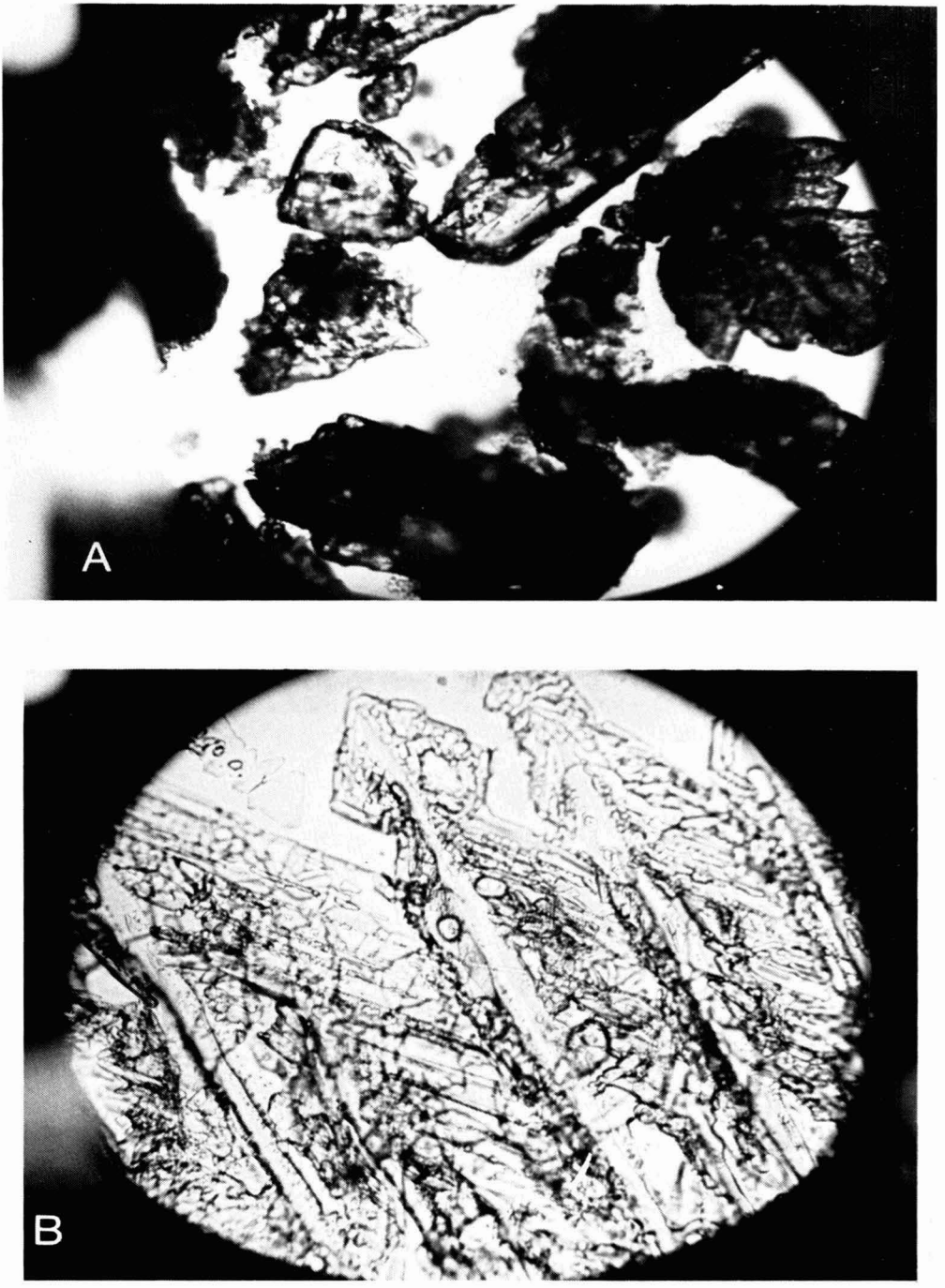

FIG. 5(A). Calcium tartrate × 80; (B) cobalt tartrate × 80.

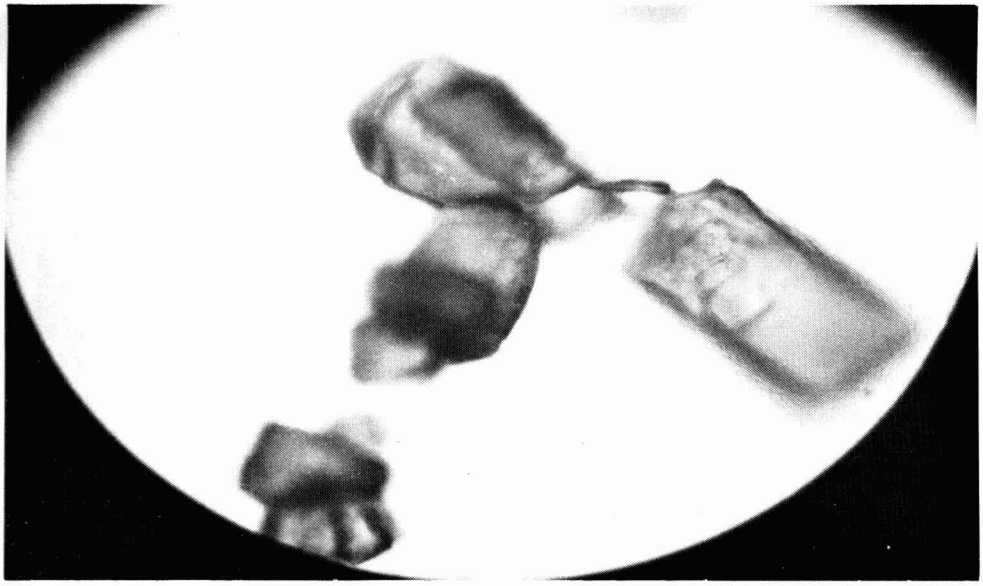

FIG. 6. Copper tartrate × 80.

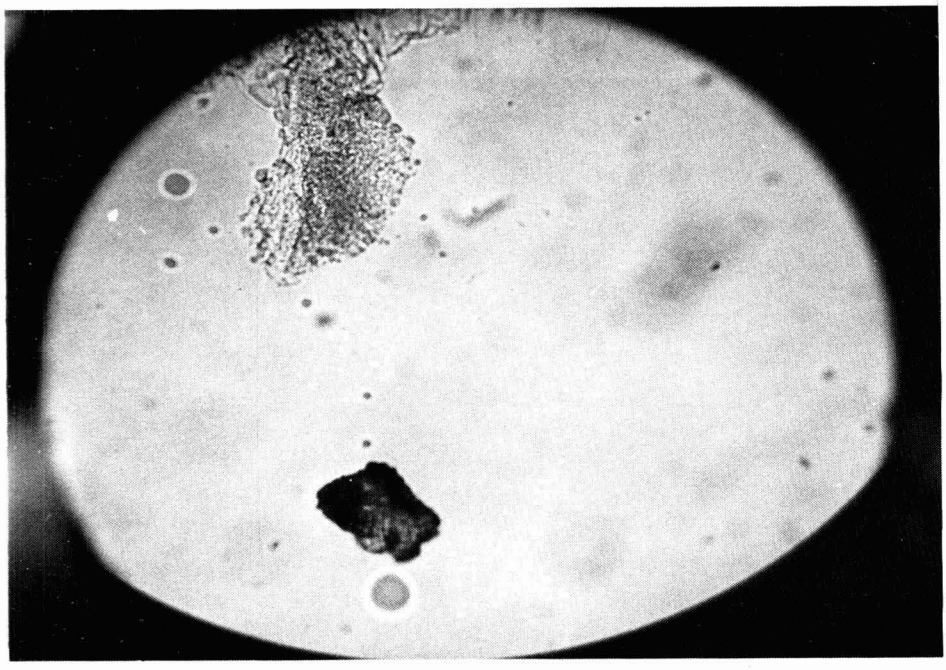

FIG. 7. Nickel tartrate × 80.

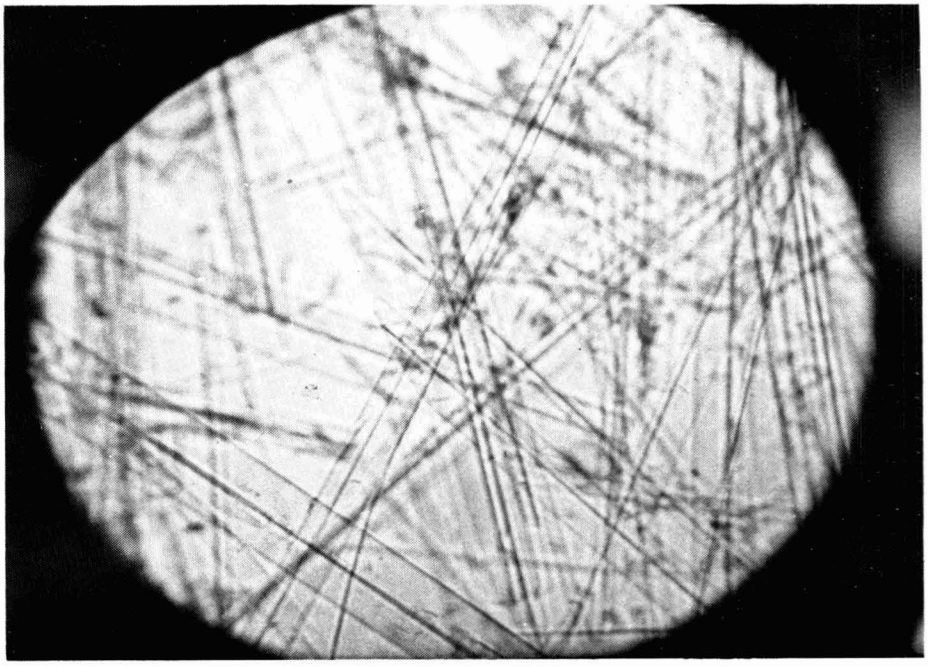

FIG. 8. Copper oxalate × 80.

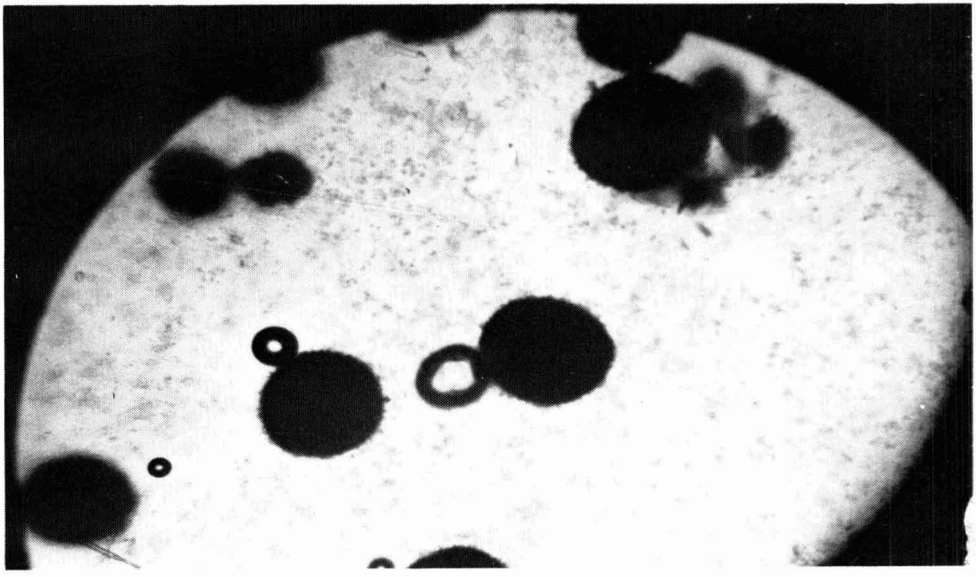

FIG. 9. Cobalt oxalate × 80.

SUMMARY

Simple procedures for the preparation of Liesegang rings and for growing single crystals in gels are described. Both are eye-catching demonstrations. They are also easy home experiments that can become a fascinating hobby.

ACKNOWLEDGMENT

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A Fluorometric Method for the Determination of Nitrilotriacetic Acid

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INTRODUCTION

Because of pollution and toxicological implications (*4, 6, 7, 11, 16, 18, 19*), there has been a continuing need for the determination of the detergent builder NTA (nitrilotriacetic acid). The presently developed methods: polarography (*1–3, 8*), gas chromatography (*5, 12, 18*), ion exchange chromatography (*9*) and colorimetry (*17*) are often too lengthy, insensitive, or may require costly equipment. The proposed indirect fluorometric method for the determination of NTA, which is based upon the reaction of NTA with the fluorescent Ga(III)-8-hydroxyquinoline complex (*20*) is rapid, sensitive, and does not require expensive instrumentation. The standard addition method (*13*) also is incorporated into the procedure to improve the accuracy.

MATERIALS AND METHODS

Reagents

Stock Ga(III) solution, 2.297×10^{-3} *M*, was made by dissolving 80.10 mg of 99.997% pure gallium metal in a 500-ml volumetric flask containing a few milliliters of concd HNO_3 solution and a few drops of concd HCl solution and diluting to volume; the working solution, 2.297×10^{-5} *M*, was made by dilution.

An 8-hydroxyquinoline solution (oxine), 6.89×10^{-3} *M*, was made by dissolving 0.5000 g of oxine in 250 ml of deionized water in a 500-ml volumetric flask containing 3 ml of 6 *M* acetic acid and diluting to the mark with ethyl alcohol. This solution should be stored in the dark and can be kept 2 to 3 wk (*14*). The working solution, 6.89×10^{-5} M, was made by dilution of the stock solution with an equal volume mixture of water and ethanol.

The masking agent is made by dissolving 1.68 g KI, 1.39 g

$NaH_2PO_4 \cdot H_2O$ and 0.49 g NaCN in a 250-ml volumetric flask and diluting with deionized water.

An acetate buffer (pH 6) was made by dissolving 192.1 g of sodium acetate in a minimal amount of water, using heat if necessary, and adding 77.4 ml of glacial acetic acid. This mixture was transferred to a 500-ml volumetric flask and diluted to volume with water.

Reagent grade $CHCl_3$ was used.

Hydroxylamine hydrochloride (1 *M*) in water was used.

Apparatus

A Turner Model 111 fluorometer was used for all fluorescent intensity (FI) measurements with Corning 7-59 primary and 3-72 secondary filters. Wratten gray filters, when needed, were used to reduce the intensity of the excitation light. One-centimeter circular quartz cuvettes were used, but glass cuvettes could have been used.

Procedure

For a water sample containing approximately 0 to 500 ppb NTA, the following procedure should be followed. To a series of 6 clean 125 ml separatory funnels, reagents should be added as shown in Table 1; the values in Table 1 refer to milliliters of reagent added.

To the separatory funnels A to F add the Ga(III), oxine and buffer reagents, shake well for 20 sec and let stand for 2 min. Then add the $NH_2OH \cdot HCl$ solution and shake for 20 sec and let stand for 2 min. Now add the masking agent, shake for 20 sec, and let stand 2 min. Then add the sample to each separatory funnel, shake well for 30 sec and let stand for 5 min. Repeat this last step when adding the deionized water and the 10^{-5} *M* NTA solution. Then add the $CHCl_3$ to each flask, shake for 1 min and let stand 8 min. Wash the stems of the funnels with acetone, allow it to evaporate and drain a portion of the $CHCl_3$ layer into a cuvette. Adjust the fluorometer with gray filters so that the highest fluorescent sample will give a scale reading of 70 to 90 and the sample from funnel C, 10–20. Read each sample in triplicate; average the results.

RESULTS AND DISCUSSION

Calculation of Results

From a plot of fluorescence intensity (FI) vs milliliters of 10^{-5} *M* NTA, evaluate the NTA values for samples A and B.

For sample A (after subracting the amount of NTA added to the sample by the standard addition method), multiply the resultant NTA

TABLE 1

ANALYTICAL PROCEDURE

	Reagent order (ml)								
Separatory funnel	Ga(III)[a]	Oxine[b]	Buffer	$NH_2OH \cdot HCl$	Masking agent	Sample	Deionized H_2O	10^{-5} *M* NTA	$CHCl_3$
A	3.00	4.00	2.00	1.00	1.00	80.00	10.00	2.00	10.00
B	3.00	4.00	2.00	1.00	1.00	20.00	69.00	3.00	10.00
C	3.00	4.00	2.00	1.00	1.00	0.00	92.00	0.00	10.00
D	3.00	4.00	2.00	1.00	1.00	0.00	90.00	2.00	10.00
E	3.00	4.00	2.00	1.00	1.00	0.00	88.50	3.50	10.00
F	3.00	4.00	2.00	1.00	1.00	0.00	87.00	5.00	10.00

[a] Gallium(III) solution, 2.297×10^{-5} *M*.

[b] Oxine solution, 6.89×10^{-5} *M*.

concentration by 92/80, and for sample B (after making the subtraction), multiply the resultant NTA concentration by 92/20 to obtain the NTA concentration in the unknown. To obtain the concentration of NTA in the sample, average A and B.

Theory of Reaction

The reaction can be summarized as follows (*20*):

$$\underset{\text{weakly fluorescent}}{\text{Ga(III)} + 3\ \text{(oxine)}} \longrightarrow \underset{\text{fluorescent}}{\text{Ga(III)–(oxine)}_3},$$

$$\text{NTA} + \text{Ga(III)–(oxine)}_3 \longrightarrow \underset{\text{nonfluorescent}}{\text{Ga(III)–NTA}} + \underset{\text{weakly fluorescent}}{\text{oxine}}$$

Accordingly, the amount of NTA is related directly to the decrease in the fluorescence intensity. Interference may come from several sources such as: (a) a metal that forms a fluorescent complex with oxine after it is liberated from the Ga(III)–$(oxine)_3$ complex; (b) a complexing agent that will displace Ga(III) from the Ga(III.–$(oxine)_3$ complex; (d) a substance that is fluorescent itself or forms a fluorescent compound or complex with substances in the solution; (e) a substance that will combine with the Ga(III)–$(oxine)_3$ complex to shift the emission wavelength.

Of these interferences (a), (b) and possibly (c) would be most serious and probable. To minimize these effects, the use of masking agents is incorporated into the procedure.

Experimental Reaction and Extraction Conditions

The order of addition of reagents is important. The best order is Ga(III), oxine, buffer, NTA and then extraction. Any complexing agents or sample addition comes near the addition of the NTA.

Figure 1 shows the pH formation and extraction conditions for oxine. The formation and extraction conditions were determined to be optimum at pH 6.

Formation time studies are shown in Fig. 2. At least 6 min of standing was found sufficient for complete extraction.

A calibration curve is shown in Fig. 3 for the determination of NTA in the 10^{-5} *M* range.

Chloroform was employed as solvent for the Ga$(oxine)_3$ complex since previous work showed that the fluorescent Ca and Mg quinolates are not extracted (*14*) with chloroform. In addition an enhancement of the fluorescent intensity is observed when chloroform is used.

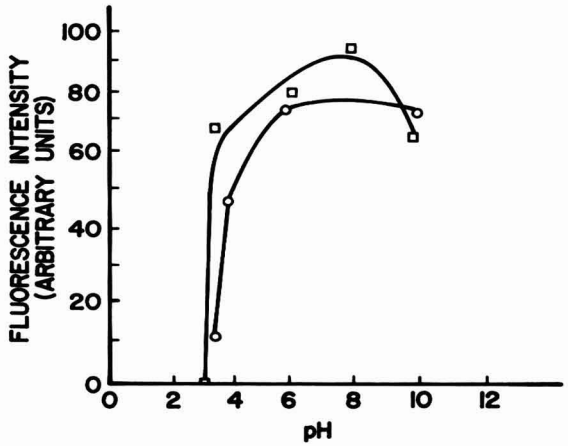

FIG. 1. Formation (○) an extraction (□) conditions for the $Ga(oxine)_3$ complex.

