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SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 32, No. 5, May 1965

AN APPARATUS FOR SIMULTANEOUS GAS EVOLUTION ANALYSIS AND MASS SPECTROMETRIC ANALYSIS

An apparatus is described which enables the gas evolution analysis (GEA) and mass spectrometric analysis (MSA) curves of a sample to be recorded simultaneously. The sample is pyrolyzed in a chamber in a dynamic helium atmosphere. The evolved products are detected in the helium gas by a thermal conductivity cell which results in the GEA curve. An inexpensive mass spectrometer is used to monitor the helium gas stream which gives the MSA curve. The advantages of the apparatus over other recent techniques are given.

W. W. WENDLANDT AND TH. M. SOUTHERN,
Anal. Chim. Acta, 32 (1965) 405–410

EXTRACTION AND FLAME SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM AND RHODIUM

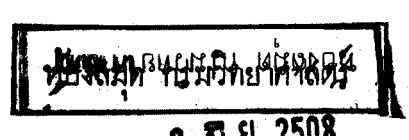
Solvent extraction methods involving toluene, chloroform, 4-methyl-2-pentanone and pentyl acetate were studied for palladium and rhodium chelates. The palladium–salicylaldoxime chelate was extracted quantitatively into 4-methyl-2-pentanone at pH 3. The rhodium–diethyldithiocarbamate chelate was completely extracted into 4-methyl-2-pentanone at pH 8. The optimum combustion conditions for each of the organic extracts were then studied. The position of maximum emission intensity in the flame mantle was determined for each chelate and solvent system; readings were taken at 363.5 mμ for palladium, and 369.2 mμ for rhodium. For palladium, when 4-methyl-2-pentanone was used instead of water as solvent, the emission intensity increased 21-fold. For rhodium, this ketone increased the sensitivity 27 times compared with water. A method is suggested for the separation and determination of palladium and rhodium in the same sample.

H. C. ESHELMAN, J. DYER AND J. ARMENTOR,
Anal. Chim. Acta, 32 (1965) 411–417

THE SPECTROPHOTOMETRIC DETERMINATION OF RHENIUM

Some 50 analytical reagents were briefly checked for use in the spectrophotometric determination of rhenium. Seven reagents were checked in detail and interferences were examined. The most promising reagents appeared to be 1-phenyl-2-thiourea and 1,5-diphenylcarbohydrazide.

E. N. POLLOCK AND L. P. ZOPATTI,
Anal. Chim. Acta, 32 (1965) 418–427



THE USE OF ANION-EXCHANGE RESIN FOR THE DETERMINATION OF TRACES OF GOLD IN COPPER

The anion exchange of gold from chloride–nitrate medium is described and applied to the determination of 0.01 to 100 p.p.m. of gold in copper metal as a separation step before photometry or activation analysis.

A. MIZUIKE, Y. IIDA, K. YAMADA AND S. HIRANO,
Anal. Chim. Acta, 32 (1965) 428–434

THE SPECIFICITY OF THE COLOUR REACTION BETWEEN AMMONIA AND 3-PHENYL-2-THIOHYDANTOIN

The colour reaction between 3-phenyl-2-thiohydantoin and ammonia is studied quantitatively. Determinations of 0.1–0.6 μmoles of 3-phenyl-2-thiohydantoin are possible with a precision close to 2%. In analyses of amino acid mixtures for glycine after conversion to 3-phenyl-2-thiohydantoin, only derivatives of serine and threonine interfere to a slight extent. The specificity of the primary colour reaction with ammonia and the structural requirements for it are discussed; a structure for the pigment species is proposed.

K. NAGENDRA NATH REDDY AND L. K. RAMACHANDRAN,
Anal. Chim. Acta, 32 (1965) 435–447

SUBSTITUTED HYDRAZONES AS TRIDENTATE CHELATING AGENTS

The compositions and stabilities of the metal chelates of two new tridentate ligands, pyridine-2-aldehyde-2-quinolylhydrazone and pyridine-2-aldehyde-2-thiazolylhydrazone are reported. Cationic bis-chelates, which have been reported with similar ligands, are not formed during chelation with these ligands. The copper reaction, in which both mono- and bis-chelates are formed, is discussed in detail.

M. L. HEIT AND D. E. RYAN,
Anal. Chim. Acta, 32 (1965) 448–455

A NEW METHOD OF RADIOACTIVATION ANALYSIS BASED ON THE QUANTITATIVE ISOTOPE DILUTION PRINCIPLE

The simple quantitative isotope dilution method proposed, allows the errors due to flux fluctuation and self-shielding, normal to ordinary radioactivation analysis, to be avoided. Three different methods are discussed. The most satisfactory method from a theoretical viewpoint involves division of the irradiated sample into two parts; in one part, an amount m is extracted and the radioactivity a is measured. An amount of carrier M is added to the other part, an amount m is again extracted and its radioactivity a' is measured. The unknown amount M_x is then calculated from $(a/a') - 1 = M/M_x$. A method for the determination of traces of copper and silver in metallic tin and zinc based on this principle is described.