Acetate buffer is used in the procedure as in previous work (*14*), and also in this work it was observed that it produced an enhancement of fluorescence over no buffer or over a buffer such as potassium acid phthalate.

Four masking agent mixtures were tested for the reduction of interferences; each mixture contained three of the following four ions: iodide, fluoride, phosphate and cyanide. The mixture of iodide, phosphate and cyanide gave near linear results and was incorporated into the procedure.

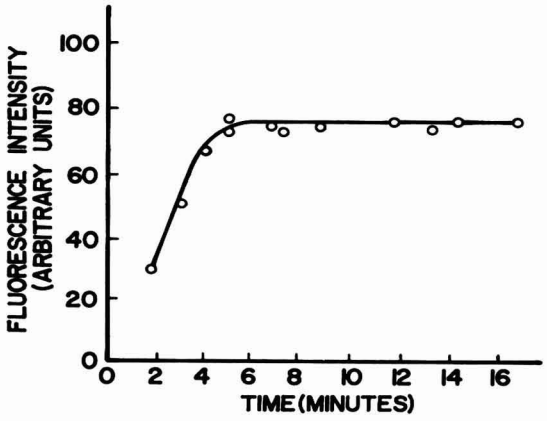

FIG. 2. Extraction time studies for the $Ga(oxine)_3$ complex.

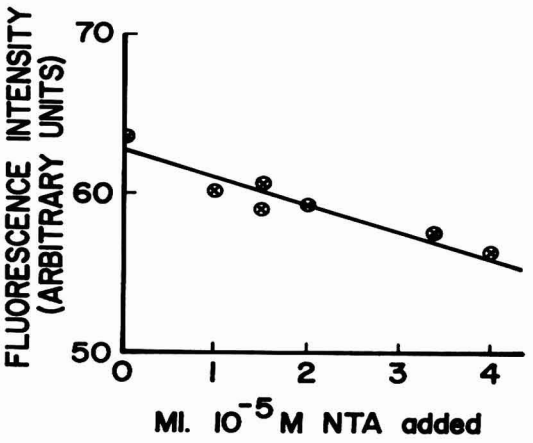

FIG. 3. Calibration curve for the standard addition determination of NTA; average of triplicate measurement of all points.

Gray filters were required to reduce the fluorescence intensity of strongly fluorescent samples; better precision was observed in the procedure when the gray filter was used to decrease the intensity of the excitation light.

Table 2 shows the precision of the extraction procedure. The standard deviation of each point on the curve is less than 5%.

TABLE 2

STANDARD DEVIATION STUDY OF THE EXTRACTION PROCEDURE

10^{-5} *M* NTA added[a] (ml)	No. of similar points determined	Standard deviation of FI of points
0	3	0.58
0	3	2.35
0	3	2.00
0	3	1.00
3	3	1.00
3	3	1.73
3	3	0.58
3	3	1.53
5	3	0.00
5	3	0.58
5	3	1.16
5	3	1.16

[a] Milliliters added in the standard addition procedure to the aqueous layer to make a volume of approximately 100 ml.

TABLE 3

EFFECT OF INTERFERING SUBSTANCES ON THE $Ga(oxine)_3$ PROCEDURE FOR DETERMINING NTA

Foreign compounds present	NTA	
	Added	Found
0.01 *M* $ZnCl_2$, 0.01 *M* NaF	3.0×10^{-5}	5.5×10^{-5}
0.01 *M* $CaCl_2$, 0.01 *M* $Fe(NO_3)_3$, 0.01 *M* Na_2SO_4[a]	7.0×10^{-5}	FI quenched
0.01 *M* NaCl, 0.01 *M* NaCN, 0.01 *M* K_2HPO_4	None	None
0.01 *M* $MgSO_4$, 0.01 *M* $ZnCl_2$, 0.01 *M* Na_2SO_4	7.0×10^{-5}	4.0×10^{-5}
0.01 *M* $Fe(NO_3)_3$, 0.01 M $NaHCO_3$[a]	None	FI quenched
0.01 *M* NaF, 0.01 *M* NaCN, 0.01 *M* K_2HPO_4	3.0×10^{-5}	3.5×10^{-5}
0.01 *M* NaCN, 0.01 *M* NaF, 0.01 *M* $CaCl_2$	7.0×10^{-5}	9.6×10^{-5}
0.01 *M* Na_2SO_4, 0.05 *M* $MgSO_4$	None	1.5×10^{-5}
0.01 *M* K_2HPO_4, 0.01 *M* NaCN, 0.01 *M* NaCl	None	-1.0×10^{-5}
0.01 *M* $NaHCO_3$, 0.01 *M* NaCN, 0.01 *M* $CaCl_2$	3.0×10^{-5}	1.5×10^{-5}
0.001 *M* $Fe(NO_3)_3$ [NH_2OH used]	2×10^{-5}	2×10^{-5}
0.01 *M* $Fe(NO_3)_3$ [NH_2OH used]	2×10^{-5}	2.3×10^{-5}

[a] In the above studies, $NH_2OH \cdot HCl$ was not used to mask any interference.

Table 3 shows the effect of interfering ions on the $Ga(oxine)_3$ extraction procedure. Iron interferes greatly in the procedure. However it is masked by hydroxylamine hydrochloride when iron is present in less than 0.01 *M* concentration.

As reported previously for the standard addition method (*13*), and

TABLE 4

DETERMINATION OF THE BEST MOLE RATION OF ADDED NTA IN THE STANDARD ADDITION METHOD AND DETERMINATION OF NTA IN A WATER SAMPLE

NTA (ml) added[a] by standard addition procedure	Mole ratio of NTA in standard to unknown	NTA (ppm)		Relative error (%)
		Found	Present	
none	0.00	0.130	0.0446	190
1.00	0.57	0.102	0.0446	130
3.00	1.72	0.0515	0.0446	15
5.00	2.86	0.0336	0.0446	−25

[a] Duplicate chemical samples, each of which is also read in duplicate in the fluorometer.

as shown in Table 4, a minimum amount of interference is observed in this determination when the concentration ratio (on a mole basis) of added standard to sample is 2. Using this concentration ratio, the relative error would be 10% or less when analyzing tap water samples containing NTA in the range of 10^{-7} to 5×10^{-7} M. This concentration ratio of added standard to sample might vary slightly for a different sample matrix.

The detection limit at 95% confidence is 0.197 ppb, and the sensitivity is 10.6 ppb^{-1} using previously proposed criteria (*10, 15*). For sensitivity γ, the equation is:

$$\gamma = dI/S_I dC,$$

where C is the concentration, I is the scale reading and S_I is the standard deviation of points from the regression line. The detection limit, which is related to the reciprocal of the sensitivity, indicates the minimum concentration that will produce a discernible change in the signal intensity and is given by the equation:

$$\mathrm{DL} = \frac{t(n-1,\ 1-\alpha)}{\alpha},$$

where t is the distribution for $n-1$ measurements at a confidence level of $100(1-\alpha)\%$.

SUMMARY

The procedure described in this paper can be used to determine trace amounts of nitrilotriacetic acid (NTA) in tap water samples with a relative standard deviation of 10% or less. The procedure does not use expensive equipment, is relatively fast and can be used for routine determination of NTA.

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Microdetermination of Phosphate with EDTA

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A few indirect complexometric methods for the determination of the phosphate ion are known (*2, 6–8, 12, 13*). Beside being time consuming, owing to the hours or days required for complete precipitation of the phosphate, these methods cannot always be applied to microanalysis due to the solubility product of the precipitate.

A suitable method for the microdetermination of phosphate is described, based on the instantaneous formation of the yellow orthophosphate, Ag_3PO_4 ($K_{sp} = 1.3 \times 10^{-20}$) (*9*), resulting from the reaction of silver nitrate with a slightly alkaline or neutral solution of the phosphate sample. In this reaction the phosphate ion is completely precipitated almost instantaneously in the presence of an excess of silver nitrate. However, it is necessary that Cl^-, Br^-, I^-, vanadate, molybdate, chromate, sulfate and tungstate ions be absent so as to avoid co-precipitation.

The freshly formed silver orthophosphate dissolves in an ammoniacal solution of potassium cyanonickelate as used by Flaschka and Huditz (*3*) and Flaschka (*4*) for dissolving silver halides:

$$2Ag_3PO_4 + 3K_2Ni(CN)_4 \longrightarrow 3K_2Ag_2(CN)_4 + 3Ni^{2+} + 2PO_4^{3-}.$$

Two phosphate ions coexist with three of nickel in the solution. The freed nickel is titrated with EDTA to a visual end-point with murexide as indicator. The required time for a determination of phosphate in solution is about 1 hr, while the classical phosphomolybdate and magnesium pyrophosphate procedures need several hours and in many cases a microbalance has to be used.

EXPERIMENTAL METHODS

The following procedure requires the phosphate solution to be neutral or very slightly alkaline.

Reagents

a. $AgNO_3$ solution. Prepared by stirring 30 g of $AgNO_3$ with 100 ml of cold water and filtering the solution. Store it in a dark brown bottle.

b. Murexide indicator. As murexide solution is only stable for 1 or 2 days (*1*), it is preferable to dilute the indicator with solid sodium chloride and to add the finely powdered solid mixture to the liquid to be titrated (*5*).

c. Potassium cyanonickelate. $K_2Ni(CN)_4$ solution: Titrate a measured portion of 0.2 *M* nickel sulfate solution, with 1 *M* potassium cyanide solution until murexide changes from yellow to purple. Repeat the titration and average the results. Mix equivalent amounts of the two solutions, add some ammonia, and dilute with water to a cyanonickelate concentration of about 0.1 *M*. The resulting solution, if sufficiently alkaline, is stable for some months (*10, 11*).

d. 0.01 M EDTA. Dissolve 3.721 g of disodium EDTA dihydrate in distilled water in a 1000 ml graduated flask and complete the volume up to the mark. Generally it is not necessary to check the titer, but if desired, this can be done by titrating a known volume of the EDTA solution using a known molar solution of nickel sulfate and murexide as an indicator. The end point is indicated by the change in color of the solution from purple to yellow. In case a 0.001 *M* EDTA solution is needed, it can be prepared by diluting the 0.01 *M* with water in the ratio of 1 : 10.

e. Wash solution (saturated aqueous solution of Ag_3PO_4). Stir mechanically during 1 hr about 5 g of freshly precipitated silver orthophosphate in 1 liter of water. Let the solid matter settle down and then decant the clear supernatant liquid.

Procedure

Pipet a known volume of the phosphate solution and make it neutral or slightly alkaline (NH_4OH or HNO_3) so as to have a phosphate content of 1–5 mg. Add water if necessary to bring the volume to about 50 ml. Then in a dark corner of the laboratory, add about 5 ml of the silver nitrate solution, stir well and allow the precipitate to settle. Add a drop or two of the $AgNO_3$ solution to the clear supernatant liquid and check that no cloudiness is produced after addition of silver nitrate. In this case all the phosphate has been precipitated. If not, add more reagent for complete precipitation.

Filter the precipitate through a fine sintered glass filter with a capacity of 15–25 ml. Wash the precipitate three or four times with the wash solution, then once with water and discard filtrate and washings. Place the filter and precipitate in a beaker containing suf-

ficient potassium cyanonickelate to cover the filter. Warm gently while stirring until all the yellow precipitate has dissolved. Remove the filter carefully from the beaker and rinse it well with water, collecting the washings in the beaker.

The nickel displaced by the silver in the complex, is titrated with EDTA solution, using murexide as indicator. The end point is indicated by the sudden change in color from yellow-orange to purple.

Calculations

Let us consider the reaction after dissolution of the silver orthophosphate in the potassium cyanonickelate according to the equation given above. We conclude that $2PO_4^{3-}$ correspond to $3Ni^{2+}$ as the end products of the reaction. In other words, 189.914 g of PO_4^{3-} correspond to 176.13 g of nickel. As 1 ml of 0.01 *M* EDTA is equivalent to 0.5871 mg of Ni, it thereby results, that 1 ml of 0.01 *M* EDTA corresponds indirectly to 0.633 mg PO_4^{3-}, or 1 ml of 0.001 *M* EDTA is equivalent to 63.3 μg PO_4^{3-}.

When the end point is reached, the number of ml 0.01 *M* EDTA used is multiplied by 0.633 to obtain the phosphate content of the sample (mg), or in the case of titration with 0.001 *M* EDTA by 63.3 to obtain the phosphate content in micrograms.

RESULTS AND CONCLUSIONS

The above described procedure gives very satisfactory results and a microdetermination of phosphate in minerals, soils, fertilizers, biological samples, pharmaceutical products, and organo-phosphorus compounds after the phosphate is in solution, can be carried out in about 1 hr. The accuracy is about 1% (see Table 1). The described method has the advantage of not requiring previous removal of Fe, Al, Ni,

TABLE 1

TITRATION OF SODIUM PHOSPHATE WITH 0.01 *M* EDTA

PO_4^{3-} taken (mg)	PO_4^{3-} found (mg)	Difference	
		mg	%
50.0	50.2	+0.2	0.4
40.0	40.1	+0.1	0.3
30.0	29.9	−0.1	0.3
20.0	20.1	+0.1	0.5
10.0	10.1	+0.1	1.0
5.0	5.1	+0.1	2.0

TABLE 2

TITRATION OF SODIUM PHOSPHATE WITH 0.001 *M* EDTA

PO_4^{3-} taken (μg)	PO_4^{3-} found (μg)	Difference	
		μg	%
5000	5050	+50	1
4000	4030	+30	1
3000	3050	+50	2
2000	2040	+40	2
1000	1020	+20	2
500	510	+10	2
300	310	+10	3
200	190	−10	5
100	110	+10	10

Co, Mn, Zn, Cd, Pb, Ca, Sr, Ba, and Mg, as is the case for the usual gravimetric methods.

The procedure can also be used for microquantities of phosphate, by titrating the freed nickel with a 0.001 *M* EDTA solution. In this case 1 ml of the titrant will correspond to 63.3 μg PO_4^{3-} (see Table 2).

SUMMARY

An indirect microdetermination of phosphate via EDTA titration is described, which can be applied to minerals, soils, fertilizers, biological samples, drugs and organo-phosphorus compounds. The method is based on the precipitation of phosphate as the very insoluble silver orthophosphate ($K_{sp} = 1.3 \times 10^{-20}$), dissolution of this precipitate in a solution of potassium cyanonickelate and titration of the nickel displaced by silver. The phosphate content is obtained indirectly by multiplying the number of ml of the titrant by a factor. The method takes about an hour after the phosphate is brought into solution. The accuracy is about 1% for samples containing 5–50 mg PO_4^{3-} and about 3–5% for samples with 100 to 5000 μg PO_4^{3-}.

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Rapid Thin-Layer Chromatographic Separation of Cocaine from Codeine, Heroin, 6-Monoacetylmorphine, Morphine and Quinine on Microscope Slides

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Previous publications have described several thin-layer chromatographic procedures for the separation of cocaine from a number of other compounds. Thus, Fiebig, Felczak and Janicki (*3*) separated cocaine from atropine, homatropine and hyoscine by thin-layer chromatography on alkaline silica gel plates, while Emerson and Anderson (*2*) reported thin-layer chromatographic separation of cocaine from a number of analgesic drugs including morphine and codeine, also using silica gel plates in an atmosphere of ammonia. Other investigators have employed thin-layer chromatography for the separation of cocaine from procaine and quinine (*1*, *6*) and from morphine, dextromethorphan, diamorphine, cyclizine and dipipanone (*4*, *5*).

In this paper a method of separation of cocaine from codeine, heroin, 6-monoacetylmorphine, morphine and quinine by thin-layer chromatography on sodium hydroxide impregnated silica gel microscope slides has been presented.

MATERIALS AND METHODS

Reagents

The narcotics were supplied by the US Government.

Potassium iodoplatinate reagent. 10 ml of 10% (w/v) potassium chloride solution added to 250 ml of 4% (w/v) potassium iodide solution and the mixture diluted to 500 ml with water.

Silica gel G "Merck" containing 13% calcium sulfate as binder.

NaOH, 1.0 *N* aqueous solution

Acetone–benzene (50:50) mixture.

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

Preparation of Microscope Slide Plates

The procedure used, involved a modification of the method reported by Peifer (*7*). The microscope slides, measuring 7.5 × 2.5 cm, were cleaned in dilute detergent solution, rinsed in methanol and then thoroughly dried before use. The slides were covered with silica gel G by dipping into a stirred suspension containing 45 g of silica gel G in 115 ml of a solution composed of 1.0 *N* aqueous sodium hydroxide solution, methanol and chloroform (10:40:50).

Two slides were placed back-to-back and dipped into the well-stirred suspension and quickly withdrawn. When the solvent had partially evaporated the edges of the slides were cleaned, and the slides were separated and heated on a wire-screen set at $\frac{1}{4}$ in. above a hot plate for 5 min with the hot plate set at about one third its heating capacity. The plates were allowed to cool to room temperature after which they were spotted with 5 $\mu g/\mu l$ of the test mixture containing cocaine, quinine and the selected morphinoid alkaloids dissolved in methanol.

Development and Detection

The plates were developed in microscope slide jars (9.5 × 5.5 cm) equipped with lids and containing 2 ml of acetone–benzene solvent mixture (50:50). Prior equilibration was unnecessary and up to three slides could be placed in the tank per run. The development time was 3 min. At the end of this period the chromatoplates were removed from the solvent system and the solvent front was marked immediately. The solvent was allowed to evaporate at room temperature, after which, the chromatoplates were sprayed with potassium iodoplatinate reagent for the detection of cocaine, quinine and the selected morphinoid alkaloids used.

RESULTS AND DISCUSSION

Table 1 records the R_f values of cocaine and the individual alkaloids which were selected for these studies. These results show that by employing the procedure of thin-layer chromatographic separation described herein, cocaine is efficiently separated from codeine, heroin, 6-monoacetylmorphine, morphine and quinine. Furthermore, the results recorded in Table 2 demonstrate that when a mixture containing cocaine, codeine, heroin, 6-monoacetylmorphine, morphine and quinine was chromatographed using the present procedure, only two distinct spots were separated, with cocaine alone being one such spot, and showing rapid migration. All the alkaloid materials of the mixture moved negligibly from the point of application, and they appeared as a single large spot on the microchromatoplate. Thus, the

TABLE 1

R_f VALUES OF SINGLE COMPONENT ALKALOIDS AND COCAINE USING BASIC SILICA GEL G ON MICROSCOPE SLIDE CHROMATOPLATES AND ACETONE–BENZENE (50:50) AS DEVELOPING SOLVENT

Compounds	R_f
Cocaine	0.79
Heroin	0.07
6-Monoacetylmorphine	0.07
Codeine	0.02
Morphine	0.02
Quinine	0.02

procedure as described, affords a rapid efficient method of separation of cocaine from codeine, heroin, 6-monoacetylmorphine, morphine and quinine. As low as 1 $\mu g/\mu l$ of cocaine may be detected by the procedure in the presence of up to 10 $\mu g/\mu l$ of each of the selected morphinoid alkaloids and quinine. Furthermore, because of the efficiency of separation of cocaine from the morphinoid alkaloids and quinine, the former may also be efficiently eluted after its separation.