N. SUZUKI AND K. KUDO,
Anal. Chim. Acta, 32 (1965) 456–464

DETERMINATION OF THE SULFHYDRYL AND DISULFIDE GROUPS IN PROTEINS BY METHYLATION, TREATMENT WITH RANEY NICKEL AND INFRARED SPECTROMETRY

A method is described for the determination of the total and accessible free SH-groups in proteins, based on the methylation of the SH-groups in non-hydrolysed proteins with dimethyl sulfate, followed by hydrolysis, additional methylation (for the total free SH-groups) and treatment with Raney nickel. The methane released is measured by infrared spectroscopy. Methionine must be determined separately and a correction made. The relative error is 3%, and the time necessary for one determination is about 2 h (excluding the time for hydrolysis). The method may also be applied to the determination of accessible SH-groups of homogenates of internal organs. If the protein hydrolysate and the non hydrolysed protein are reduced before methylation, the total and accessible disulfide groups can also be determined.

M. KRSTEVA, B. V. ALEXIEV, C. P. IVANOV AND B. YORDANOV,
Anal. Chim. Acta, 32 (1965) 465–471

AN AUTOMATIC TITRATION CONTROL UNIT

A unit is described for the automatic termination of coulometric or volumetric titrations in conjunction with a suitable valve millivoltmeter and motorized buret or constant current generator. Two values of potential can be preset on the instrument, in either rising or falling sequence. On reaching the first potential, the endpoint is approached by pulsed addition of the titrant; the titration is terminated at the second potential. The *on* and *off* time cycles for the pulsing circuit are independently variable from 0.1 to 1.0 sec *on* and 3 to 15 sec *off*.

K. C. STEED AND F. FRANSMAN,
Anal. Chim. Acta, 32 (1965) 472–476

THE NATURE OF LIGHT

PART II. THE RELATIONSHIP BETWEEN PHOTONS, ELECTRONS AND POSITRONS

It is proposed that a photon is composed of a positive and a negative charge. The relationship between photons, electrons and positrons is discussed. A mechanism of the production of coherent light by lasers is also proposed.

The nature of light is intimately concerned with the Special Case of the Theory of Relativity. However the model proposed provides no clue to explain why this theory should hold.

J. W. ROBINSON,
Anal. Chim. Acta, 32 (1965) 477–484

STEAM DISTILLATION METHODS FOR DETERMINATION OF AMMONIUM, NITRATE AND NITRITE

Steam distillation methods of determining ammonium, nitrate, and nitrite in the presence of alkali-labile organic nitrogen compounds are described. They involve the use of magnesium oxide for distillation of ammonium, ball-milled Devarda alloy for reduction of nitrate and nitrite to ammonium, and sulfamic acid for destruction of nitrite. The methods are rapid, accurate and precise, and they permit nitrogen isotope-ratio analysis of ammonium, nitrate, and nitrite in tracer studies using ^{15}N-enriched compounds. They give quantitative recovery of ammonium, nitrate and nitrite added to soil and plant extracts, and appear suitable for analysis of biological materials.

J. M. BREMNER AND D. R. KEENEY,
Anal. Chim. Acta, 32 (1965) 485–496.

o-AMINOBENZENETHIOL: AN OXIDIMETRIC INDICATOR FOR HYDROGEN ION AND REAGENT FOR METALS

(Short Communication)

H. E. AFFSPRUNG AND R. E. MITCHELL,
Anal. Chim. Acta, 32 (1965) 496–499.

AN APPARATUS FOR SIMULTANEOUS GAS EVOLUTION ANALYSIS AND MASS SPECTROMETRIC ANALYSIS

WESLEY W. WENDLANDT AND THOMAS M. SOUTHERN
Department of Chemistry, Texas Technological College, Lubbock, Texas (U.S.A.)
(Received August 3rd, 1964)

Because of the wide variation in the weight-loss curves obtained by thermogravimetry, ROGERS, YASUDA AND ZINN[1] introduced the technique of gas evolution analysis (linear pyrolysis). With this technique, a small amount of sample is pyrolyzed in a dynamic helium or air atmosphere, at a linear heating rate, and the evolved pyrolysis gases are detected, as a function of time or temperature, by a thermal conductivity detector. A similar apparatus has been described by VASSALLO[2] for studying the thermal degradation of polymeric materials.

The combination of gas evolution analysis (GEA) with other thermal techniques such as differential thermal analysis (DTA), thermogravimetric analysis (TGA) and so on, would be expected to yield especially useful results in that the advantages of each technique could be utilized. As a result of this, several DTA–GEA apparatus designs have been described[3,4], as well as the manufacture of commercial instruments. The combined techniques allow a more complete interpretation of the DTA curve, such as distinguishing between a phase transition and a decomposition reaction involving a gaseous product, and so on. An excellent example of the use of the DTA–GEA technique has been described by WENDLANDT AND STURM[5].

One important disadvantage still persists in the simultaneous DTA–GEA technique, however. The GEA curve indicates that a gaseous product is evolved but cannot determine its composition. A solution to this problem has been to pass the evolved gases into a gas chromatograph equipped with a suitable separation column. Such a method is cumbersome in that several different columns are sometimes required for one analysis and a complete determination cannot be made on one pyrolysis experiment. A more reasonable approach to the analysis of the GEA products is to lead the evolved product gases directly into a mass spectrometer. Since most of the evolved gases are molecules having a molecular weight under 100, a rather simple and inexpensive mass spectrometer may be employed. Such an apparatus is described here and will be designated as a gas evolution analysis (GEA)–mass spectrometric analysis (MSA) apparatus. Not only does the simultaneous GEA–MSA technique have definite advantages over analysis by gas chromatographic methods, but it enables the sample to be pyrolyzed at atmospheric pressure in an inert or other atmosphere instead of the *in vacuo* conditions of the usual mass spectrometric techniques[6]. The *in vacuo* conditions of pyrolysis are difficult to correlate, at times, with thermal properties obtained by other methods.