Preliminary studies have also indicated that aspirin and caffeine do not interfere with the separation of cocaine by the procedure. Thus, when a solution of aspirin in methanol was chromatographed utilizing the procedure, it failed to migrate from the point of application, as detected by exposure of the microchromatoplates to iodine vapor (*8*). Caffeine, however, did migrate, showing an R_f value of 0.46 as identified by chloramine-T reagent (*8*). Although aspirin and caffeine were not included in the mixture analyzed by the procedure, nevertheless,

TABLE 2

R_f VALUES OF SEPARATED COMPONENTS FROM A MIXTURE CONTAINING COCAINE, QUININE AND SELECTED MORPHIMOID ALKALOIDS, USING BASIC SILICA GEL G ON MICROSCOPE SLIDE CHROMATOPLATES AND ACETONE–BENZENE (50:50) AS DEVELOPING SOLVENT

Compounds	R_f
Cocaine	0.80
Heroin	0.06
6-Monoacetylmorphine	0.06
Codeine	0.06
Morphine	0.06
Quinine	0.06

these preliminary studies suggest strongly that the method may allow efficient separation of cocaine from mixtures containing, in addition to codeine, heroin, 6-monoacetylmorphine, morphine and quinine, aspirin and caffeine.

SUMMARY

A thin-layer chromatographic method is reported for the separation of cocaine from codeine, heroin, 6-monoacetylmorphine, morphine and quinine on microscope slides. The method involves the use of sodium hydroxide-impregnated silica gel G microchromatoplates and acetone–benzene (50:50) as developer. Cocaine separates as a distinct spot while quinine and the morphinoid alkaloids separate collectively as one large spot near the point of application. Preliminary data suggest that the procedure will separate cocaine from aspirin and caffeine, and also from mixtures containing aspirin, caffeine, quinine, codeine, heroin, 6-monoacetylmorphine and morphine.

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Microchemical Urinalysis

IX. Determination of Hydroxyproline in Urine[1]

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The rate of urinary excretion of hydroxyproline is related to the active metabolic state of collagen. It is estimated that 57% of the body collagen is present in the bone (*7*); thus, the excretion of hydroxyproline should vary directly with the conditions that affect the bone tissue. Although the turnover of collagen is slower than other proteins under normal conditions, it appears to increase in bone injuries or bone diseases (*6*). Hydroxyproline excretion has also been shown to increase during prolonged bed rest (*2*).

Of the total amino acid content in collagen, about 14% is hydroxyproline. This amino acid is found almost exclusively in collagen and not in any other body protein, except for small amounts present in elastin (*1*). The determination of urinary hydroxyproline excretion rate is thus a direct measure of the dynamics of bone collagen metabolism.

In our recent papers in this series (*3, 4*) we described the quantification of calcium and citric acid in urine. Since the urinary excretion rate of these compounds is also related to bone metabolism in stressful situations, it is appropriate that we now similarly deal with the quantification of hydroxyproline.

Our method is a modification of that described by Prockop and Udenfriend (*8*). The procedure described here was found to be sensitive to 0.025 μmoles/ml or about 3 μg/ml hydroxyproline, and has a dynamic range up to at least 1 μmole/ml or 131 μg/ml. This is about a 40-fold concentration range of hydroxyproline. Good reproducibility and recovery of urinary hydroxyproline is achieved using 1 ml samples.

MATERIALS AND METHODS

Reagents

1. Concentrated hydrochloric acid from Baker and Adamson.
2. Dowex-charcoal mixture. (a) Dowex anion exchange resin, AG1-X8, 200–400 mesh, chloride form, Bio-Rad No. 3840. (b) Norit

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A, Pfanstiehl Laboratories, Inc. Use 20 g resin and 10 g Norit A and wash the mixture with 100 ml of 6 *N* hydrochloric acid 3 times, or until the effluent is no longer yellow. Rinse and dry with 1:1 ethanol:ether solution.

3. Phenolphthalein (MW, 318.31) from Eastman Organic Chemicals. Prepare 1% phenolphthalein solution by dissolving 1 g of phenophthalein in 100 ml of absolute ethanol.

4. Potassium hydroxide, Mallinckrodt (MW, 56.11). Prepare a 5 *N* solution by dissolving 140.3 g of potassium hydroxide pellets in water and dilute to 500 ml in a volumetric flask. Make 1:5 dilution for 1 *N* potassium hydroxide.

5. Potassium chloride, Mallinckrodt (MW, 74.55).

6. Borate buffer, pH 8.7: (a) Boric acid, J. T. Baker (MW, 61.844). (b) Potassium chloride, Mallinckrodt (MW, 74.55). Dissolve 61.84 g boric acid and 225 g potassium chloride in 800 ml water as much as possible. Bring pH to 8.7 with 5 *N* potassium hydroxide and dilute with water to 1 liter. Solution will be clear only when pH nears 8.7.

7. DL-Alanine, Eastman Organic Chemicals (MW, 89.10). Prepare 10% alanine solution by dissolving 25 g DL-alanine in 225 ml water and bring pH to 8.7 with 5 *N* potassium hydroxide. Dilute to 250 ml with water in a volumetric flask. Keep in refrigerator. Solution is stable for not more than 1 mo.

8. Oxidizing reagent. (a) Chloramine-T, Eastman Organic Chemicals ($CH_3 \cdot C_6H_4 \cdot SO_2$ NCl Na·3 H_2O, MW, 281.68). (b) Cellosolve, or ethylene glycol monomethyl ether, Mallinckrodt (MW, 76.09).

The oxidizing reagent is prepared fresh daily by dissolving 2.8 g of chloramine-T in 50 ml of cellosolve.

9. Sodium thiosulfate, Mallinckrodt ($Na_2S_2O_3 \cdot 5\ H_2O$, MW, 248.18). Make 3.6 *M* solution by dissolving 893.4 g of sodium thiosulfate in 1 liter of warm water.

10. Toluene, Mallinckrodt (MW, 92.14).

11. Hydroxy-L-proline, Cal Biochem Lot 45105 No. 3980 (MW, 131.1). Make a standard stock solution by dissolving 131.1 mg hydroxyproline in water and diluting it to 10 ml in volumetric flask. This standard contains 100 μmoles/ml. Working standards of 0.125, 0.250, 0.500, 0.750 and 1.0 μmole/ml, respectively, are prepared by dilution from the stock solution.

12. Ehrlich's reagent. Three substances are required to prepare this reagent: (a) concentrated sulfuric acid, Baker and Adamson (MW, 98.08); (2) absolute ethanol, Commercial Solvents Corporation, Gold Shield, 200 proof; (c) *p*-dimethylaminobenzaldehyde, Mallinckrodt, mp 73–75°C. In one beaker, add 13.7 ml of concentrated sulfuric acid to 100 ml of absolute ethanol slowly, stirring continuously. In another

beaker, add 100 ml of absolute ethanol to 60 g of *p*-dimethylaminobenzaldehyde and stir thoroughly. Then slowly add ethanol–sulfuric acid solution and stir until dissolved. Avoid overexposure to atmospheric moisture. Store in brown bottle in the refrigerator and redissolve any precipitate present by heating in warm water before each use. This reagent is stable for about 1 mo or longer. If necessary, the *p*-dimethylaminobenzaldehyde can be purified before use (*5*).

Equipment

1. Serological pipets: 1, 2, 5, and 10 ml.
2. Repipets: 1, 5, and 10 ml.
3. "Grunbaum" pipets: 1 ml. Items 2 and 3 available from Labindustries, 1802 2nd Street, Berkeley, Calif.
4. Disposable pipets: 15 cm long, Pasteur-type.
5. Screw cap tubes, Kimax: 16×125 mm, 25×200 mm and 13×100 mm.
6. Pressure cooker, 15 psi.
7. Mechanical shaker.
8. Vortex mixer.
9. Water bath.
10. Spectrophotometer. Beckman DB-G or DU can be used.
11. Centrifuge.

Procedure

1. Hydrolysis. One milliliter of urine in screw-cap tubes (16×125 mm) is diluted with 1 ml of distilled water. To this solution, add 2 ml of concentrated hydrochloric acid and mix. The test tubes are tightly capped before being placed in a pressure cooker (use 15 psi), and the samples are autoclaved for 3 hr at 124°C. The hydrolyzed samples are cooled to room temperature for analysis, or they can be frozen and stored at −20°C.

To the hydrolyzed samples add 4 ml of distilled water, and add Dowex-charcoal mixture (from 0.5 to 1 ml) to each test tube with spatula. The tubes are put on a mechanical shaker for 5 min, after which they are centrifuged for 15 min at 2000 rpm. From the centrifuged samples, transfer 4 ml of supernatant to large test tubes (25×200 mm).

At this time, 4 ml samples of blanks and working standards are also placed in large test tubes and treated in the same manner as the unknowns in the following steps of the procedure.

2. Oxidation. Add 1 drop of 1% phenolphthalein solution to each tube, followed by 2.5 ml of 5 *N* potassium hydroxide. After mixing

the solution on a Vortex mixer, 1 *N* potassium hydroxide is added dropwise until a faint pink color appears. Although the color of the solution from one tube to the other may not be identical, subsequent addition of buffer will bring the pH to 8.7. The solution is saturated with 2 g potassium chloride. To this solution add 2 ml of borate buffer (pH 8.7) and then 1 ml of 10% alanine solution. The alanine is added to stabilize the reaction conditions during oxidation. The sample is then oxidized by adding 2 ml of chloramine-T solution at room temperature, and after 20 min the oxidation reaction is stopped by adding 6 ml of 3.6 *M* sodium thiosulfate solution.

3. Extraction. To each tube, add 10 ml of toluene. Put on a mechanical shaker for 2 min and not longer. Toluene is removed by aspiration and discarded. Care must be taken not to remove any of the aqueous phase, even at the risk of retaining a small fraction of the toluene phase. The tubes are tightly capped and placed in boiling water bath for 30 min and cooled under running tap water. Again, 10 ml of toluene are added to each tube, which is then shaken for 2 min.

4. Color development. Three milliliter of toluene phase are transferred to a new set of test tubes (13×100 mm). Exactly 1.2 ml of Ehrlich's reagent are added, and the solution is rapidly mixed on a Vortex mixer. Allow the solution to stand for 15 min before reading at 560 nm on a spectrophotometer.

5. Calculation. The concentration of hydroxyproline in the unknown urine is directly proportional to the concentration of hydroxyproline in the standard.

RESULTS AND DISCUSSION

The previously described method for nanogram amounts of hydroxyproline in tissues by Grunbaum and Glick (*5*) could not be utilized on urine because of the inherent presence in the latter of nonspecific chromophores. To eliminate this interference, the hydrolyzed urine was subjected to shaking with a Dowex–charcoal mixture. In this process, the interfering substances are adsorbed and the supernatant becomes colorless. Shaking of the hydrolysate with the resin–charcoal mixture, rather than using a chromatographic column, was by far the simpler means to achieve purification.

The basic principle in the analysis of hydroxyproline involves the oxidation of the imino acid to pyrrole-2-carboxylic acid and pyrrole, and the subsequent formation of a chromophore with *p*-dimethylaminobenzaldehyde. Since the first oxidation products, Δ'pyrrole-4-hydroxy-2-carboxylic acid and pyrrole-2-carboxylic acid, are insoluble in toluene, interfering substances are removed from the

solution by mixing it with toluene, which is then discarded by aspiration. The solution is heated for the formation of pyrrole, which is extracted into toluene.

It appears that at this stage only the pyrrole derived from hydroxyproline remains to react with the rather nonspecific *p*-dimethylaminobenzaldehyde. Thus, the eventual chromophore is derived from hydroxyproline alone.

In Table 1 the absorbance is shown to be a rectilinear function of the hydroxyproline concentration over a 40-fold range. Duplicate determinations generally did not differ by more than ±1%. Recoveries of hydroxyproline added to urine varied between 94 and 99%.

Results of hydroxyproline analyses performed on 531 urine samples obtained from pig-tailed monkeys in a wide variety of experimental conditions gave a mean value of 70 μg/ml, with a range of 3 to 250 μg/ml. The corresponding hydroxyproline urinary rates computed from the urine concentration and urine volume output rate were a mean of 0.74 mg/hr, with a range of 0.04 to 3.36 mg/hr.

Hydroxyproline analyses were also performed on 29 urine samples from a group of human subjects at rest and after heavy exercise. In this series the mean concentration of urinary hydroxyproline was 48 μg/ml, with a range of 12 to 75 μg/ml. The mean excretion rate for the group was 1.87 mg/hr, with a range of 0.79 to 3.28 mg/hr.

In order to estimate the amounts quantitatively in the lower concentration ranges, it was necessary to use a 1 ml aliquot of urine. However, because of the sensitivity of the method described, down to 3 μg/ml, much smaller aliquots of urine could be used when the urine concentration of hydroxyproline was high.

TABLE 1

ABSORBANCE OVER A 40-FOLD INCREASE IN CONCENTRATION OF HYDROXYPROLINE

μmoles	μg	OD[a]		Av OD
0.025	3.3	0.030	0.029	0.030
0.050	6.6	0.059	0.056	0.058
0.100	13.1	0.116	0.120	0.118
0.125	16.4	0.140	0.152	0.146
0.250	32.8	0.291	0.293	0.292
0.500	65.6	0.558	0.560	0.559
0.750	98.3	0.834	0.838	0.836
1.000	131.1	1.098	1.115	1.107

[a] Absorbance of duplicate samples.

The procedure described permits the simultaneous analysis of 35 urine samples of hydroxyproline by a single technician in one working day.

SUMMARY

A simplified procedure is described for the determination of hydroxyproline in human or monkey urine. In this procedure 1 ml of urine is subjected in succession to hydrolysis, oxidation, extraction and color development. During these steps impurities and interfering substances are eliminated, thus resulting in a chromophore due to hydroxyproline alone. Thirty-five urine samples can be analyzed in one working day.

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Design and Construction of Cells for Long-Path Photometers

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A previous publication dealt with the design and construction of a long-path spectrophotometer (*1*). The cells described in that paper served adequately as long as predominantly aqueous solutions were employed during the evaluation of the performances of the instrument. When applied in actual trace analysis, shortcomings became evident when the procedure involved solvent extractions. Some solvents, especially chloroform and methylisobutyl ketone, attacked the common epoxy glue employed for assembling the cells. The window affixed to one end and the photodetector cemented into the other end came loose. Investigations were initiated to find ways of improving the design and construction of the cells.

The most ideal situation would be to have an all-glass cell. Fusing flat windows to the end of a capillary proved unfeasible with the means commonly at hand in a laboratory. However, for cell bodies with an inner diameter of about 4 mm or larger, the following procedure has produced suitable devices.[1]

ALL-GLASS CELL

Make T-joints at the ends of the body tube at a distance appropriate for the desired length of cell. Cut the body tube near the T-joints and grind the end surface down close to the joints. Heat one end of the cell and seal with a small bead of molten glass. Gently puff into the tube until the bead is flattened on the inside. Proceed in the same manner at the other end. Cool the cell slowly. Grind the ends flat and polish to a smooth and optically clear surface.

With the inner sides of the cell ends not optically flat, it is advisable to position such cells in the instrument so that the incoming light is focused onto the entrance window. In this way, excessive loss of light is avoided.

[1] Don Lillie, glassblower for Georgia Institute of Technology.

For cells with capillary bodies, cementing windows to the ends has proven to be the simpler approach and the cells perform adequately when the appropriate glue is employed. Two sealants have been found to possess the required properties: Omniseal[2] and CIBA 6005.[3]

CEMENTED CELLS

a. Employing Omniseal. Make T-joints to the body tube, cut near the joints and grind down as close as possible to the joints. Apply an amount of cement just sufficient to thinly cover the ground surface. Press a piece of thin glass (microscope cover glass) onto the end. Closely inspect the area and make sure that no cement has been squeezed onto the inner surface of the window. If the window is not clear at the lumen of the tube, remove the glass and repeat the procedure with less cement. When a complete seal and a satisfactorily clear window results, place the cell upright (with the window on top) into an oven at about 275°C for 3 hr. Allow the cell to cool and repeat the foregoing procedure at the other end of the cell. Allow the cell to cure for 3 days in the oven at the above temperature. Remove any of the window glass protruding from the sides of the body tube by gently grinding it away.

b. Employing CIBA 6005. Mix 100 parts by weight of resin with 28 parts of hardener. Use this cement in exactly the same manner to affix the windows as described above. The curing is complete after 24 hr at room temperature.

Cells assembled in either of the two ways were filled with chloroform and showed no weakening of the seals after a period of 5 days. No deterioration was noted from other solvents including ethanol, acetone and methyl isobutyl ketone.

When a IN2175 photoduodiode or similar devices with highly directional sensitivity to light are employed, a loss of light intensity by a factor of up to 3 or 4 is noted when capillary cells with two windows are used. If this loss cannot be tolerated and stronger light sources or higher output amplification are undesirable, it is best to omit one window and to resort to the incorporation of the detector into the cell body as described for the original model (*1*). If the photodetector, of course, cannot tolerate the high curing temperature required for the Omniseal, the CIBA 6005 or an analogous cement has to be used. If not available, the following possibility provides a way out. The detector is fitted with Teflon tape into the flared

[2] Omniseal available from Curtis Associates, San Diego, California.

[3] CIBA 6005 available from Industrial Adhesives, Inc., 2793 East Ponce de Leon Drive, Decatur, Georgia.

end of the capillary tube in such a way as to provide a complete seal against liquid penetration. The detector is then secured at the exit end with a drop of any cement suitable for that particular purpose.

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Determination of Micro Amounts of Calcium by a ^{45}Ca-Gravimetric Procedure

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INTRODUCTION

Numerous gravimetric techniques have been employed for the quantitative determination of calcium (*1*). Of these, only the Clark–Collip permanganate titration procedure was widely used for measuring calcium (Ca) in biological tissues (*3*). Although this method can provide accurate quantitative determinations of Ca, its usefulness is limited by a lack of sensitivity, the presence of interfering substances in tissue, and the need for technical expertise. Over the last 15 yr a number of investigators have tried to develop a simple, precise, and sensitive replacement for the Clark–Collip method. Such attempts have included modifications of the Clark–Collip procedure itself (*8*), various complexometric reactions (*2, 7*), flame photometry (*4, 5*), and atomic absorption (*6, 9, 10*); and while many of these techniques have certain advantages over the Clark–Collip method, only atomic absorption spectrophotometry (AAS) has received general acceptance. Since the first application of AAS to biological fluids (*9*), equipment and procedural changes have enabled AAS to supplant the Clark–Collip method for the measurement of tissue Ca (*6, 10*). This paper describes a new gravimetric procedure involving the use of radioactive calcium (^{45}Ca). This new method offers many of the advantages of AAS, and it should be of special interest to those laboratories without atomic absorption equipment.

Solubility product considerations indicate that when a small unknown amount of calcium (Ca_u) is added to a solution relatively high in oxalate (C_2O_4), the concentration of calcium remaining in solution after complete precipitation ($[Ca_s]$) is independent of Ca_u but is dependent upon the oxalate concentration ($[C_2O_4]$) and the solubility product constant (K_{sp}) for calcium oxalate (CaC_2O_4). Furthermore, if a known amount of ^{45}Ca and associated carrier calcium (Ca_c) is initially added to Ca_u, then the resultant specific activity of the sample

([^{45}Ca activity/$Ca_u + Ca_c$]) is equal to the specific activity of Ca_s ([supernatant activity]/[Ca_s]). Mathematically this can be expressed as

when $[C_2O_4] >> [Ca_u]$, then after complete precipitation $[Ca_s] = \frac{K_{sp}}{[C_2O_4]}$, and when ^{45}Ca is added to Ca_u it holds that $\frac{^{45}\text{Ca activity}}{Ca_u + Ca_c} = \frac{[\text{supernatant activity}]}{[Ca_s]}$.

Substituting for $[Ca_s]$ and rearranging, we can write

$$Ca_u = \frac{(^{45}\text{Ca activity})(K_{sp})}{[\text{supernatant activity}][C_2O_4]} - Ca_c.$$

If the amount of ^{45}Ca (and Ca_c) added to various Ca_u samples is constant and if $[C_2O_4]$ is maintained at a uniform level, then the above relation can be simplified to the linear equation.

$$Ca_u = A \frac{1}{[\text{supernatant}]} + B \qquad \text{or}$$

$$\frac{1}{[\text{supernatant activity}]} = M(Ca_u) + N.$$

(A, B, M, and N are constants related to K_{sp}, $[C_2O_4]$, ^{45}Ca activity and Ca_c.)

It is this inverse relationship between supernatant activity and calcium content which forms the basis for our analytical procedure.

MATERIALS AND METHODS

In the following studies all reactions were carried out in HCl acid-rinsed 25-ml Pyrex flasks agitated by a water-bath shaker (New Brunswick Scientific) set for 25°C and 300 shakes per min. Water for all studies was glass distilled. To each vessel was added sequentially the following: 400 nCi of $^{45}CaCl_2$ (New England Nuclear, 10 mCi/mg), 0–300 μg Ca as $CaCl_2$ (Mallinckrodt Chemicals), and 26.8 mg $Na_2C_2O_4$ (Mallinckrodt). The final volume in all cases was 4 ml. After CaC_2O_4 precipitation was complete, the contents of each flask were passed through a 0.22 μm cellulose filter (Millipore, GSWP). A 1-ml aliquot of each filtrate was then measured for ^{45}Ca activity by liquid scintillation (Packard Tricarb, model 3320). The cocktail formula was as follows: 5.5 g 2,5-diphenyloxazole (PPO), 0.10 g 1,4-bis [2-(5-phenyloxazolyl)]-benzene (POPOP), 333 ml Triton X-100, and

toluene to make 1 liter. Quench correction was performed by external standardization.

The necessary time for complete precipitation of CaC_2O_4 was determined by varying shaking times from 0 to 6 hr. A 5-hr period was used for all other studies. The influence of magnesium (Mg) was measured by establishing Mg/Ca ratios of 0–64 with several different Ca concentrations. Mg as $MgCl_2$ (Mallinckrodt) was added to the flask before the $Na_2C_2O_4$, and Ca recoveries were then determined. The interaction of phosphate (PO_4) was judged in a similar fashion. Plasma Ca analyses were made with plasma obtained from New Zealand white rabbits, and in these studies 0.4 ml of plasma was used in place of the $CaCl_2$. Comparison measurements were made with an Instrumentation Lab Model 5 atomic absorption spectrophotometer.

RESULTS

Although standard gravimetric procedures involving CaC_2O_4 usually allow 30 min for complete precipitation (3), the dilute solutions employed in this study required considerably more time. Figure 1 indicates that when the Ca concentration was 1.25 μg/ml, 5 hr of shaking were needed for the filtrate ^{45}Ca activity to reach stable levels. This time was chosen for all subsequent studies, even though solutions with greater Ca content reached equilibrium much sooner. For example, with a Ca concentration of 50 μg/ml, 30 min of shaking was adequate.

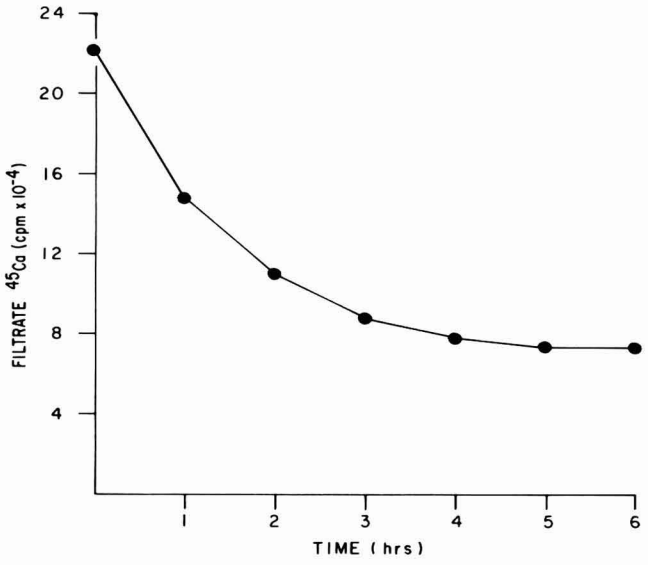

FIG. 1. Calcium oxalate precipitation time. The Ca concentration was 1.25 μg/ml.

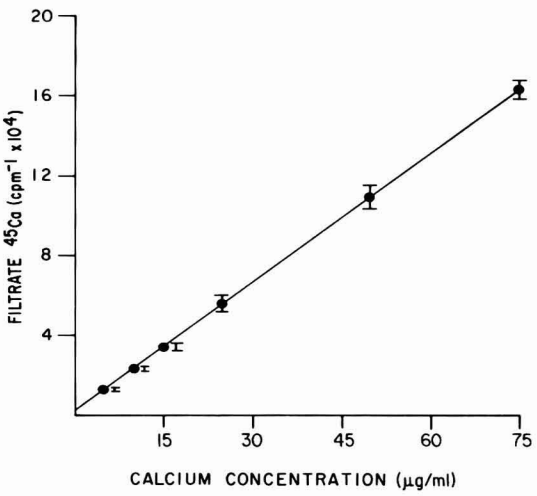

FIG. 2. Calcium standard curve: 5–75 μg/ml. Each point represents the mean of five separate determinations and the brackets indicate the 95% confidence limits. The highest concentration (75 μg/ml) has been corrected for a change in oxalate concentration by the precipitation of calcium oxalate.

Figure 2 represents a typical standard curve generated over a 15-fold concentration range. As expected from previously discussed theoretical considerations, a linear plot of the reciprocal of filtrate ^{45}Ca activity versus Ca content does not pass through the origin. This reflects the small but significant amount of carrier Ca associated with the ^{45}Ca added to each sample prior to precipitation. The precision obtainable with this assay (brackets indicate 95% confidence limits) is independent of the Ca concentration, and the coefficient of variation at each point is about ±2.5%.

The sensitivity of the method was estimated by utilizing more dilute Ca solutions. Figure 3 represents a typical plot obtained with Ca concentrations from 0 to 5 μg/ml. The slight curvilinear appearance of this graph indicates that below 5 μg/ml the inverse relationship between Ca content and filtrate radioactivity is no longer strictly linear. There are several possible reasons for this phenomenon. Perhaps with low Ca levels some CaC_2O_4 crystals formed during precipitation are too small for effective filtration, thus increasing filtrate radioactivity. A more plausible explanation, however, is that the solubility characteristics of CaC_2O_4 are altered. The solubility may be affected by the formation of coordination complexes or by an increase in the K_{sp} for CaC_2O_4 in the nanomolar concentration range of Ca. Regardless of the actual explanation for the nonlinearity, the reproducibility in this range is similar to that previously discussed, indicating

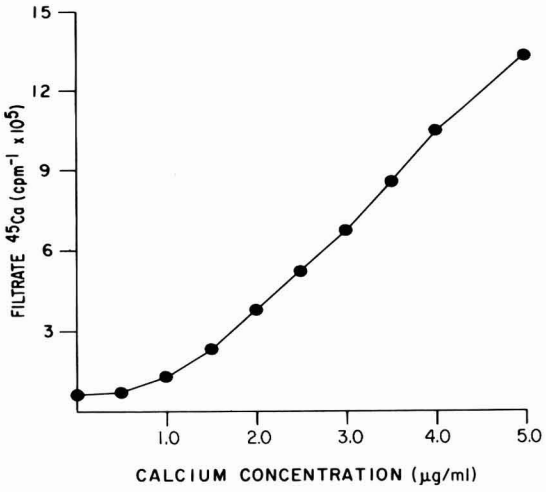

FIG. 3. Calcium standard curve: 0–5 μg/ml. Each point represents the mean of five separate determinations.

that the method as described is suitable for analysis of Ca concentrations as low as 1 μg/ml.

This procedure is essentially a simple gravimetric analysis, and only those substances which can interfere with the precipitation of CaC_2O_4 have the potential to affect adversely Ca determinations. Of the ions normally present in tissue, only Mg and PO_4 were considered to have any possible influence on our technique. Table 1 illustrates the effect of Mg on Ca recoveries. Here, the Ca content was maintained at 1.25 μg/ml while Mg was varied to establish Mg/Ca molar ratios of 0–64. Since similar quantitative findings were obtained with other Ca concentrations, the results listed here are representative. As long as the Mg/Ca ratio is 16:1 or less, the influence of Mg is negligible. The exact mechanism by which Mg produces errors at the higher ratios is uncertain; however, it is not due to lowering the C_2O_4 titre by the formation of MgC_2O_4. Because the K_{sp} for MgC_2O_4 is 10^5 higher than that for CaC_2O_4, very little if any Mg salt is formed.

The influence of PO_4 was evaluated in much the same manner as was Mg. With the Ca concentrations employed in this study, PO_4/Ca molar ratios in the range of 100:1 had no effect on Ca recoveries. For example, with a Ca level of 5 μg/ml and a PO_4/Ca molar ratio of 132:1, the mean Ca determination was only 1.6% less than the control. As long as the pH is less than 11, PO_4 appears to be of little concern. With more basic solutions, however, the formation of the relatively insoluble $Ca_3(PO_4)_2$ will be favored. This

TABLE 1
EFFECT OF MAGNESIUM ON CALCIUM RECOVERY

Mg/Ca	Mean CPM	Actual Ca concentration (μg/ml)	Calculated Ca concentration (μg/ml)	% Error
0	41026	1.250	—	—
4	41527	1.250	1.236	−1.12%
8	41211	1.250	1.247	−0.24%
16	41705	1.250	1.232	−1.44%
32	43333	1.250	1.186	−5.12%
64	46333	1.250	1.110	−11.20%

possibility must be taken into account if extremely basic media are employed.

The analytical method as described is intended for tissue Ca measurements after dry ashing. However, the applicability of the technique to the direct determination of untreated plasma was examined. Table 2 contains several plasma Ca values obtained with our procedure as well as several determined by AAS. As shown, our procedure gave Ca levels consistantly 10% lower than the AAS levels. The reason for this discrepancy was investigated. Recovery measurements of Ca additions to plasma were found to be essentially 100% (99.0 ± 3.1). This tended to rule out some nonspecific protein interference as the causative agent. Plasma aliquots were also measured by both methods before and after dry ashing. The ashing completely removed the noted discrepancy between the two methods. It appears that some tightly bound Ca measured by AAS is not available for precipitation with C_2O_4; therefore, treatment designed to destroy or denature pro-

TABLE 2
DIRECT PLASMA CALCIUM DETERMINATIONS:
COMPARISON WITH ATOMIC ABSORPTION

Animal	^{45}Ca method ± SD[a] (meqiv/l)	Atomic absorption[b] (meqiv/l)	% Difference
1	4.92 ± .19	5.50	−10.5%
2	4.76 ± .12	5.30	−10.2%
3	5.15 ± .14	5.69	−9.5%
4	6.20 ± .18	6.88	−9.9%

[a] Each value represents the mean of 5 separate determinations; 0.4 ml plasma was used for each measurement.

[b] Each value represents the mean of 5 separate determinations; 0.2 ml plasma was used for each measurement.

tein is required if quantitative measurements of plasma Ca are to be obtained with the present procedure.

DISCUSSION

The gravimetric method described here is based upon simple physical laws and chemical considerations. As such, there are a number of alterations that can easily be made to suit particular analytical situations. For example, the useful range of the ^{45}Ca technique is in part limited by the C_2O_4 concentration, since it must remain essentially unaltered by CaC_2O_4 precipitation. Increasing the level of C_2O_4 permits greater amounts of Ca to be measured. Furthermore, the precision at high Ca values is dependent upon the filtrate radioactivity, and increasing the amount of ^{45}Ca maintains good reproducibility.

The absolute sensitivity of the method is a function of the reaction volume, which is why all results are given in terms of concentration. With the 4-ml volumes used in this study, the minimal amount of Ca that could be accurately measured was 4 μg (1 μg/ml). By reducing the reaction volume to 0.4 ml, the sensitivity can be increased 10-fold with little or no loss in precision. Suitable alterations of reaction constituents and volumes in fact should allow for measurements of Ca samples as small as 100 ng (2.5 nmol).

One possible drawback of the procedure as reported is the long time period employed for CaC_2O_4 precipitation. If this should pose a problem, the reaction time can be minimized by altering at least two factors, the Ca concentration and the temperature of the reaction. When samples are relatively high in Ca, the time required for complete precipitation is considerably less than 5 hr. Another way of shortening the precipitation time is to heat the reaction vessel during shaking. Even with very dilute solutions, it was observed that heat greatly increases the precipitation rate. It must be kept in mind, though, that the K_{sp} is temperature-dependent, and poor temperature regulation could cause significant errors. Regardless of the precipitation time, this technique compares favorably with other methods insofar as manipulation time is concerned. Total working time per sample determination is between 1 and 2 min.

One final note should be made concerning the general usefulness of this procedure for the measurement of substances besides Ca. The relationship upon which the ^{45}Ca method is based applies to any reaction that forms an insoluble compound and obeys the mass law. Therefore, many substances, both organic as well as inorganic, may be measured by this technique, provided a radioactively labeled form is available.

SUMMARY

Described is a new gravimetric calcium (Ca) micromethod involving the use of ^{45}Ca. The technique is based upon an inverse relationship between Ca content and supernatant radioactivity following Ca oxalate precipitation and filtration. The procedure is sensitive (1 μg/ml), precise (coeff of var. $\pm 2.6\%$), and is largely free from interfering substances normally present in biological tissues.

ACKNOWLEDGMENTS

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Application of a pH-Stat to Ultramicrohydrogenation Analysis

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Analysis of organic compounds by catalytic hydrogenation in micro- and ultramicroscale, mainly for the determination of unsaturation, has long drawn considerable attention of analytical chemists. Recently, as little as 0.56 μmole of octene-1 has been successfully analyzed by Curran and Curley (*2*), by means of a combination of null-point pressure measurement and coulometry, with $\pm 6\%$ precision and -1% accuracy. In the present work, it was aimed to develop an analytical method which is comparable in precision and accuracy with the above but with instrumentation less complicated. A good part of this aim was realized by incorporating a commercial pH-stat in the system and making use of a very simple optical pressure sensor.

The principle of the operation of the present system is simple. The reaction of the unsaturated compound with hydrogen is carried out in a detachable compartment of the reduction apparatus. The minute negative pressure change produced by the consumption of hydrogen is detected by the pressure sensor, which thereby generates a dc error signal. This signal fed into the pH-stat actuates the syringe buret, analogously to the operation of the pH-stat in pH control, and a volume of pure solvent is delivered into the reduction apparatus, until the pressure change is just compensated and the error signal disappears. The syringe buret is provided with a potentiometer that gives a dc output proportional to the volume that has been delivered, and the recording of this signal against time makes it possible to record the progress of reaction. This was found quite useful for the detection of the completion of the reaction, detection of any irregularities, and the measurement of the magnitude of the background absorption of hydrogen by the catalyst.

The test showed that the present system works fine for several representative unsaturates, of which sample sizes ranged from 0.78 to

15.6 μmoles. The precision and accuracy obtained was very close to those reported for the combination of null-point pressure measurement with coulometry (*2*), despite the difference of instrumental sophistication.

EXPERIMENTAL METHODS

Chemicals. The unsaturates were all of reagent grade supplied by Tokyo Kasei Chemicals, and were used as received or after drying in desiccator. The catalyst was usually 20 mg of 10% palladium–charcoal (Engelhard Japan), except in a single experiment (Expt 6) in which 20 μmoles of chloroplatinic acid reduced with sodium borohydride, according to the direction by Brown (*1*), was the catalyst. Tank hydrogen was used without purification.

Apparatus. The schematic of the system is shown in Fig. 1. The all-glass reduction on apparatus (Fig. 2) is similar to that by Brown (1). The detachable flask in which the reaction is carried out has a side arm for sample injection, which is closed with a silicone rubber stopper of the type sold for gas chromatographic use. The assembled glass apparatus is immersed in the constant-temperature bath, except the tip of the side arm for sample injection and the connections to the optical pressure sensor, buret and the hydrogen in- and outlets. The exposed part of the connection to the sensor is shielded from the change of ambient temperature by polystyrene foam sheath. The total volume of the reduction apparatus was about 40 ml.

The pH-stat was TOA Dempa Type HS-2a. As many of the commercial pH-stats are, it consists of a pH meter, a control box and an automatic syringe buret, and may be used either as a pH controller or a recording titrator. The volume signal was recorded with a TOA Dempa EPR-2T recorder, at chart speeds ranging from 1 to 20

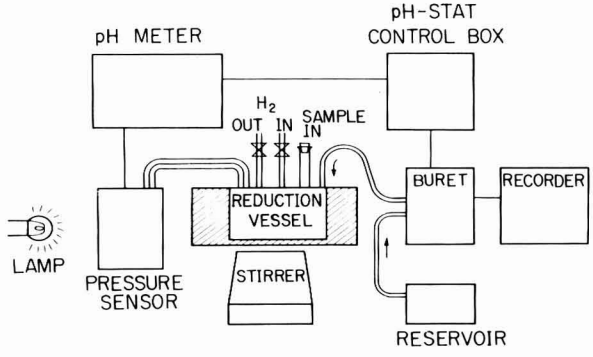

FIG. 1. Schematic of instrumentation for hydrogenation analysis.

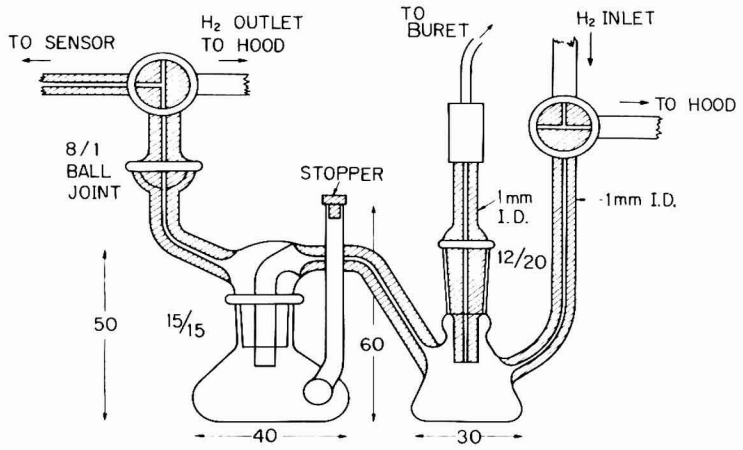

FIG. 2. The reduction apparatus. The detachable flask on the left is for carrying out the hydrogenation, and the other on the right is the receiver of the solvent delivered by the buret.

mm/min. A 1 ml syringe buret was used throughout the work; larger syringes up to 20 ml is available for micro- and macroscale work.