EXPERIMENTAL

GEA–MSA apparatus

A schematic diagram of the apparatus is given in Fig. 1, while the pyrolysis chamber is illustrated in Fig. 2.

The GEA part of the apparatus consisted of a pyrolysis chamber, S, furnace, FU, furnace temperature controller, C1, sample temperature recorder, R2, thermistor

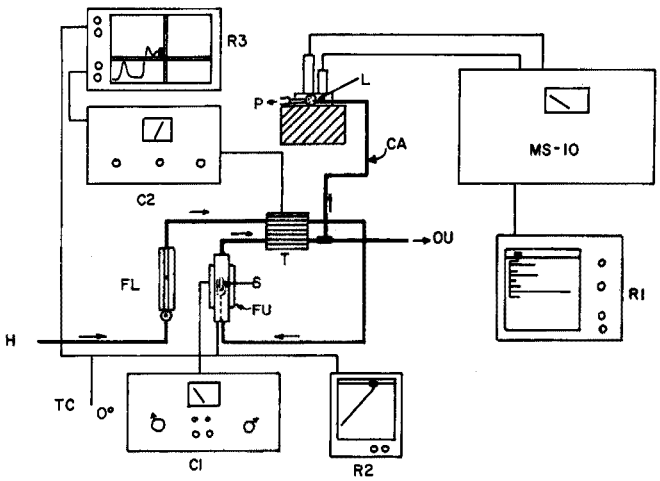


Fig. 1. Schematic diagram of GEA–MSA apparatus.

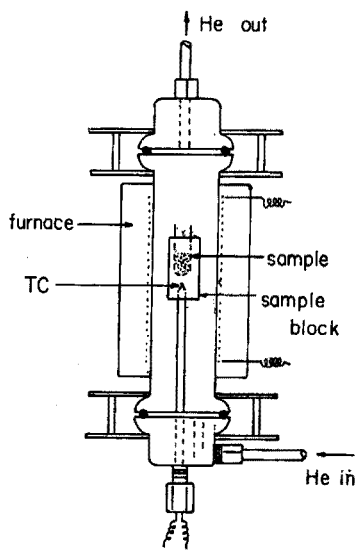


Fig. 2. Pyrolysis chamber for GEA–MSA apparatus.

thermal conductivity cell, T (Gow-Mac Instrument Company, Madison, N.J., Model 9680), bridge circuit, C2 (Gow-Mac design), and a X-Y recorder, R3. Helium, from a cylinder and pressure regulator, is passed through the reference side of the thermal conductivity cell at a flow-rate controlled and measured by a flowmeter, FL, into the pyrolysis chamber, and then into the sample side of the cell. The gas emerging from the cell passes through a "T" connection which contains the end of a 4-ft. length of 0.013-ID stainless steel capillary tubing, CA. The other end of the tubing is connected to the mass spectrometer inlet system which also contains a molecular leak, L. A portion of the gas stream is thus introduced into the spectrometer while the remainder is pumped out to the atmosphere by a mechanical pump at P. The gases are then analyzed by the Associated Electrical Industries Ltd. Model MS-10 mass spectrometer (distributed in the U.S.A. by Picker X-ray Corp., White Plains, N.Y.), the output of which is displayed on recorder R1.

The pyrolysis chamber consisted of a Pyrex glass tube, 17 cm long by 2.5 cm in diameter, which was terminated on both ends by a 2.5-cm inner diameter "O"-ring joint. Two "O"-ring joints, identical to the glass joint, were machined from aluminum and attached to the glass joint by metal clamps. All metal tubing connections and internal thermocouple connections were made by Conax connectors fastened into the aluminum joints. The glass tube was wrapped with asbestos paper, wound with enough Nichrome heater wire to give a total resistance of about 25 ohms, and then insulated with additional asbestos paper to give a layer about 0.8 cm thick. The sample block consisted of an aluminum cylinder, 1.7 cm in diameter and 2.5 cm in length, containing a glass cup sample holder, 0.8 cm in diameter and 1.5 cm in length. The entire sample holder assembly rested on a two-holed ceramic insulator tube (3 mm diameter) which held a Chromel-Alumel thermocouple. Thus, sample temperature, not furnace temperature, was measured. The pyrolysis furnace temperature rise was controlled by a motor-driven variable transformer, which has previously been described[7].

The metal tubing from the pyrolysis chamber to the thermal conductivity cell, the thermal conductivity cell, and the capillary tubing, were all maintained at about 80° by means of external heating jackets.

Procedure for pyrolysis

From 80–120 mg of sample was placed into the sample container and the pyrolysis chamber assembled and made gas-tight. Helium was flushed through the system at a flow-rate of 200 ml/min for about 5 min. During this time, the thermal conductivity cell bridge circuit was allowed to stabilize at a bridge current of 8 mA and a residual gas mass spectrum was determined over the mass range to be studied. The mass spectrometer could be scanned continuously from m/e 12 to 45 or 18 to 100 at a rate of one complete scan in 3.5 min when a 10^{10}-ohm electrometer tube and rapid scan motor were employed.