The pressure sensor shown in Fig. 3 was constructed in this laboratory. Its optical part (Fig. 3a) has a glass–polyethelene U-tube supported by a small wood block. The latter has a pair of vertical holes for the U-tube, and also a pair of horizontal ones each intersecting the vertical one. One end of each horizontal hole serves as the light window, and the other end as the cavity for a cadmium sulfide cell. The light source was a 5 W frosted tungsten lamp placed at 15 cm dis-

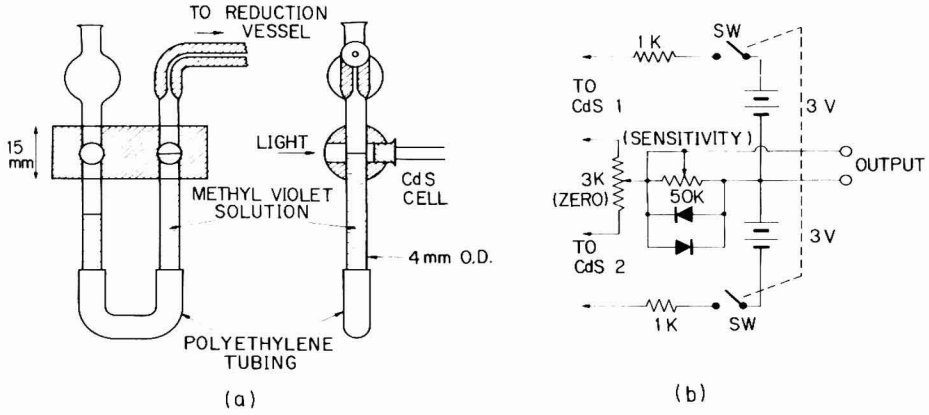

FIG. 3. The optical sensor for pressure change: (a) the optical part and (b) electrical part. The two diodes are for protection of pH meter.

tance from the sensor. The U-tube was partly filled with a solution of 0.02% methyl violet in 50% isopropanol, just to the lower end of the wood block. The electrical part of the sensor is a simple bridge, in which the two cadmium sulfide cells and the resistors form the two arms (Fig. 3b). As is explained in later section, one of the cadmium sulfide cells which faces the leg of the U-tube which is in direct connection with the reduction apparatus senses the change of the level of the colored solution, and the other cadmium sulfide cell works as the reference. The output of the bridge is connected to the pH meter, in place of the usual pH electrodes.

The performance of the pressure sensor was tested as in the following. The zero control of the sensor is put in its center position, and also the sensitivity control in its middle range. The apparatus, including the sensor, are set up as in Fig. 1, with the reduction apparatus empty and with its hydrogen outlet open. At this stage, the colored solution in the U-tube is at the same level in the two legs, and the light reaches freely to the cadmium sulfide cells. The position of the 5 W lamp is then finely adjusted so that the output of the sensor is approximately zero on the pH meter. The zero input corresponded to an indication of pH 7, in our pH meter. A small amount of air is drawn out of the vessel by means of a syringe to let the colored solution in one of the legs of the U-tube blind the light window. This corresponds to the maximum output of the sensor, and the sensitivity control of the sensor is adjusted so that the pH meter indicates pH 1 or 13. Now the control of the pH-stat is turned from "stand by" to "titrate" position and the recorder is started. As the pure solvent in the buret is slowly delivered into the reduction vessel, the pressure gradually increases. The titration is stopped when pH meter again indicates pH 7. An S curve, as shown in Fig. 4, is usually obtained on the recorder chart, if the sensor is working properly. In the example of Fig. 4, the maximum sensitivity of the sensor to the pressure change was found at about pH 10, where the sensitivity was about 2 pH units (120 mV)/μl of solvent injected. Since the nominal precision of the pH-stat was $\pm$0.015 pH, this sensitivity seemed more than enough for a ultramicrodetermination. Despite its high sensitivity, the sensor was found reasonably stable, except for a small zero drift over hours, which mattered little since the determination was usually completed within minutes. The drifts may be corrected using the zero control.

The operation of the pH-stat in the actual determination is essentially the same as that in its use in pH control. The direction of the selector switch is chosen so that the decrease of the volume inside the reduction apparatus actuates the buret to restore it, and the "pH set"

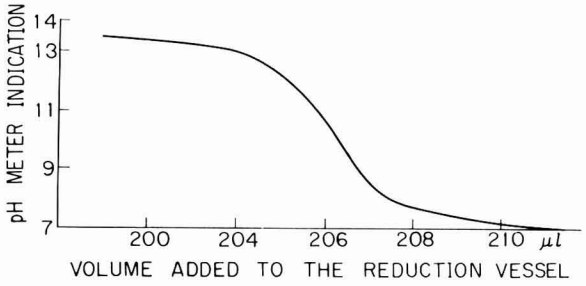

FIG. 4. Typical response of the optical sensor toward the addition of pure solvent to the reduction apparatus. Deflection of 1 unit pH on the pH meter corresponds to a 59 mV output change.

dial which selects the pH to be maintained is set at the pH value where the sensitivity of the sensor is at its maximum, i.e., pH 10 in the example of Fig. 4.

A Haake ultrathermostat with a thermometer-type regulator and on–off heater control was used to circulate 27°C water in the constant-temperature bath. To improve the temperature regulation, the heater power was reduced to 40 W and the cooling water was supplied from a constant-head reservoir. The obtained precision was better than 0.01°C and was satisfactory. In our set, a 0.01°C temperature drift should cause a 1.3 μl error in the result.

PROCEDURE

Among many possible variations, a typical one is described here. Four milliliters of isopropanol, 20 mg of 10% palladium–charcoal and a stirring bar is placed in the detachable reduction flask. The apparatus is set up and the buret is filled with isopropanol. Hydrogen is slowly passed through the glass apparatus, directing the stream to the hood. The whole set is equilibrated with the stirrer on for 1 to 2 hr. Hydrogen stream is stopped, and the reduction apparatus is isolated from outside except through the pressure sensor, by means of stopcocks. A small volume of hydrogen is drawn out from the side arm using a syringe, until the pH meter indication passes the preset pH value. The pH-stat is put into "pH control" operation, and the recorder is turned on. The buret should automatically deliver an amount of isopropanol until the pH meter indicates the preset pH. The whole set is again let stand in this state for 20 min, during which the absorption of hydrogen by the catalyst is recorded. The rate of this background absorption b, on which the correction is based, is calculated from the recorder trace.

If the background absorption was found small enough for the in-

tended analysis (see Discussion below), the actual determination can start. The sample solution to be injected in the reduction apparatus should be more concentrated than 0.05 *M* if it is expected to absorb 1 mole of hydrogen/mole, for the net decrease of the volume would be nearly zero if its concentration is 0.04 *M*. The injection of the sample solution is carried out with the tip of the microsyringe touching the wall of the side arm. The first reading of the volume from the four-digit counter on the buret is made immediately after the injection (V_1). After a few moments, the buret starts to work compensating the volume decrease due to the reaction, and the progress of the reaction is recorded as a volume–time curve on the chart. The completion of the reaction is readily found from the chart. The second reading of the volume (V_2) is then made, and it is noted if the background absorption is uniform and small as it was before the injection. If it is, this is the end of the first determination, and the consumed volume of hydrogen is calculated as the sum of the volume difference $V_2 - V_1$ and the injected sample volume. For determinations in ultramicroscale, however, this is not always the case, and additional volume readings are necessary for background correction. After the second reading, several readings (V_3, V_4 . ., etc.) are made at approximately 15 sec intervals, each time marking off the recorder trace. The corrected volumes are calculated as $V_2 - V_1 - bT_2$, $V_3 - V_1 - bT_3$ . . ., etc. Here b is the rate of the background absorption (μl/min) and T is the time (min) elapsed between the injection and the respective volume reading. If the reaction is complete at the time of the volume readings, and the background absorption is constant, the corrected volumes should be practically constant. The consumed volume of hydrogen is the sum of the mean value of the corrected volume and the sample volume.

After the second injection, only one reading after the completion of the reaction is usually necessary, since the optimum time for volume reading is now known provided no irregularities are found in the recorded curves. Injections of aliquots of a particular sample may be repeated usually more than dozen times, if desired. Reductions of different unsaturates over the same catalyst is not objectionable, if no irregularities such as the increase of the background absorption or the slowing down of the reaction is not found on the recorder chart. To save the time required for equilibration, it is convenient to have two identical glass apparatus and use them alternately for equilibration and determination.

The calculation of the moles of hydrogen consumed requires knowledge of pressure inside the reduction apparatus. With our set, the difference of the levels of the colored solution in the two legs of

the U-tube was 14 mm when the determination was going on. Since the density of the colored solution was approximately 0.9, the inside pressure was 0.9 mm lower than the observed atmospheric pressure.

RESULTS AND DISCUSSION

Since the interest of the present work was chiefly on the performance of the instrumental system, only several representative unsaturates which are known to be quantitatively reduced under essentially the same conditions were employed. The results of the determinations are shown in Table 1. For the smallest sample size of 0.7815 μmole of octene-1, the precision was $\pm 7.0\%$, but it steadily improved with larger sample sizes and was $\pm 0.5\%$ for 15.63 μmole. The same trend was found for cyclohexene too. These results were obtained by addition of samples as 0.1 to 0.2 *M* solutions in isopropanol.

When Table 1 is compared with Table 4 of Ref. (*2*), which was obtained by much more sophisticated instrumentation, it is known that the present method is at least comparable in precision and accuracy with the other, for similar sample sizes. Apparently the error in measurement of small volume changes is not the limiting factor, but the relative magnitude of the background absorption seems to be responsible.

In this connection, several observations on the background absorption were made during this work. With 20 mg of 10% palladium–charcoal and 4 ml isopropanol, the rate of the background absorption was typically 10 μl after 10 min of equilibration. It steadily decreased with time to a value between several tenths of a microliter and a few microliters, after 1 to 2 hr. Essentially the same trend was found for Brown's platinum catalyst. Fortunately for the present system which is unable to deal with a positive pressure change, a positive background drift was never observed in the presence of either of the catalysts.

The correction method employed in the present work for the background correction, as well as the graphical method by the previous workers (*2*), is based on the assumption that the background absorption rate is constant before, during and after the reaction. Although this assumption seems usually adequate for practical purposes, an apparent deviation from it was experienced in the following instance. In the repetitive injection of 0.1010 *M* sorbic acid, the corrected volume of apparent hydrogen consumption increased steadily from 100.3 to 105.3 μl during seven injections and reactions. Examination of the recorded curves showed that the background absorption rate noticably increased after three determinations, and that this is responsi-

TABLE 1

TYPICAL RESULTS OF ULTRAMICROHYDROGENATION ANALYSIS

Expt no.	Compound	Theor. H_2 consumption/mole	Compound taken (A) (μ moles)	No. of detns.	Compound found (B) (μ moles)	(B/A) × 100	SD relative to B
1	Octene-1	1	0.7815	4	0.800	102.4	7.0
2		1	0.9804	5	0.953	97.2	5.8
3		1	1.094	4	1.084	99.1	2.6
4		1	1.563	6	1.575	100.8	3.9
5		1	1.961	5	1.941	99.0	2.6
6		1	4.902	3	4.930	100.6	0.5
7		1	10.94	4	11.08	101.3	0.3
8		1	15.63	6	15.76	100.8	0.5
9	Cyclohexene	1	1.024	8	1.031	100.7	3.6
10		1	2.561	7	2.577	100.7	1.7
11		1	10.24	6	10.30	100.6	0.5
12	Sorbic acid	2	2.525	3	2.522	99.9	1.5
13	*t*-Cinnamic acid	1	5.108	4	5.137	100.6	3.3
14	Nitrobenzene	6	2.523	3	2.559	101.4	2.2

ble for the above results. After equilibrating the catalyst for 1 hr, the background absorption rate reverted to the original value and the repeated determination showed 100.2 μl consumption. The results shown as Expt 11 in Table 1 are based on the first three injections. The impurity which might be present in the sample of sorbic acid may perhaps be responsible but, all the same, the importance of scrutinizing the recorded curves for any irregularities is to be stressed.

CONCLUSION

Analytical results at least comparable with a more sophisticated method were obtained by a relatively simple instrumentation including a commercial pH-stat and a simple optical pressure sensor, for the sample sizes ranging from 0.78 to 15.6 μmoles of several representative unsaturates. There is no reason to suppose scaling up this method would not make an excellent micro- or macromethod, at least for laboratories with readily available pH-stat. No alterations are necessary for the pH-stat except replacing the pH electrodes by the optical sensor.

A pH-stat is in itself a servomechanism that seems to have many potential applicabilities to various types of chemical analysis and control.

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The Determination of Lanthanides in Organometallic Complexes by the Closed Oxygen Flask Method

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INTRODUCTION

The quantitative determination of the lanthanide content in organometallic compounds becomes a necessity for those species whose characterization by common physical techniques is hampered by various factors (*4*). Since all organolanthanides are air- and moisture-sensitive to varying degrees, the customary procedures involved in metal estimation, namely either the dry combustion of a sample in oxygen to the corresponding metal oxide or the wet combustion of the sample by an appropriate acid medium in a Kjeldahl flask, become extremely cumbersome. Moreover, the dry combustion technique is totally inapplicable if metals other than the rare earths are also present in the compound to be analyzed. We now report that the desired lanthanide determinations can be carried out simply, accurately and rapidly if the analytical sample is initially decomposed by means of the oxygen flask.

Since 1955 considerable attention has been focused on the analysis of nonmetals by Schöniger's oxygen flask method (*7, 14, 15*). However, despite its apparent utility for the analogous determination of metals, this combustion technique has only been infrequently employed (*1, 8, 12, 13, 16*) during the estimation of both main group (e.g., Mg, Ca, Ba, and Sn) and transition metals (e.g., Zn, Cd, Hg, Mn, and Co). Furthermore, the metal-containing species which were studied were primarily air-stable coordination compounds, and thus true organometallic complexes (i.e., those containing direct metal–carbon bonds) have largely been neglected. Our studies, described in this paper, represent the first extensive application of the oxygen-flask technique during the analysis of a class of these latter industrially important complexes.

Accounts of the basic principles, methods and problems of metal analysis by the Schöniger method have been published (*1, 8*).

EXPERIMENTAL MATERIALS AND METHODS

Reagents and Apparatus

All reagents used were of analytical grade or comparable purity. The titrations were carried out with either a Gilmont micrometer burette (Cole-Parmer catalog number 7876) having a 2.5000 ml capacity and capable of measuring to 0.0001 ml, or a Mettler E457 micrometer burette having a 5.000 ml capacity and capable of measuring to 0.001 ml, as required. The hydrogen-ion concentration in the various solutions was monitored by an Orion model 801 digital pH meter.

Procedure

Since all of the compounds examined were sensitive in differing degrees to both air and moisture, all manipulations of the solid species prior to combustion were performed in a glove bag (*9*) filled with prepurified nitrogen. Analytical samples were prepared by placing 5–10 mg of the organolanthanide complex into preweighed adhesive cellulose containers (*2*) fitted with a filter paper lining. The containers were then sealed and their weight was determined either by the procedure outlined by Pickhardt and co-workers (*9*), or by direct weighing in the nitrogen atmosphere on a Cahn electronic balance. The samples were then ignited in a 500-ml oxygen flask charged with 10 ml of either 1 *N* HCl or 1 *N* HNO_3 as the absorbent solution. After combustion was complete, the flask was shaken thoroughly for 10 min, and then the stopper and platinum gauze were rinsed with distilled water. During this operation it was noted that the solubilities of the lanthanide residues in the absorbent solutions diminished as the atomic weight of the metal increased. Thus, for example, the La, Sm, and Gd oxides readily dissolved in the acid solution whereas the corresponding Dy, Er, and Yb species required gentle heating to effect complete dissolution. Once the metal oxides were dissolved, the contents of the oxygen flask were then washed into a 150-ml beaker, the total volume of the solution at this stage being approximately 50 ml.

The most rapid and simple means of final determination involved the titrimetric evaluation of the lanthanides with 0.01 *N* or 0.005 *N* EDTA. Two types of titration environments were utilized and the end points of the titrations were ascertained by the three methods outlined below.

A. A basic medium with Eriochrome Black T as indicator. The hydrochloric acid absorbent solution was neutralized at room temperature with 1 *N* NH_4OH, with sodium tartrate being added at pH 6 to prevent precipitation of hydroxide derivatives of the metals. An

appropriate NH_4Cl–NH_4OH buffer solution was next introduced to maintain the pH of the solution in the optimum range of 8.3–8.6. The solution was then heated to ~80°C, and was titrated directly at this temperature in the presence of Eriochrome Black T. Throughout this titration, stringent adherence to the specified pH range [narrower than that previously reported for analogous determinations (*3*)] was found to be mandatory because the indicator was very sensitive to changes in the hydrogen-ion concentration, especially when small amounts of metals such as ytterbium were being titrated. For example, at pH 8.8 the end points were attained very slowly and the recoveries of the heavier lanthanides were never quantitative. Below pH 8.3, on the other hand, the color change of the indicator near the end point was very poor.

B. An acidic medium with Xylenol orange as indicator. The pH of the hydrochloric acid absorbent solution was adjusted to approximately 4 with 1 *N* NH_4OH. While the solution was gently warmed, an appropriate CH_3COONa–CH_3COOH buffer was added to maintain the pH of the solution in the range 4.8–5.5. This environment was found to be the most satisfactory of all for obtaining good end points in the direct titration of the warm solution with Xylenol Orange as indicator. If the pH was allowed to increase above 5.8, not only were repeated false end points prematurely observed, but also the reddish tint of the indicator itself obscured the desired color change at the true end point. Our findings thus substantiated recent reports [cf. (5)] that the true working pH range of Xylenol Orange in such complexometric titrations is actually lower than has been previously claimed (*6*).

C. An acidic medium with the end point being detected potentiometrically. One normal nitric acid was used as the absorption medium during the initial combustion. The pH of the final absorbent solution was adjusted to 4.3–5.0 with 1 *N* NaOH. Then 5 ml of an acetate buffer (pH 4.8) and four drops of a 10^{-3} *M* solution of the mercury–EDTA complex were added. The resultant solution was heated to 80°C and was titrated while hot with EDTA, the end point being detected potentiometrically with a mercury electrode (*10, 11*). A calomel electrode filled with a saturated solution of KNO_3 was employed as a reference.

No difficulties were experienced with this method and all titrations were easily performed. Moreover, the presence of chlorine in the organolanthanide sample did not interfere with the mercury indicator electrode. Hence, because of its general applicability, especially when very small (< 1 mg) amounts of metal were to be determined, and less rigorous experimental conditions, this procedure eventually became the method of choice.

TABLE 1

ANALYSIS OF VARIOUS ORGANOLANTHANIDE COMPLEXES

Compound	% Lanthanide	
	Theoretical	Found
Tris(cyclopentadienyl)lanthanum, $(C_5H_5)_3La$	41.56	41.47
Tris(cyclopentadienyl)samarium, $(C_5H_5)_3Sm$	43.51	43.21
Tris(methylcyclopentadienyl)samarium,[b] $(C_6H_7)_3Sm$	38.79	38.64
Tris(methylcyclopentadienyl)gadolinium, $(C_6H_7)_3Gd$	39.84	39.81
Tris(methylcyclopentadienyl)dysprosium, $(C_6H_7)_3Dy$	40.64	40.69
Tris(cyclopentadienyl)erbium, $(C_5H_5)_3Er$	46.12	46.34
Bis(cyclopentadienyl)erbium chloride, $(C_5H_5)_2ErCl$	50.23	50.47
Bis(cyclopentadienyl)ytterbium chloride,[a] $(C_5H_5)_2YbCl$	51.08	51.31
Tricarbonyltris(cyclopentadienyl)erbiumtungsten,[b] $(C_5H_5)_2ErW(C_5H_5)(CO)_3$	26.54	26.42
Tricarbonyltris(cyclopentadienyl)tungstenytterbium,[a] $(C_5H_5)_2YbW(C_5H_5)(CO)_3$	27.20	27.04

[a] Determined by method A.
[b] Determined by method B; all others determined by method C.

The closed oxygen flask technique was also successfully utilized during the analysis of more complex organometallic compounds. For instance, the compounds which contained both tungsten and a lanthanide were initially burned in the usual manner. The absorbent solution was then simply boiled, the tungstic acid was removed from the hot solution by filtration, and the lanthanide content of the filtrate was determined by one of the methods described previously.

RESULTS AND DISCUSSION

Good-precision end points were obtained with all the methods of final determination as shown by the representative data displayed in Table 1.

The results indicate that the closed oxygen flask method provides a general means of decomposing very reactive compounds which contain direct metal–carbon bonds. Moreover, the flask method expedites the desired metal analyses in those cases for which a rapid final method of determination is known or can be developed. In this connection it should be noted that when small amounts (i.e., 1–3 mg) of metals are being analyzed, conditions different from those employed for large-scale determinations may be required. For example, our studies reveal that for the use of Eriochrome Black T as an indicator in lanthanide–EDTA titrations, the hydrogen-ion concentration range in which the indicator–metal complex is not too inert and the protonated form of the indicator does not interfere is, unexpectedly, quite

small when microdeterminations of metals are performed.

Certainly the closed oxygen flask method is invaluable as the first step during analysis of the lanthanide content of all types of organolanthanide complexes. We are presently investigating the applicability of the described procedures to more intricate organometallic entities, and details of these studies will be reported in subsequent publications.

SUMMARY

A simple and rapid method for the determination of lanthanides in organolanthanide complexes is based on the closed oxygen flask method of combustion. Although a number of methods of final determination have proved to be satisfactory, the preferred procedure involves the following steps. The combustion products are absorbed in 1 *N* HNO_3, interfering metal elements are removed if necessary, the pH of the solution is adjusted to 4.8, and the desired lanthanide element is evaluated by a direct potentiometric titration with EDTA. The accuracy of the final results is good and the method has been applied during the analysis of several air- and moisture-sensitive cyclopentadienyl derivatives.

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Indirect Atomic Absorption Spectrophotometric Determination of Arsenic

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INTRODUCTION

An indirect method has been used for the determination of arsenic based on the formation of arsenomolybdic acid, its extraction in methyl isobutyl ketone (MIBK) and its use in the determination of molybdenum (and indirectly arsenic) by atomic absorption spectroscopy. Danchik and Boltz (*1*) have reported a similar method for the determination of arsenic in pure solutions. In the present study, interference from other ions that form heteropoly acids with molybdic acid was overcome by prior separation of arsenic from these ions using diethylammonium diethyldithiocarbamate (DDDC) as a complexing agent and diethyl ether as solvent. Arsenic has been determined in the presence of phosphate, silicate, and germanium (IV). The method has also been used for the determination of arsenic in organic material.

MATERIALS AND METHODS

Apparatus

A Perkin–Elmer Model 303 atomic absorption spectrophotometer fitted with a potentiometric recorder, a standard molybdenum hollow cathode lamp, and a standard 10-cm air–acetylene premix burner was used in this study.

Reagents

All materials used were of reagent grade or better.

Determination of Arsenic in Pure Solutions

Known amounts of arsenic (V) in 10 ml 0.5 *N* HNO_3 were treated with 1 ml 1% ammonium molybdate solution and the solutions were allowed to stand for 15 min for complete formation of arsenomolybdic

acid. These solutions were extracted with 10 ml MIBK and the MIBK layer washed once with 10 ml 0.5 *N* HNO_3. MIBK extracts were fed to the burner of the atomic absorption spectrophotometer and absorption due to molybdenum was measured. The molybdenum 3133 Å line and a reducing air–acetylene flame were used. A calibration curve constructed from the readings obtained was used to determine arsenic in unknown solutions.

Determination of Arsenic in Presence of Other Metals

The method used for selective extraction of arsenic DDDC complex is similar to that described by Morrison and Freiser (*4*). Arsenic (V) was reduced to arsenic (111) since only arsenic (111) is complexed by DDDC. Two milliliter 20% KI solution were added to 20 ml of arsenic solution in 5–6 *N* HCl and the mixture was heated for a few min at 40°C. Next, 0.5 ml 5% $NaHSO_3$ solution and 10 ml 1% aqueous DDDC solution were added, the mixture was cooled to room temperature and was extracted with 20 ml diethyl ether. The ether extract was washed twice with 10 ml 2 *N* HCl solution and evaporated on a steam bath. The residue was treated with 10 ml concd. HNO_3, followed by evaporation of the HNO_3 on a hot plate. The residue was then dissolved in 10 ml 0.5 *N* HNO_3 solution and arsenic was determined in this solution by the method described for the determination of arsenic in pure solutions.

Determination of Arsenic in Organic Material

Ten grams of organic material was treated with 10 ml 10% magnesium nitrate solution; the material was dried in an air oven and then ashed at 600–700°C. The ash was dissolved in 20 ml 5–6 *N* HCl solution. Arsenic was determined in this solution by the method described for the determination of arsenic in the presence of other metals.

RESULTS AND DISCUSSION

The indirect method for the determination of arsenic was found to have a sensitivity of 0.04 ppm (concentration of metal that gives 1% absorption).

The DDDC–organic solvent combination has been shown to be very selective in extracting arsenic from strongly acidic solutions (*3–5*). In view of this only a restricted study of interferences has been made in the present investigation. When 1 ppm of arsenic was determined according to the recommended procedure, no interference was caused by 100 ppm of phosphate, silicate and germanium (IV).

The recommended method described above was used for the determination of arsenic in feed grade wheat. Samples of feed grade wheat

were also analysed by arsine–silver diethyldithiocarbamate method (*2, 6*). Both methods indicated presence of 0.85 ppm of arsenic in feed grade wheat.

SUMMARY

Arsenic has been determined indirectly by atomic absorption spectroscopy by estimating molybdenum in a solution of arsenomolybdic acid in methyl isobutyl ketone. Interference from other ions has been eliminated by prior extraction of arsenic as the diethyldithiocarbamate in diethyl ether. Arsenic has been determined in the presence of phosphate, silicate and germanium (IV). The method has also been used for the determination of arsenic in organic material.

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MICROCHEMICAL JOURNAL **18,** 181–189 (1973)

Micro- and Ultramicrodetermination of the Formyl and Isonitrile Groups in the Presence of Other Acyl Groups

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INTRODUCTION

There are only a few published methods for the determination of the formyl group in organic compounds. All of these methods are based on the hydrolytic cleavage of the formyl group, followed by the quantitative determination of the liberated formic acid either as the free acid or a suitable derivative (*1, 16, 19, 26*). Only one of these methods (*16*) describes the determination of the formyl group in the presence of the acetyl group.

In order to obtain formic acid quantitatively, one must devise hydrolytic conditions that will assure the quantitative cleavage of the formyl group as well as the quantitative separation of the liberated formic acid from the products of hydrolysis. This is not an easy task especially when one is dealing with microgram amounts.

Acid hydrolysis of the acyl group is achieved by nonvolatile acids such as 33% sulfuric or 25% *p*-toluenesulfonic acid. When the formyl group is present, the use of sulfuric acid leads to the partial decomposition of the formic acid (*18, 22, 27*).

The hydrolytic cleavage of the formyl group is easily achieved using sodium hydroxide solution. In some cases, such cleavage can be accomplished at room temperature (*18*). When alkali is used for the hydrolysis, the reaction mixture must be acidified before distillation. Distillation can be accomplished either at normal or reduced pressures or by using steam distillation.

None of the above-mentioned methods of distillation, including the use of Wiesenberger's apparatus (*28*), led to quantitative results in our experience. Therefore the distillation method of Grant (*12*) was tried. This method is based on the use of a special apparatus in which the distillation is conducted in a closed system at room temperature at

TABLE 1
EXISTING METHODS FOR THE DETERMINATION OF THE FORMYL GROUP.

Author	Method of		
	Cleavage of the formyl group	Separation of formic acid	Determination of formic acid
Alicino (*1*)[a] Steyermark (*26*)	Acid hydrolysis (25% *p*-toluene-sulfonic acid)	Distillation in vacuo (50–60 mm)	Iodometric titration
Kan *et al.* (16)	Basic hydrolysis (*N* NaOH)	None	indirectly $HgCl_2 \xrightarrow{HCOOH} Hg_2Cl_2$ Hg_2Cl_2 determined iodometrically
Laksmi and Ramachandran (19)	Hydrazinolytic cleavage	Formic hydrazide	Colorimetrically as *N*-formyl-*N'*-(2,4-dinitrophenyl)-hydrazine

[a] Alicino and Steyermark determined the formyl group according to the method for acetyl group determination of Elek and Harte (*9*).

pressures below 10μ. The distillate condenses at $-78°C$, and the recovery of formic acid is of the order of 99–100%.

Method A

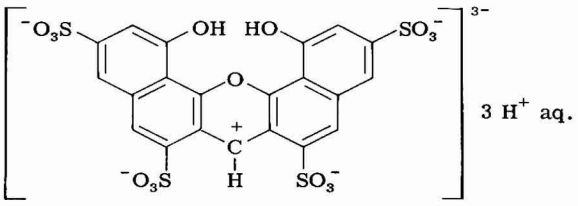
The microdetermination of the formyl and isonitrile groups is based on the decomposition of formic acid to carbon monoxide and water. To our knowledge, this well-known reaction has not been adapted for convenient analytical use (*6*). Using 60% sulfuric acid at reflux for 0.5 hr, a quantitative cleavage of the formyl group occurs and is followed by the decomposition of the formed formic acid. For the determination of carbon monoxide, the method of Schütze (*25*) and Zimmermann (*29*) was selected, using the oxidation of carbon monoxide by iodine pentoxide, followed by gravimetric determination of the carbon dioxide.

This gravimetric micromethod requires relatively large amounts of material ($\sim$30 mg) for compounds of molecular weight around 1000 ($\sim$3% of formyl group).

Method B

The ultramicromethod of determination of the formyl and isonitrile groups is based on the basic hydrolysis (1 *N* sodium hydroxide) of the sample. The hydrolysate is acidified, and the quantitatively formed formic acid is distilled in the closed system and then reduced to formaldehyde using magnesium turnings and hydrochloric acid (*7, 11*).

The amount of formaldehyde is determined spectrophotometrically by measuring the color intensity at 570 nm generated by reaction with chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) (*8, 21*). It has been suggested that the color is due to the formation of (I) (*15*)

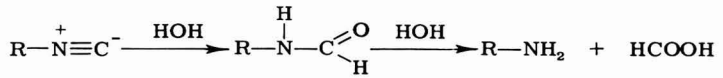


This method represents a modification of the formic acid determination by Grant (*13*).

The colorimetric ultramicromethod described here requires only very small amounts of material. The sample to be analyzed should not contain over 20 μg of formic acid formed after hydrolysis. (The color intensity follows Beer's law in the range 1–20 μg/5 ml.) Although as little as 1 μg of formic acid (0.2 μg/ml) may be determined, the most

convenient amount is 5–10 μg/5 ml (1–2 μg/ml).

Both of these methods allow the determination of the formyl group in the presence of other volatile acyl groups, and can be used for determination of isonitrile groups. The isonitrile group yields formic acid according to the following reactions:



Interferences (*2–5, 17, 20, 23, 24*).

Gravimetric method. Any fragment or group present in the compound to be analyzed or contamination which yields formic acid or carbon monoxide by acid hydrolysis will interfere.

Colorimetric method. Any fragment or group present in the compound to be analyzed or contamination which yields formic acid or formaldehyde by alkaline hydrolysis will interfere.

As another interference, for example, the possibility of the ring formation arising from the formyl group should also be taken into consideration.

EXPERIMENTAL METHODS

Method A. Gravimetric Microdetermination

Reagents. 60% Sulfuric acid
Nitrogen
Ascarite (8–20 mesh)
Magnesium perchlorate (anhydrous)
Activated iodine pentoxide (Schütze contact)
4,4′-Methylenebis(*N,N′*-dimethylaniline), granulated

Apparatus and procedure. Figure 1 shows the apparatus which consists of a 25-ml pear-shaped flask connected to a small condenser. Nitrogen, which is swept through the system at 15 ml/min, provides an inert atmosphere and mixes the solution. During a 30-min digestion of the substance in 2 ml of 60% sulfuric acid, the reaction vessel is heated in an air bath with a microburner to gentle boiling. The carbon monoxide thus formed passes through the ascarite tube to the oxidation tube where it is oxidized at room temperature to carbon dioxide with the Schütze contact, and finally is transferred to the absorption tube. After an additional 15 min of cooling time and continuous sweeping with nitrogen, the reaction is completed. The absorption tube is then disconnected and the absorbed carbon dioxide is determined gravimetrically. The tube is handled similarly to the absorption tube in carbon and hydrogen analysis. The determination is compared against a standard sample. A small blank of approximately 0.01 mg was found.

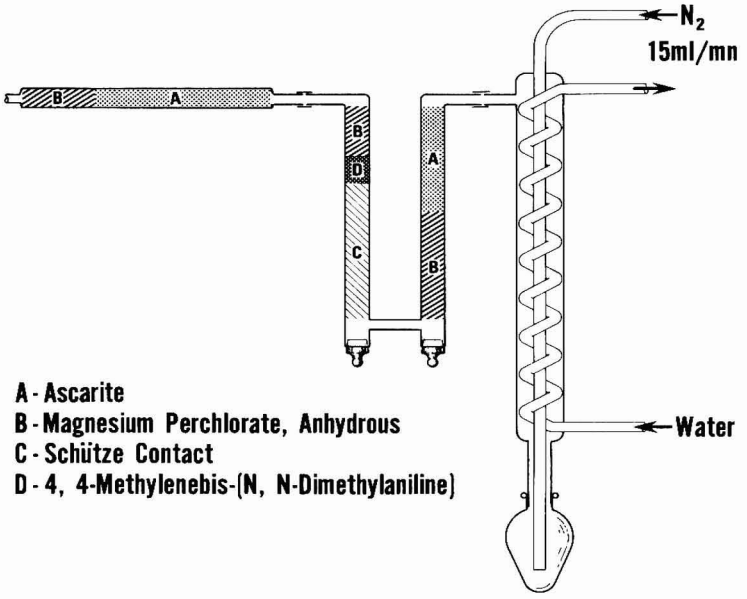


FIG. 1. Apparatus for hydrolysis of the formyl group and decomposition of formic acid.

Calculations.

$$\% \text{ formyl group} = \frac{0.6594 \times \text{mg } CO_2 \times 100}{\text{mg sample wt}},$$

$$\% \text{ isonitrile group} = \frac{0.5912 \times \text{mg } CO_2 \times 100}{\text{mg sample wt}}.$$

Method B. Colorimetric Ultramicro Determination

Reagents. 1 *N* Sodium hydroxide solution
1 *N* Ethyl alcoholic sodium hydroxide solution 50%
4 *N* Hydrochloric acid solution
1 *N* Hydrochloric acid solution
Magnesium turnings 3–5 mm long and 1–3 mm wide
Concentrated hydrochloric acid

Chromotropic acid reagent. A solution of 0.3 g of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid), Practical, Matheson, Coleman and Bell, purified according to the procedure described by Bricker and Johnson (*4*) in 10 ml of water and 90 ml of concentrated sulfuric acid (analytical reagent grade). Store in a dark glass stoppered bottle.

Procedure. An appropriate amount of sample is dissolved in 1 *N* NaOH (1.5 ml of this solution should not produce over 20 μg of formic acid after hydrolysis). If the substance is insoluble in sodium

TABLE 2
RESULTS USING GRAVIMETRIC METHOD

Substance	MW	Sample (mg)	CO_2 (mg)	Formyl group (%)	
				Theory	Found
Formylhydrazine (practical grade)	60.06	11.629	8.33	48.32	47.26
N-Formylaniline (formanilide)	121.13	12.478	4.49	23.96	23.74
N-Formylindoline	147.17	10.956	3.25	19.72	19.58
2-Formylamino-3-hydroxybenzoic acid	181.14	12.411	2.80	21.99	21.54
N-Formyldiphenylamine	197.23	8.979	1.99	14.72	14.70
N-Formyldiethylaminomalonate	203.19	14.061	3.03	14.28	14.21
α-Formylamino-β-(6-chloroindol-3)-propionic acid	266.69	15.14	2.24	14.63	14.42
Vincristine sulfate	923.02	32.957	1.572	3.14	2.81
				Isonitrile group	
$C_{16}H_{24}O_8(NC)_2$	396.39	23.54	5.34	13.14	13.41

hydroxide solution at room temperature or upon heating, the hydrolysis is performed in 1.5 ml 1 *N* ethanolic sodium hydroxide. In such a case, after the completion of hydrolysis, the reaction mixture is evaporated to dryness in a closed system. Hydrolysis is conducted in a 50-ml round flask (14/20) for 30 min under reflux (heating mantle, 130–140°C). After cooling of the hydrolysate to room temperature, 0.5 ml of 4 *N* hydrochloric acid is added. When the sample is evaporated to dryness, 2 ml of 1 *N* HCl is added. (The total volume should be approximately 2 ml).) Then, formic acid is distilled in the closed system shown in Fig. 2. Flask C containing the hydrolysate is immersed in a dry ice–acetone mixture. After evacuation to approximately 10μ stopcock D is closed and the apparatus is disconnected from the vacuum pump. Tube A is now placed in a mixture of dry ice–acetone (Dewar flask) and flask C is removed from the cooling bath. The distillation at room temperature is carried to dryness and the distillate is condensed at −78°C. This operation requires 1:5–2.0 hr. Twelve distillations may be conducted simultaneously in a 25-cm diameter Dewar flask. The distillate containing formic acid in tube A is then immersed in an ice bath on a shaker, and

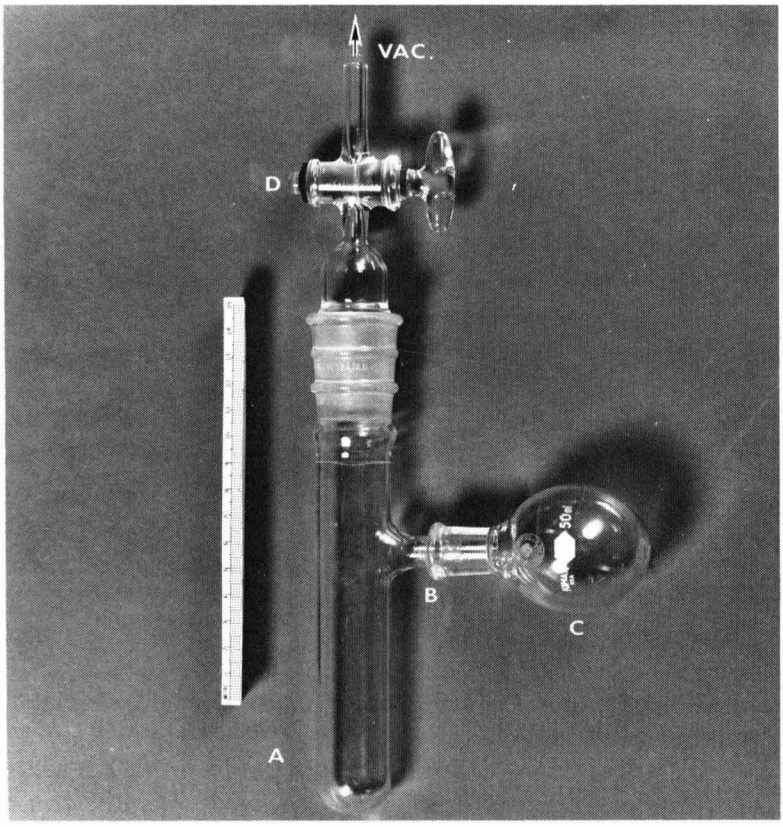


FIG. 2. Apparatus for low-temperature vacuum distillation in a closed system.

the reduction to formaldehyde is achieved using 80 mg of magnesium turnings and 0.5 ml concentrated hydrochloric acid. The acid is added in ten 0.05-ml portions at 2-min intervals. After the addition is complete and the magnesium has not completely reacted, the vessel is shaken for an additional time. After the completion of the reduction, 1.5 ml of chromotropic acid reagent is added. The mixture is then heated in a glycerine bath at 98–100°C for 60 min. After cooling, the reaction mixture is diluted to a total volume of 5 ml with distilled water and the absorbance is read at 570 nm against the reagent blank.