The flow-rate was then lowered to 50 ml/min and the heating cycle of the furnace begun. Usually, a furnace heating-rate of 4–6°/min was employed, but this could be varied at will. The heating-rate curve was recorded on the strip-chart recorder, R2, while the gas evolution curve was recorded on X-Y recorder R3. Several modes of operation of the output of the mass spectrometer are possible. The various m/e peaks can be recorded continuously on recorder R1 at a rate of one scan

every 3.5 min. Or, the spectrometer can be set on one *m/e* value and the output recorded as a function of time on recorder R1 or of temperature on recorder R3. Usually, the latter method was employed after an initial run had been performed to determine the composition of the evolved gases in the GEA curve peaks. If several different products were evolved during the pyrolysis, multiple runs were made each at a different *m/e* value.

RESULTS AND DISCUSSION

The utility of the apparatus is illustrated by studying the thermal dissociation of $[Cu(NH_3)_4]SO_4 \cdot H_2O$. ANOUS[8] has previously shown that *in vacuo*, $[Cu(NH_3)_4]SO_4$ loses one mole of ammonia per mole of complex beginning at 30° and a second mole at 55°. At still higher temperatures, greater than 90°, decomposition of $Cu(NH_3)_2SO_4$ occurs with the gradual formation of a dark-grey to brown product, accompanied by the sublimation of ammonium sulfate. In a more recent investigation, PFEIFER[9] postulated the following dissociation sequence:

$$[Cu(NH_3)_4]SO_4 \cdot H_2O \xrightarrow{>175^\circ} CuSO_4 \cdot 2NH_3 \cdot H_2O \xrightarrow{>210^\circ}$$
$$CuSO_4 \cdot 2NH_3 \xrightarrow{>305^\circ} CuSO_4 \cdot NH_3 \xrightarrow{>374^\circ} CuSO_4$$

From the crystal structure of $[Cu(NH_3)_4]SO_4 \cdot H_2O$, as reported by MAZZI[10], it is highly unlikely that the thermal dissociation proceeds as given by PFEIFER[9]. The complex consists of layers of $Cu(NH_3)_4^{2+}$ ions bonded to layers of sulfate ion tetrahedra by water molecules. Thus, it is unlikely that the ammonia should be evolved before the water.

The gas evolution curve and the mass spectrometer curves are given in Fig. 3. The GEA curve consists of 6 maxima with peak temperatures of 145°, 175°, 285°,

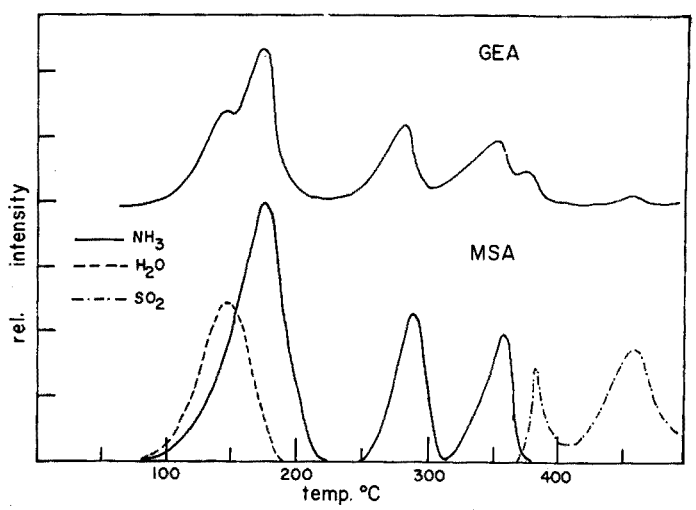


Fig. 3. GEA and MSA curves of $[Cu(NH_3)_4]SO_4 \cdot H_2O$; heating rate of 6.0°/min; sample size of 80 mg; helium flow-rate of 50 ml/min.

355°, 380°, and 460°, respectively. By itself, the GEA curve is impossible to interpret although "intelligent guesses" may be made. All that can be definitely concluded is that each peak represents the evolution of a gaseous product which may be water, ammonia, or other substances.

The MSA curves, however, enable a complete interpretation to be made of the thermal dissociation process. The first peak, at 145°, is due to the evolution of water. This peak was recorded by a pyrolysis scan at a *m/e* of 18. The next 3 peaks, at 175°, 290°, and 360°, are due to the evolution of ammonia, as revealed by a pyrolysis scan at a *m/e* of 17. No correction was made for the contribution of water to the ammonia peaks. The 382° and 460° peaks were due to the evolution of sulfur dioxide; these were caused by the decomposition of anhydrous copper sulfate to $CuSO_4 \cdot CuO$[11].

Thus, it is seen that the interpretation by PFEIFER[9] is entirely erroneous. The thermal dissociation of $[Cu(NH_3)_4]SO_4 \cdot H_2O$ takes place according to the sequence:

$$[Cu(NH_3)_4]SO_4 \cdot H_2O(s) \rightarrow [Cu(NH_3)_4]SO_4(s) + H_2O(v)$$

$$[Cu(NH_3)_4]SO_4(s) \rightarrow Cu(NH_3)_2SO_4(s) + 2NH_3(g)$$

$$Cu(NH_3)_2SO_4(s) \rightarrow Cu(NH_3)SO_4(s) + NH_3(g)$$

$$Cu(NH_3)SO_4(s) \rightarrow CuSO_4(s) + NH_3(g)$$

$$CuSO_4(s) \rightarrow CuO \cdot CuSO_4(s) + SO_2(g) + \tfrac{1}{2}O_2(g)$$

Not a great amount of significance can be placed upon the peak maxima temperatures since they are dependent on the pyrolysis apparatus experimental parameters such as sample size, carrier gas flow-rate, furnace heating-rate, and so on[1].

Thus, the use of the GEA–MSA technique enables the exact interpretation of thermal decomposition reaction involving low molecular weight products. It is unlikely that quantitative data can be obtained because of the difficulty in regulating the pressure in the mass spectrometer. However, quantitative data are easily obtained by other chemical methods such as sample collection on various absorbents and others. The qualitative identification of gas mixtures is, however, easily carried out with this apparatus. There appears to be no reason why the MSA technique could not be coupled with other thermal methods such as TGA, DTA, high temperature X-ray, pyrolytic methods, and others. Such an attempt has been made with DTA[12], but the sample was pyrolyzed *in vacuo* causing a reduction in the resolution of the DTA peaks.

The support of this work by the U.S. Atomic Energy Commission and the Robert A. Welch Foundation is gratefully acknowledged.

SUMMARY

An apparatus is described which enables the gas evolution analysis (GEA) and mass spectrometric analysis (MSA) curves of a sample to be recorded simultaneously. The sample is pyrolyzed in a chamber in a dynamic helium atmosphere. The evolved products are detected in the helium gas by a thermal conductivity cell which results in the GEA curve. An inexpensive mass spectrometer is used to monitor the helium gas stream which gives the MSA curve. The advantages of the apparatus over other recent techniques are given.

RÉSUMÉ

Les auteurs décrivent un appareil permettant l'enregistrement simultané des courbes obtenues par l'analyse de gaz pyrolysés, ainsi que celles de l'analyse spectrométrique de masse. Les produits formés sont détectés à l'aide d'une cellule de conductivité thermique. L'échantillon est pyrolysé dans une atmosphère d'hélium. On décrit les avantages de cet appareil, par rapport à d'autres techniques récentes.

ZUSAMMENFASSUNG

Es wird eine Apparatur beschrieben, die die gleichzeitige Aufzeichnung von Kurven bei der Gasentwicklungsanalyse (GEA) und der massenspektrometrischen Analyse (MSA) gestattet. Die Probe wird in einer Kammer in einer strömenden Heliumatmosphäre pyrolisiert. Die entwickelten Produkte werden im Helium mit einer thermischen Leitfähigkeitszelle nachgewiesen und als GEA-Kurve registriert. Ein Massenspektrometer kontrolliert den Heliumgasstrom und registriert die MSA-Kurve. Es werden die Vorteile der Apparatur gegenüber anderen neueren Techniken angegeben.

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EXTRACTION AND FLAME SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM AND RHODIUM*

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Very little information is available in the literature on the application of flame photometry to the analysis of the platinum metals. GILBERT[1] has stated: "That no one has used flame photometry for determining the platinum metals — at least ruthenium, rhodium, and palladium — is the more surprising in view of the analytical difficulties that beset these elements".

In the present investigation a flame spectrophotometric method for palladium and for rhodium was developed. Palladium was isolated from most of the other elements by a single extraction with a 4-methyl-2-pentanone solution of salicylaldoxime, after which the organic phase was aspirated directly into the flame. Rhodium was then extracted from the aqueous phase into 4-methyl-2-pentanone, as the diethyldithiocarbamate chelate.

The method of GILCHRIST AND WICHERS[2] has been used for many years in the analysis of the platinum metals. However the flame spectrophotometric method for palladium and rhodium has the advantage of speed and can be used for samples containing them in the microgram range. The colorimetric method of FORSYTHE *et al.*[3] gives excellent results for palladium, but the procedure is time-consuming.

The effects of the following experimental variables were studied: the flows of oxygen and acetylene and their optimum ratio, the emission intensity from different regions of the flame mantle, and the effects of several cations and anions.

EXPERIMENTAL

Apparatus

The flame spectrophotometer and associated equipment were previously described[4].

Reagents

Standard solutions of palladium and rhodium were made from spectrographically analyzed palladium(II) chloride and rhodium(III) chloride (J. Bishop and Co.). The weight of the chloride needed to furnish 500 mg of the metal was calculated from the assay value; this weight was dissolved in 0.025 *M* hydrochloric acid and diluted to 500 ml with the same solvent.