The relationship between the absorption intensity and quantity of formic acid is established by measuring the color produced by a series of standards containing 0–20 μg of formic acid subjected to the same procedure as the sample.

SUMMARY

Gravimetric and colorimetric micromethods for determination of formyl and isonitrile groups are reported. The gravimetric method is

TABLE 3
RESULTS USING COLORIMETRIC METHOD

Substance	MW	Formyl group (%)	
		Theory	Found
Formylhydrazine (practical grade)	60.06	48.32	47.11
N-Formylaniline (formanilide)	121.13	23.96	23.76
N-Formylindoline	147.17	19.72	19.63
N-Formyldiphenylamine	197.23	14.72	14.59
N-Formyldiethyl-aminomalonate	203.19	14.28	14.18
Folinic acid	473.44	6.13	6.04
Vincristine sulfate	923.02	3.14	2.76
		Isonitrile group	
$C_{16}H_{24}O_8(NC)_2$	396.39	13.13	12.47

based on a quantitative cleavage of the formyl group and decomposition of formed formic acid to carbon monoxide by means of 60% sulfuric acid. Carbon monoxide is oxidized and the formed carbon dioxide is determined gravimetrically.

The colorimetric ultramicromethod is based on the basic hydrolysis of the formyl group. After acidifying the hydrolysate, the quantitatively formed formic acid is distilled under special conditions. The acid is then reduced to formaldehyde. Its quantity is determined spectrophotometrically with chromotropic acid.

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The Comparison of Hydrogen-Bond Dissociation of *cis*-9-Octadecenoic Acid and *trans*-9-Octadecenoic Acid in the Inactive Solvents by Means of IR Absorption Spectroscopy

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The comparison of selected *cis*- and *trans*-9-octadecene derivatives in the aspects of influence of their functional groups and geometrical configuration of isomers on the course of autoxidation was the matter of our previous papers (*2–5*). On the basis of the performed investigations it was stated, that the character of functional group and configuration of isomer essentially influenced the kinetics and mechanism of the autoxidation process. Because different functional groups and different configurations variously influence intermolecular relations in the mass of oxidized substance, the supposition was made that the strength and character of hydrogen bond is related with the mechanism and kinetics of this process. Therefore, in this paper we tried to establish the degree of dissociation of hydrogen-bond multimers in a function of concentration in the inactive solvents for the following substances: *cis*-9-octadecenoic acid and *trans*-9-octadecenoic acid. The applied technique of measurements was ir absorption spectroscopy.

By reason of the complicated multimer structures, which are possible to occur in the case of the a/m substances, as well as the lack of the adequate theoretical models, the degree of dissociation was accepted as:

$$\alpha = \frac{\epsilon_{10}}{\epsilon_{11}}$$

where ϵ_{10} is the maximum value of molar extinction coefficient of the monomer O–H band measured in the acid solution, and ϵ_{11} is the maximum value of molar extinction coefficient of the monomer O–H band for an indefinitely diluted solution of acid in the same solvent.

EXPERIMENTAL

For our investigations the following substances were used: *cis*-9-octadecenoic acid (R.C.B., Belgium) and *trans*-9-octadecenoic acid (Koch-Light Lab., Ltd., England). Both these substances were of a high purity (98–100%). The inactive solvents used in our experiment were: carbon tetrachloride for ir spectroscopy (Lachema, Czechoslovakia) and *n*-hexane for ir spectroscopy (R.C.B., Belgium).

The measurements were performed on a double-beam UR-20 spectrophotometer (Carl Zeiss, Jena, East Germany), employing an LiF prism and NaCl trays. Spectra of the stretching O–H vibrations in the region of monomer absorption band were run for the 3-mm thick trays. The following conditions of registration were applied: velocity 64 cm^{-1}/min, width 100 cm^{-1}/40 mm.

The obtained results are shown in Tables 1 and 2.

Besides we show the molecular extinction coefficient curves of the monomer O–H bands for the examined series of concentrations of both substances in carbon tetrachloride and *n*-hexane.

As an explanation it must be added, that the spectrophotometrically registered monomer bands were graphically separated from multimer bands in the below presented way.

This procedure was necessary to prepare Figs. 1–4 and to calculate ϵ_{max} and α values.

TABLE 1
cis-9-OCTADECENOIC ACID

Solvent	Concentration [M]	Monomer O–H band		Degree of dissociation α
		ν_0 [cm^{-1}]	ϵ_{max} [1/mole · mm]	
Carbon tetrachloride	1.21	3537	–	–
	0.72	3537	0.60	0.17
	0.43	3537	0.73	0.21
	0.121	3537	1.77	0.51
	0.072	3538	2.35	0.68
	0.043	3538	2.73	0.79
n-Hexane	1.21	3547	0.30	0.17
	0.72	3547	0.34	0.19
	0.43	3547	0.42	0.23
	0.121	3548	0.91	0.51
	0.072	3548	1.30	0.72
	0.043	3549	1.52	0.84

TABLE 2
trans-9-OCTADECENOIC ACID

Solvent	Concentration [*M*]	Monomer O–H band		Degree of dissociation α
		ν_0 [cm^{-1}]	ϵ_{max} [l/mole · mm]	
Carbon tetrachloride	1.21	3537	0.52	0.15
	0.72	3537	0.58	0.17
	0.43	3538	0.76	0.22
	0.121	3538	1.51	0.44
	0.072	3538	3.45	1.00
	0.043	3538	2.98	0.86
n-Hexane	1.21	this concentration in *n*-hexane not available		
	0.72	3547	0.35	0.19
	0.43	3547	0.46	0.26
	0.121	3548	0.93	0.52
	0.072	3548	1.45	0.81
	0.043	3549	1.66	0.92

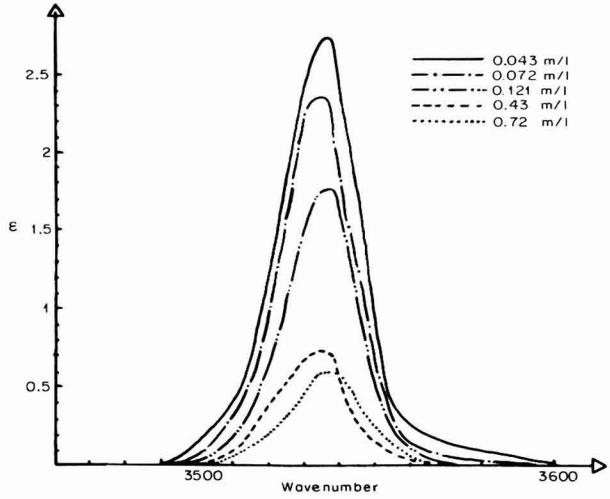

FIG. 1. The molar extinction coefficient values of the monomer O–H bands versus wavenumber values for a series of concentrations of *cis*-9-octadecenoic acid solutions in carbon tetrachloride.

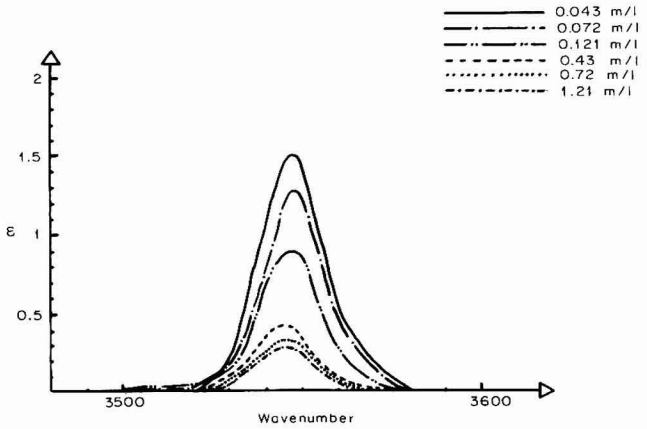

FIG. 2. The molar extinction coefficient values of the monomer O–H bands versus wavenumber values for a series of concentrations of *cis*-9-octadecenoic acid solutions in *n*-hexane.

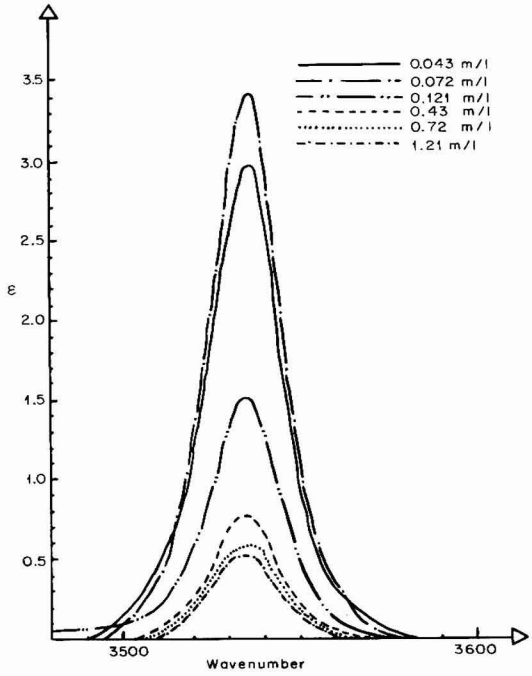

FIG. 3. The molar extinction coefficient values of the monomer O–H bands versus wavenumber values for a series of concentrations of *trans*-9-octadecenoic acid solutions in carbon tetrachloride.

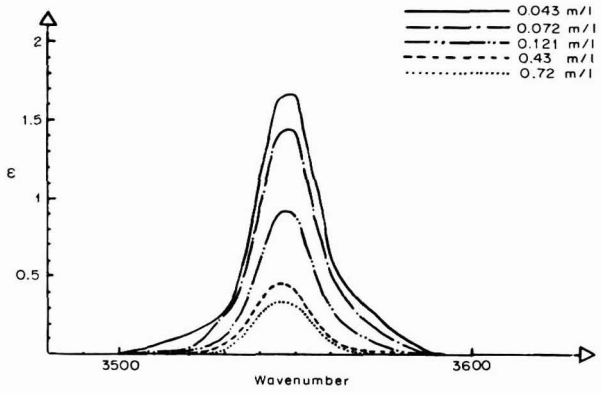

FIG. 4. The molar extinction coefficient values of the monomer O–H bands versus wavenumber values for a series of concentrations of *trans*-9-octadecenoic acid solutions in *n*-hexane.

DISCUSSION

As it proceeds from data placed in the Tables 1 and 2, and from the Figs. 1–4, *cis*-9-octadecenoic acid exhibits somewhat smaller degree of dissociation in the inactive solvents than *trans*-9-octadecenoic acid. In the case of the most diluted samples, i.e., with the 0.043 *M* samples in carbon tetrachloride, the number value of dissociation degree of *cis*-9-octadecenoic acid equals 0.79, and the analogical value of *trans*-9-octadecenoic acid equals 0.86. Similarly one may

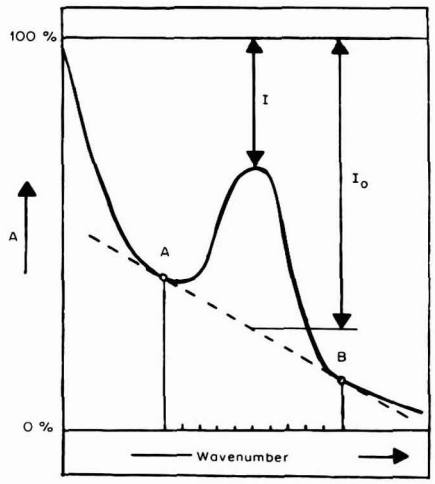

FIG. 5. The graphical isolation of monomer band; the part of curve confined between points A and B was recalculated according to the Lambert–Beer law to get the extinction coefficient values instead of absorption.

compare the discussed values for the *n*-hexane solutions: for the most diluted sample of *cis*-isomer the dissociation degree equals 0.84, while for the analogical sample of *trans*-isomer 0.92. The solutions of *trans*-9-octadecenoic acid in carbon tetrachloride have an additional, characteristic feature: already at the concentration of 0.072 *M* one observes almost complete dissociation for this series (Table 2). In all the other cases the greatest approach to the complete dissociation was obtainable only with the smallest concentration of the examined solution.

The influence of carbon tetrachloride and *n*-hexane on the maximum molar extinction coefficient values of examined substances agrees with the observations of another authors (*1*), dealing with other substances. Namely carbon tetrachloride solutions show the higher values of this coefficient, than *n*-hexane solutions. It is certainly connected with the polarity of these solvents and with the geometry of their structures.

SUMMARY

The comparison was made of the dissociation degree values for the hydrogen-bond multimers of two geometrical isomers: *cis*- and *trans*-9-octadecenoic acids. The measurements were performed for the series of solutions of the discussed substances in the inactive solvents: carbon tetrachloride and *n*-hexane. The applied technique was ir absorption spectroscopy. The general statement is that *trans*-9-octadecenoic acid in both carbon tetrachloride and *n*-hexane shows greater tendency towards dissociation of multimers than *cis*-9-octadeconoic acid.

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MICROCHEMICAL JOURNAL 18, 196–202 (1973)

Book Reviews

Analytical Chemistry: Key to Progress on National Problems. Edited by W. WAYNE MEINKE and JOHN K. TAYLOR. National Bureau of Standards Special Publication 351, National Bureau of Standards, United States Department of Commerce, Washington, D. C., 1972. × + 470 pp. $3.50.

The book is the proceedings of the 24th Annual Symposium on Analytical Chemistry sponsored by Analytical Chemistry, the American Chemical Society Division of Analytical Chemistry, and the National Bureau of Standards Analytical Chemistry Division held at Gaithersburg, Md., June 16–18, 1971.

The book is composed of seven chapters, the first being a reproduction of the paper published by Dr. G. E. F. Lundell in 1933 on the subject of chemical analysis. The six following chapters are composed of the invited papers and the panel discussions which followed each paper. The titles of these six papers are "Opportunities for Analytical Chemistry in Solid State Research and Electronics," "Analytical Problems in Biomedical Research and Clinical Chemistry," "Analytical Problems in Agricultural Science," "Analytical Problems in Air Pollution Control," "Analytical Problems in Water Pollution Control," and "Problems in Applications of Analytical Chemistry to Oceanography."

Each chapter is of the review type, being rich in references, and each chapter ends with detailed discussions on the subject matter by six leading analytical chemists, which includes the speaker and the Chairman of the session. The book is very informative and there are numerous amusing cartoons to illustrate points under discussion. It will make a valuable addition to the collection of any analytical chemist as well as to all libraries.

AL STEYERMARK, *Department of Chemistry, Newark College of Arts and Sciences, Rutgers University, Newark, New Jersey 07102*

Absorption Spectra in the Ultraviolet and Visible Region. Vol. 16. Edited by L. LÁNG; collaborators: A. BARTECKI, G. HORVÁTH, J. SZÖKE, AND G. VARSANYI. Academic Press, New York, 1972. 423 pp. $29.50.

This is a loose-leaf collection of absorption spectra and data in the ultraviolet and visible regions, mostly in the 200–400 nm range, but many extending farther, even up to 1000 nm. Two hundred organic compounds are included; some are organometallic and some are silicon containing. A separate sheet is used for each compound. The front of each sheet shows the name of the compound, its structure, and the spectrum (log ϵ vs nm). The back of each sheet gives the empirical formula, molecular weight, boiling or melting point, solvent or solvents used, concentration, cell length, instrument employed, and a table giving log I_0/I for each wavelength at which readings were made.

A very convenient table of contents, in booklet form, is included. It is divided into six sections: "Figures (Diagrams)," "Subject Index," "Formula Index," "Author Index," "Figure (Diagram) Index" (different from the first named inasmuch as the

authors are listed), and "Literature." The headings in the "Contents" are in German, English, and Russian.

This book will make a valuable addition to the collection of anyone in the fields of analytical and/or organic chemistry and to libraries.

AL STEYERMARK, *Department of Chemistry, Newark College of Arts and Sciences, Rutgers University, Newark, New Jersey 07102*

Quality Control in the Pharmaceutical Industry. Vol. 1. Edited by MURRAY S. COOPER. Academic Press, New York, 1972. xi + 245 pp. $17.00.

Volume 1 of this multivolume treatise on quality control in the pharmaceutical industry deals with subjects which include sterility testing, quality control of antibiotics, design of facilities, pharmaceutical preservatives, quality control in hospital pharmacy, safety testing of pharmaceuticals, quality control of biological and pharmaceutical products. This volume discusses subjects in sufficient detail that the scientist can broaden his knowledge of technical areas outside his own speciality. The book is written in a manner which will enable both the scientist and administrator to benefit from this practical and comprehensive guide to various aspects of the quality control of pharmaceutical products.

The book does lack continuity and it is unfortunate that the editor did not present an introductory chapter that would group the chapters according to associated subject matter. The fact that the series is geared to the needs of both the scientist and administrator presents some difficulty; i.e., this volume was not very chemically oriented. If volume 1 is an indication of the future volumes the series will have little appeal to the analytical research chemist. I did, however, find the chapters on preservatives in pharmaceutical products and safety testing of pharmaceuticals particularly interesting. The book has value in allowing the specialist to familiarize himself with the broad function of quality control in the pharmaceutical industry and for this reason the book would be an excellent addition to the libraries of technical and administrative personnel in the pharmaceutical industry.

DAVID F. TOMKINS, *Hoffmann-La Roche Inc., Nutley, New Jersey 07110*

Ultrapurity: Methods and Techniques. Edited by M. ZIEF and R. SPEIGHTS. Dekker, New York, 1972. xv + 699 pp. $37.50.[1]

This is an outstanding book presenting the successful work for the first time to bring together the four essential techniques and interrelated parameters of ultrapurity: preparation, handling, containment, and analysis. It is intended not only to provide the preparation methods and analytical techniques for ultrapure materials required for electronics industry but also to reveal a new challenge in making ultrapure chemicals available for a broad spectrum of scientific disciplines such as clinical chemistry, geochemistry, ceramics, environmental analysis and oceanography. The book thus reflects the continuing advancements in the preparation of ultrapure chemicals, the explosive growth in developments pertaining to the handling and containment of these materials, as well as the necessity for complete analysis.

[1] Available from Research Materials Information Center, Solid-State Division, Oak Ridge National Laboratory, P.O. Box *X*, Oak Ridge, Tenn. 37830.

In format, the book consists of four main parts: preparation, handling, containment, analysis, and one appendix part which provides the information sources for ultrapurification and characterization of ultrapure materials. For convenience, the book is further subdivided into 22 chapters. At the end of each chapter, a list of references pertinent to that particular topic is given. A total of 973 references which cover the literature up to the year of 1971 has been cited. Both author and subject indexes are available for convenient and effective use of the text.