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Procedure

Use 20 ml of a solution containing palladium(II) chloride and rhodium(III) chloride. The concentration of the palladium and the rhodium should be between 5 and 100 μg/ml. Add a known volume (usually 20.0 ml) of a 1.5% (w/v) solution of salicylaldoxime in 4-methyl-2-pentanone and agitate briefly. Finally adjust the pH of the aqueous phase to 3.2 $\pm$ 0.2 with 0.5 *M* aqueous ammonia. The base should be added in small portions and with thorough mixing. Let stand for 1 h with occasional shaking. Quantitatively separate the two phases. Reserve the aqueous phase for the determination of rhodium. Aspirate the organic phase into the flame and record the palladium line emission at 363.5 mμ and the flame background at 362.0 mμ. Bracket the sample with suitable aliquots of the standard palladium solution which have been carried through the same extraction procedure.

Add 20.00 ml of 4-methyl-2-pentanone and 15 ml of an 8% (w/v) solution of sodium diethyldithiocarbamate in demineralized water to the aqueous phase that was reserved for the determination of rhodium. Shake for 1 min, and then add 2 *M* ammonium acetate buffer, pH 8, in three 5-ml portions and shake for 5 min. Let stand for 2 h with occasional shaking. Decant the organic phase into the sample cup. Aspirate the organic phase into the flame and record the rhodium line emission at 369.2 mμ and the flame background at 367 mμ. Bracket the sample with suitable aliquots of the standard rhodium solution which have been carried through the diethyldithiocarbamate extraction procedure.

RESULTS AND DISCUSSION

Slit-width

With the line-rich spectra of palladium and of rhodium, the minimum slit-width that is compatible with the sensitivity requirements should be used. With a photomultiplier tube sufficient emission intensity was obtained with a slit-width of 0.030 mm, which corresponded to a band width of 0.42 mμ at one half of maximum emission intensity. Little additional resolution is gained by the use of a narrower slit-width[5].

Oxygen and acetylene flows

The effect of increasing acetylene flow-rate, at constant oxygen flow-rate, upon the emission intensity of palladium is shown in Fig. 1. A slight maximum in emission intensity occurred from 2.6 to 3.0 cu.ft./h for acetylene, while the oxygen flow-rate was maintained at 6.0 cu.ft./h. The optimum ratio of oxygen to acetylene flows was from 2.3 to 1 down to 2.0 to 1. A similar effect was noted for other oxygen flow-rates.

The emission intensity of the palladium 363.5 mμ line as a function of oxygen flow-rate at constant acetylene flow rate was also studied. The emission readings were erratic for oxygen flows less than 5 cu.ft./h, but increased pronouncedly as the oxygen flow-rate was increased from 5 to 8 cu.ft./h; additional increase in oxygen flow produced no significant change.

Figure 2 shows the effect upon the palladium emission intensity produced by changing the oxygen and acetylene flow-rates while maintaining the flow ratio at 2:1. The emission intensity of the palladium line increased about 30% as the oxygen flow-

rate was increased from 5 to 10 cu.ft./h. This increase in oxygen flow-rate approximately tripled the aspiration rate of the organic extract.

The emission intensity of the rhodium 369.2-mμ line as a function of acetylene flow-rate at constant oxygen flow is shown in Fig. 1. With the oxygen flow-rate

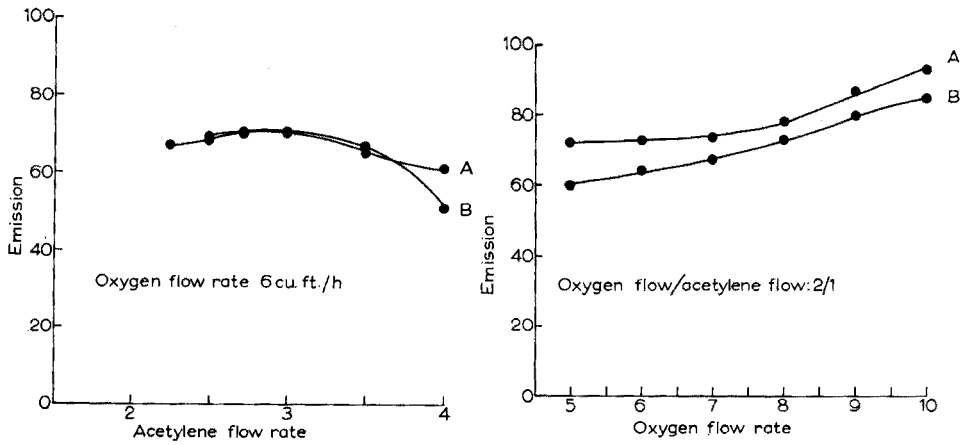

Fig. 1. Intensity of emission as a function of acetylene flow-rate at constant oxygen flow. (A) Palladium chelate (20 μg Pd per ml 4-methyl-2-pentanone). (B) Rhodium chelate (10 μg Rh per ml 4-methyl-2-pentanone).

Fig. 2. Intensity of emission as a function of oxygen and acetylene flows at the optimum combustion ratio. (A) Palladium chelate (20 μg Pd per ml 4-methyl-2-pentanone). (B) Rhodium chelate (10 μg Rh per ml 4-methyl-2-pentanone).

constant at 6 cu.ft./h, the maximum emission intensity was obtained when the acetylene flow-rate was 2.5–3.0 cu.ft./h. Increasing the oxygen flow from 5 to 10 cu.ft./h at a constant acetylene flow-rate of 3 cu.ft./h had only a very slight increasing effect upon the emission intensity of rhodium.