Part I describes in detail the preparation methods for ultrapure materials such as alkali halides, organic solvents, pure metallic sodium and potassium, phosphorus pentoxide, proteins, *p*-xylene, isopropylbenzene, ultrapure chemicals, ultrapure water, and cholesterol. Almost all modern important preparation and analytical techniques are effectively utilized and critically discussed. Primarily, these include liquid–liquid extraction, ion-exchange, precipitation, synthesis, high temperature technique, progressive freezing, zone-melting, fractional crystallization and fractional distillation, sublimation, membrane ultrafiltration technique, partial freezing, spectroscopic methods, differential scanning calorimetry, polarography, photometric and complexiметric titrations, and various chromatographic methods (such as GLC, preparative-GLC, TLC, frontal-analysis and dry-column chromatographies).

Specifically, it is of great worth to look at a few more things in the first part. First, a particular emphasis has been placed on the purification of alkali halides as the chapter occupied about one-third of the total volume of the part (Part I consists of 11 chapters, 199 pp., 579 references, 28 tables, and 45 figures). This is useful and logical since alkali halides have found technologically important applications in optical components, scintillation counters, and as solvents for electromechanical process. Second, the chapters on the purification of organic solvents by adsorptive filtration for uv spectroscopy, preparation of ultrapure chemicals by fractional distillation, preparation of ultrapure water by a train of purification equipment and methods although are very common, much modification and improvement on the methods and techniques have been successfully done. Furthermore, the chapter on the membrane ultrafiltration technique for purification of proteins is also found very attractive and useful for biological materials, polymer solutions or latexes, colloids, and pigment dispersion. Finally, the development of dry-column chromatography as a purification technique for dye mixture, lipid mixture, azobenzenes, alkaloids, and heterocyclic substances is very challenging and possesses potential applications in the future.

Part II deals with handling (2 chapters, 82 pp., 80 ref., 20 tables, and 13 figs.). In this part, one chapter considers the contamination problems in trace-element analysis and ultrapurification, another chapter disscusses the airborne contamination. These provide an evaluation of potential sources of contamination encountered in container materials, laboratory apparatus, chemical reagents and solvents, those introduced by physical and chemical manipulation of samples, and environmental effects (such as temperature and humidity) during manufacturing and packaging, etc. Whenever possible, suggestions for eliminating or minimizing specific errors in these areas were also offered. Thus, it is expected that, once contamination hazards have been recognized and evaluated, successful trace analysis and purification work can be more meaningfully accomplished.

Part III is concerned with containment (3 chapters, 116 pp., 149 references, 22 tables, and 37 figures). In this part, a particular attention was paid to the general understanding of the nature of the glass surface and the reactions between the surface of glass container and various chemicals so that a prediction of the effect of the glass on the ultrapure solutions can be made. A general discussion on the physical, chemical (resistance to chemicals), and transport (of gases and ions) properties that are significant in the use of vitreous silica as a container material and that can be important parameters

regarding the purity of the container itself and ultrapurity of the chemicals, etc., is provided. Besides, some criteria such as compatibility, corrosion rate, degree of contamination, specific requirements or conditions of use, passive resistance and active usefulness, etc., for evaluation of a ceramic as a container are also presented.

Part IV considers analytical techniques (6 chapters, 182 pp., 408 references, 15 tables, 20 figures). Both classical "wet" methods and modern instrumental techniques are recommended. A complete evaluation of a high-purity chemical involves the determination of the overall purity and the minor trace constituents, the performance of general tests (such as residue on ignition), and the measurement of physical properties (for examples, conductivity and melting point). Several chapters describe separately the selective and effective use of the instrumental methods of analysis, for examples, emission spectroscopy, flame spectroscophotometric trace analysis, neturon activation analysis, visible spectrophotometry and coulometric titrations. However, the discussion on the use of mass spectrometry was neglected. This is very regretful. In the reviewer's opinion, mass spectrometry should be included as an integral part in the text, since it is also one of the most powerful tool in modern trace analysis.

Part V, Appendix (55 pp.), provides the best information sources[1] of ultrapurification and characterization for ultrapure materials. The general information sources include national and international information services, specialized information centers, and new journals. The information sources for ultrapurification cover the literature from 1969 to 1965 and some pre-1965. The information sources for characterization have been extensively reviewed and subdivided into several categories. These are miscellaneous, activation analysis, neutron diffraction, mass spectrometry, optical methods, electron diffraction and microscopy, X-ray diffraction and spectroscopy, electron-microprobe analysis, measurement of electrical properties, thermal methods, resonance methods, Mössbauer effect, field emission and field ion microscopy, etc.

In summary, this book is one of the best books ever found in a decade or longer. It is eventually a representative of the splendid cooperation of the leading authorities who have devoted considerable time and effort to the preparation of their specific fields of chapters. Throughout the book, the treatment of subject matter is comprehensive and logical. Much information and materials were presented in easily comparable and readable tabular forms, and the preparation and analytical procedures in quickly followed and understandable schematic diagrams and figures. There is no significant typographical error. Both editors and authors are to be congratulated on having done such an excellent job. The book is therefore highly recommended to those material and synthetic scientists, analytical chemists, and research workers as an invaluable reference book or encyclopedia for ultrapure materials. Because of high cost of the book, libraries should keep one copy on the shelves. However, the individuals may retain it as a high priority for purchasing when finances permit.

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MATERIALS Science Toxicology Laboratories,
The University of Tennessee, Medical Units, Memphis, Tennessee 38103

Organic Electronic Spectral Data, Vol. VIII. Academic Press, New York, 1972. XIV + 695 pp. $37.50.

Volume VII of this established series was reviewed in *Microchemical Journal* **17,** 370 (1972), and very little additional comment is needed.

Volume VIII covers only the year 1966 and is consequently less bulky than the last

few volumes that covered two years each. The total number of journals abstracted is 102 (compared with 101 for Vol. VII), but the selection has been improved by dropping several and adding others.

ROBERT M. SILVERSTEIN, *State University of New York, College of Environmental Science and Forestry, Syracuse, New York 13210*

Handbook of Commercial Scientific Instruments, Vol. 1. Edited by CLAUDE VEILLON. Dekker, New York, 1972. xvi + 174 pp. $11.75.

This series is intended to acquaint scientists with available scientific instrumentation. The volumes will give an objective evaluation of commercial scientific instrumentation to assist the scientist in selecting an instrument that will fit his needs. Specifications, descriptions, schematic drawings, photographs, approximate prices, and accessories of several types of instruments will be included in the handbook volumes.

Volume 1 of this series presents information on atomic absorption instruments. This volume includes all the popular instruments sold in the U.S. plus several foreign-made instruments. After a brief introduction in which theoretical and instrumental aspects are discussed, the book describes each manufacturers products individually. The book makes an attempt to evaluate the instrument objectively and includes some comments about the applicability of each instrument to various situations. In a summary chapter the author compares models of similar prices and makes general recommendations.

Although objectivity was intended, the book is based on material obtained from manufacturers or their representatives. One can not help being somewhat skeptical about such information especially when the author has not personally used many of these instruments. The author did point out the importance of many of the capabilities of each instrument and presents the information in a form that is easily understood. The most obvious shortcoming of any book of this type is that it is out of date shortly after publication. Fortunately, manufacturers usually update existing instrumentation and frequently use the same basic designs; therefore, the book can be of general value for several years. The book will assist a reader contemplating the purchase of an atomic absorption instrument to make a selection suited to his purposes and budget and for this reason it will be a fine addition to scientific libraries.

DAVID F. TOMKINS, *Hoffmann-LaRoche Inc., Nutley, New Jersey 07110*

Interpretation of Mass Spectra of Organic Compounds. By MAYNARD C. HAMMING AND NORMAN G. FOSTER. Academic Press, New York, 1972. xiv + 695 pp. $37.50.

Two professional mass spectrometrists have given us the benefits of their extensive experience and expertise. Again we are forcibly reminded of the debt the modern organic chemist owes to the sophisticated practitioners of mass spectrometry in the petroleum industry—both authors having this background.

Interpretation of mass spectra is presented as a "numbers game"; the presentation of data is tabular rather than graphical. Use of the "rectangular array" in conjunction with "interpretation maps" is developed in detail.

The discussion of fragmentation reactions follows Biemann's scheme, which is based

on generalized types of cleavages and rearrangements. However, the very extensive literature references to fragmentations in the Appendix are organized by chemical classes. Many of the examples are derived from the authors' familiarity with petroleum-based compounds.

Very useful material is presented for the practicing spectrometrist concerned with details of instrumentation and sample handling. There is a good discussion of separation techniques and ancillary techniques for identification, and a brief treatment of computerizing mass spectral data.

Throughout the numerous discussions of correlation between spectra and structure, the authors are careful to point out the pitfalls involved and to warn against being entrapped by facile rationalizations.

Although the authors acknowledge the utility of the concept of charge localization at functional groups, they make little use of it in their diagrams of fragmentations. This failure, combined with extremely casual use of curved arrows and fishhooks, makes it quite difficult to follow many of the fragmentation patterns. One serious error was noted on p. 456 where the discussion is based on an incorrect structure. These deficiencies are noted in the expectation of a new edition of this major contribution to the literature of mass spectrometry.

R. M. SILVERSTEIN, *College of Environmental Science and Forestry, Syracuse, New York 13210*

Encyclopedia of Industrial Chemical Analysis. Volume 16. Edited by F. D. SNELL AND L. S. ETTRE. Wiley (Interscience), New York, 1972. XIV + 583 pp. Each volume $45.00 ($35.00 by subscription).

With Volume 16, this compendia extends the alphabetically arranged treatment of chemicals and groups of chemical products from mercury (and its compounds) through penicillin and related compounds. Intermediate subjects include methacrylate polymers; methanol; milk and milk products; molybdenum (and its alloys and compounds); morpholine; naphthalene and naphthalene derivatives; narcotics; nickel (and its compounds); niobium (and its alloys and compounds); nitriles; nitro compounds, organic; nitrogen and inorganic nitrogen compounds; oxygen; and ozone.

Contributors, for the most part, to this encyclopedia have been recruited widely from the technical staff of U. S. companies. With Volume 16, five U. S. companies, one U. S. university, one U. S. state agricultural station, and one West Germany firm are represented. Surprisingly, a single individual, F. A. Lowenheim, is credited as the author of five articles and the coauthor of one—together constituting over 46% of the text of the volume!

The 64-page article on nitrogen presents a 14-page treatment of the determination of "total nitrogen" in both compounds and metallurgical samples that is largely directed to procedures based on Kjeldahl digestion. The gasometric Dumas method is given only brief consideration as it is considered in other volumes of the work.

The 24-page article on oxygen devotes only one page to the direct determination of oxygen in organic compounds; the principles are delineated and key literature is cited.

The 68-page article on mercury and its compounds is timely now that the detection and determination of this element is established as a continuing challenge for the environmental analyst. About one-third of the article is directed to the determination of minor and trace amounts of mercury and includes an 8-page well-balanced practical treatment of the cold-vapor atomic absorption technique.

The 14-page article on ozone is welcome. Some 8 pages are devoted to the use, prop-

erties, and generation of this allotrope of oxygen. The remainder of the article considers the determination of ozone in the atmosphere and waters. The ozone treatment of water, sewerage, and effluents to reduce bacterial count or biological oxygen demand is receiving increasing attention worldwide. More analysts will, consequently, be called upon to determine residual trace ozone and to assess the efficacy of ozone treatment.

This encyclopedia continues to be an important contribution to the literature of practical chemical analysis. Analytical chemists should assure that their corporate or institutional library is indeed subscribing to the work. Four or more volumes and a general index must appear before the work is complete. Yet it is not too early to express the hope that the editorial staff not be disbanded, but rather work be initiated toward a second edition. There may be merit for the Editors to send a questionnaire to current subscribers inviting both criticism of the first edition and recommendations for supplementary volumes and a second edition.

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Molecular Evolution and the Origin of Life. BY SIDNEY W. FOX AND KLAUS DOSE, W. H. Freeman and Company, San Francisco, 1972. xi + 359 pp. $16.00.

In a series of carefully documented chapters the history of changing concepts of molecular evolution and the origin of life, the synthesis of micro- and macromolecules and their self-assembly into microsystems and a variety of interpretations of these phenomena in relation to observations on primitive life forms establish bases for a constructionistic approach rather than a reductionistic analysis of the spontaneous generation of pre-Darwinian life. These sections are followed by a discussion of the evolution (in Darwinian terms) of primitive living systems and a critique of some of the initial problems of extraterrestrial observations. An abundance of citations to the basic literature facilitates further study by the serious student.

The credentials of the authors as experts in this challenging area of biological science are impeccable and their approach in this volume is scholarly and objective. This reviewer shares Oparin's conviction, expressed in the foreword that this book "will be welcomed with great satisfaction by all readers interested in the far-reaching concepts of our world and its origins."

ROBERT A. HARTE, *American Society of Biological Chemists, 9650 Rockville Pike, Bethesda, Maryland 20014*

MICROCHEMICAL JOURNAL 18, 203–206 (1973)

Announcement

International Symposium on Microchemical Techniques—1973

The International Symposium on Microchemical Techniques—1973 will be held at University Park, Pennsylvania, U. S. A., on 19 August–24 August, 1973. This symposium, similar in tone to previous ones in 1961 and 1965, is again being conducted by The Pennsylvania State University through its College of Science. It is also being organized by the American Microchemical Society with the sponsorship of the International Union of Pure and Applied Chemistry, Commission on Microchemical Techniques, Division of Analytical Chemistry.

The Symposium will provide for an interchange of information and ideas among technologists from all parts of the world concerning new methods and techniques or unique applications in the field of microchemistry. The Symposium will take place in the informal atmosphere of fellowship and individual contact made possible by the excellent on-campus accommodations and facilities of The Pennsylvania State University.

Technical sessions dedicated to topics of timely interest, original papers, an exhibition of commercial equipment, buffet dinner, picnic supper, banquet and ladies' program will be included in the schedule of activities.

A commercial exhibition, open from Monday evening, August 20, through Thursday, August 23, in the East Halls Housing Complex, will feature equipment, apparatus specialties and chemicals of microchemical and microanalytical interest.

Inquiries about the technical program should be directed to:

Howard J. Francis, Jr.
The Pennwalt Corporation
900 First Avenue
King of Prussia, Pennsylvania 19406

Inquiries about the commercial exhibition should be directed to:

Ralph G. Lade
Rohm & Haas Company
P. O. Box 219
Bristol, Pennsylvania 19007

Inquiries concerning local arrangements, registration and housing should be directed to:

Kent R. Addis
J. Orvis Keller Building
The Pennsylvania State University
University Park, Pennsylvania 16802

The following is a list of the papers which will be presented in the technical program:

D. M. W. ANDERSON (Edinburgh): Organic Functional Group Analysis: New Directions.

P. HEIZMANN, K. KÖNIG, U. NIEDERSCHULTE, AND K. BALLSCHMITER* (Mainz): High Pressure Liquid Chromatography of Metal Chelates in Micro-trace Analysis.

F. BERMEJO-MARTINEZ* AND M. MOLINA-POCH (Santiago de Compostela): Photometric Determination of Copper with Uramyldiacetic Acid.

F. BERMEJO-MARTINEZ* AND M. MOLINA-POCH, (Santiago de Compostela): Uramyldiacetic Acid as a Chromogenic Agent in Absorptiometric Analysis.

T. A. BLUEMENFELD (New York): Specimen Collection, Interpretation of Data, and Future Applications in Clinical Microchemistry.

G. DEN BOEF*, U. HANNEMA, AND TH. J. M. POUW (Amsterdam): Coulometric Titration of Traces of Metal Ions.

A. BURGER* AND M. KUHNERT-BRANDSTÄTTER (Innsbruck): Investigations on Dissolution Involving Simultaneous Phase Changes.

D. BUTTERWORTH (Teddington): Elemental Microanalysis of the more "Difficult" Type of Organic and Organo-metallic Materials.

R. A. CHALMERS (Aberdeen): Writing the Technical Paper.

R. F. CULMO (Norwalk): Introduction to the Computerization of the Perkin-Elmer Model 240 Carbon, Hydrogen, and Nitrogen Analyzer.

R. E. DESSY (Blacksburg): Minicomputer Interfacing.

W. W. DREESZEN (Ames): Techniques of Mechanically Reproducing a Technical Paper.

P. P. WHEELER AND M. I. FAUTH* (Indian Head): Comparison of Analytical Results of the Coleman Nitrogen Analyzer vs the Perkin-Elmer 240 Automatic Elemental Analyzer for High Nitrogen Compounds.

H. A. FLASCHKA (Atlanta): Solid and Homogenous Extraction.

H. A. FLASCHKA (Atlanta): Some Investigations in Microspectrophotometry.

J. K. FOREMAN (London): Problems in the Separation, Reaction, and Determination of *N*-Nitrosamines at the Microgram per Kilogram Level in Environmental Samples.

W. FRENCH (Rochester): Legal Responsibilities When Writing a Technical Paper.

J. S. FRITZ (Ames): Use of Macro-reticular Resins in the Determination of Organic Pollutants in Potable Water.

M. GRASSERBAUER (Wien): Electron Macroprobe, An Advance in Microanalysis.

A. P. GRAY (Norwalk): Progress and Possibilities in Scanning Calorimetry.

B. GRIEPINK*, F. G. RÖMER, AND W. J. VAN OORT (Utrecht): Continuous Titrations for Ultramicrodeterminations.

M. L. DECKER, J. F. GENTILE, D. E. HARRINGTON*, AND J. B. WORTHINGTON (Painesville): Carbon, Hydrogen, and Nitrogen Analyzer On-Line Data Acquisition System.

R. C. HICKS (Rochester): Visualizing a Technical Paper.

K. HOZUMI (Kyoto): Low-Temperature Plasma Ashing as a Microchemical Technique for Studying Crystal Bodies in Plant Tissues.

G. INGRAM (Portsmouth): The Pyrolytic Identification of Organic Molecules. II. Pyrolysis of Some Acids, Alcohols, and Esters.

D. JAGNAR (Göteborg): Computers in Titrimetry.

J. L. JOHNSON (Warren): Analysis of Airborne Particulates Using the Scanning Electron Microscope, Electron Probe Microanalyzer, X-Ray Diffraction, and Chemical Methods.

M. T. KELLEY*, R. W. STELZNER, AND M. N. FERGUSON (Oak Ridge): Application of a Small Computer in Atomic Absorption Analyses of Environmental Samples.

R. KELLNER (Wien): Contribution to the Far-Infrared Spectroscopy of Microamounts of Metal Chelates.

M. KESSLER (Dortmund): Methodology and Application of pH Sensitive Microelectrodes for Intracellular Measurements.

D. F. KETCHUM (Rochester): The History of Automation in Organic Elemental Analysis.

J. KIRSCHBAUM (New Brunswick): Density Gradient Separations.

D. KLOCKOW*, H. DENZINGER, AND G. RÖNICKE (Freiburg and Schallstadt): Application of Micro-radiochemical Techniques to the Analysis of Some Air Pollutants.

R. KÜBLER (Basel): Automation, Computerization, and Organization of a Larger Elemental Analysis Laboratory.

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Calcium and the Heart

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The contractile and excitable properties of the heart muscle cell are of fundamental interest to the modern cardiologist. With the advance of the biochemical investigation of muscle, the heart can be studied not simply in terms of ultra-structure or biomechanical conjecture, but in terms of the kinetics of activity of enzyme systems disposed spatially in a particular way within the cell. In these processes a central role of calcium ions has emerged, based on their rhythmic sequestration by the sarcoplasmic reticulum and their specific effects on the contractile proteins.
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