The effect upon the rhodium 369.2-mμ line produced by increasing the oxygen and acetylene flow-rates while maintaining their ratio at 2:1 is shown in Fig. 2. An increase in the oxygen flow-rate from 5 to 10 cu.ft./h increased the emission intensity of the rhodium a little more than 30% and increased the aspiration rate of the sample almost 3-fold.

The emission intensity in the different regions of the flame mantle

The use of an adjustable burner mount made it possible to observe the emission intensities of palladium and rhodium at different heights in the flame. The positions of the burner ranged from 10 mm above normal to 10 mm below normal. The permanent position of the burner in its housing, as furnished by Beckman Instruments, is considered the normal position. The emission intensity of palladium and rhodium increased as the burner was raised from 10 mm below normal to 5 mm above normal, then decreased as the upward movement of the burner was continued. This

position of maximum emission intensity was for toluene, 4-methyl-2-pentanone, and pentyl acetate aerosols. The burner position that gave maximum emission intensity with a chloroform aerosol was 8 mm above normal.

Working curves

The working curves for palladium and rhodium shown in Fig. 3 were obtained with 4-methyl-2-pentanone solutions of their chelates. Slight self-absorption of the palladium 363.5-mμ line began at 20 μg/ml and continued up to 200 μg/ml, becoming more intense above that concentration. Self-absorption was only absent for rhodium solutions when their concentration was less than 10 μg/ml.

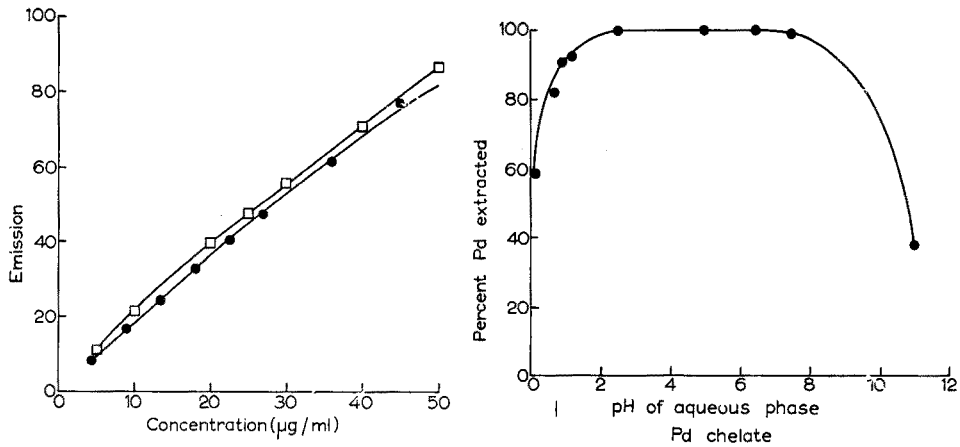

Fig. 3. Emission intensity of palladium and rhodium lines at 363.5 mμ from a 4-methyl-2-pentanone solution as a function of metal concentration. ●, Pd chelate; □, Rh chelate.

Fig. 4. Effect of pH on extraction of palladium with 1.5% salicylaldoxime in 4-methyl-2-pentanone.

Interferences

In these studies solutions containing known amounts of palladium or rhodium and the various test ions were carried singly through the extraction procedure. No attempt was made to differentiate between the effect of the interfering ion upon the extraction and upon the emission intensity of the metal. The influence of various acids upon the palladium emission intensity is shown in Table I. No serious interference was experienced from any of the acids at the concentrations tested. The effect of higher acid concentrations was not investigated because in most cases a mixture of concentrated acids is used to dissolve the palladium or rhodium sample and then the solution is evaporated to near dryness. The data contained in Table I also show that the deleterious effect of the acids upon the rhodium emission intensity was much more severe that that observed for palladium. Hydrochloric acid was the only one that was completely innocuous.

TABLE I

INFLUENCE OF VARIOUS ACIDS UPON PALLADIUM AND RHODIUM LINES

(Present, 10 μg metal per ml)

Acid tested	*Concentration (M)*	*Change in emission intensity (%)*	
		Pd	*Rh*
Acetic	0.1	0	—
	0.5	0	—
Phosphoric	0.1	0	− 4
	0.5	0	−24
Sulphuric	0.1	+1	+ 1
	0.5	0	+ 1
Nitric	0.1	−1	+ 1
	0.5	+1	− 3
Perchloric	0.1	+3	− 2
	0.5	+2	− 9
Hydrochloric	0.1	0	0
	0.5	0	0

TABLE II

INFLUENCE OF DIVERSE IONS UPON THE PALLADIUM AND RHODIUM LINES

(Present, 10 μg Pd or Rh per ml)

Interference	*Concentration (p.p.m.)*	*Change in emission intensity (%)*	
		Pd	*Rh*
Cobalt	2000	− 1.5	−11
Gold	1000	+ 2.0	− 1
Iridium	500	− 6.0	− 6
Iron	100	+12.0	− 6
Iron	10,000	− 1.0	−20
Nickel	1000	− 2.5	− 6
Osmium	1000	0	+ 2
Platinum	1000	+ 2.0	+ 5
Platinum	5000	+ 3.0	+ 2
Ruthenium	100	+ 2.5	+ 5
Ruthenium	1000	− 4.0	+ 1

Table II shows the effect of some diverse ions upon the emission intensity of palladium. It is probable that the apparent discrepancies for the two concentrations of iron and ruthenium are due to the fact that with the more concentrated solution a solid separated at the interface of the two phases during extraction and this inhibited to some extent the extraction of palladium.

The influence of some diverse ions upon the rhodium emission intensity is also shown in Table II. A large change in the emission intensity was produced by some of the cations; however, it would be simple to remove the interfering ion in most cases, *e.g.* iron could be removed by prior extraction with TTA at low pH.

Extraction of palladium

To circumvent the interference from other elements, palladium was extracted with a 1.5% solution of salicylaldoxime in 4-methyl-2-pentanone from an aqueous ammonia solution which was adjusted to pH 3.2 ± 0.2. 4-Methyl-2-pentanone was found to be superior to toluene and chloroform in combustion characteristics and superior to pentyl acetate in extraction characteristics.

The effect of the pH of the aqueous phase upon the extraction of palladium salicylaldoxime is shown in Fig. 4. The extraction is more than 99.5% complete over the pH range 2.8–6.4. The aqueous: organic volume ratio should lie between 1 and 5.

Extraction of rhodium

The extraction of rhodium diethyldithiocarbamate into 4-methyl-2-pentanone is only satisfactory in all respects when the extraction is made with the aqueous phase strongly buffered at pH 8 with ammonium acetate solution.

TABLE III

DETERMINATION OF Pd AND Rh

(Volume of organic phase, 30.00 ml)

Pd taken (μg)	*Rh taken (μg)*	*Pd recovered (%)*	*Rh recovered (%)*
300	720	97.0	99.3
300	820	98.5	99.3
300	900	99.0	98.2
600	660	98.3	100.0
600	1320	98.0	99.4
1200	660	100.0	100.0
5000	150	98.2	93.3
5000	300	98.4	92.4
5000	450	99.5	97.8

The validity of the proposed method of analysis for palladium–rhodium mixtures is shown in Table III. For the 9 samples analyzed there was no error greater than 3% for the palladium determination and only 2 of the rhodium determinations showed an error greater than 3%. The average error for the 9 palladium determinations was 1.5% and for rhodium the average error was 2.3%. The study of the emission characteristics of palladium and of rhodium from a 4-methyl-2-pentanone solution should be useful to anyone contemplating the use of a flame spectrophotometric method for these elements.

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SUMMARY

Solvent extraction methods involving toluene, chloroform, 4-methyl-2-pentanone and pentyl acetate were studied for palladium and rhodium chelates. The palladium–salicylaldoxime chelate was extracted quantitatively into 4-methyl-2-pentanone at pH 3. The rhodium–diethyldithiocarbamate chelate was completely extracted into 4-methyl-2-pentanone at pH 8. The optimum combustion conditions for each of the organic extracts were then studied. The position of maximum emission intensity in the flame mantle was determined for each chelate and solvent system; readings were taken at 363.5 mμ for palladium, and 369.2 mμ for rhodium. For palladium, when 4-methyl-2-pentanone was used instead of water as solvent, the emission intensity increased 21-fold. For rhodium, this ketone increased the sensitivity 27 times compared with water. A method is suggested for the separation and determination of palladium and rhodium in the same sample.

RÉSUMÉ

On a effectué une étude sur l'extraction de chélates de palladium et de rhodium, au moyen des solvants suivants: toluène, chloroforme, 4-méthyl-2-pentanone et pentylacétate. Le chélate palladium–salicylaldoxime est extrait quantitativement au pH 3, au moyen de la 4-méthyl-2-pentanone; le chélate rhodium–diéthyldithiocarbamate est extrait dans le même solvant, au pH 8. Une méthode par spectrophotométrie de flamme est proposée pour la séparation et le dosage du palladium et du rhodium, dans un même échantillon.

ZUSAMMENFASSUNG

Es wird die Extraktion verschiedener Palladium- und Rhodium-Chelate mit Toluol, Chloroform, 4-Methyl-2-pentanon und Pentylacetat untersucht. Mit 4-Methyl-2-pentanon wurden Pd–Salicylaldoxim und Rh–Diäthyldithiocarbamat beim pH-Wert 3 bzw. 8 quantitativ extrahiert. Für die Flammenspektralphotometrie wurden die optimalen Verbrennungsbedingungen der organischen Extrakte aufgesucht. Pd wurde bei 363.5 mμ, Rh bei 369.2 mμ gemessen. Die Verwendung des Ketons anstelle von Wasser steigert die Empfindlichkeit beim Pd um das 21-fache, beim Rh um das 27-fache. Es wird eine Methode zur Trennung und Bestimmung von Pd und Rh in derselben Probe vorgeschlagen.

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THE SPECTROPHOTOMETRIC DETERMINATION OF RHENIUM

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There is a continuing interest in the use of rhenium in refractory alloys. The analysis of rhenium seems to lend itself more suitably to spectrophotometry than to volumetric or gravimetric analysis. However, while several reagents are in use, they all suffer to a greater or lesser degree from interference by such elements as Ni, Fe, Mo, W, and Cu. The use of thiocyanate[1,2] and tetraphenylarsonium chloride[3] is well established and was not further investigated. However, other reagents of more limited use as well as reagents as yet undiscussed in the literature were investigated.

The program was two-fold in purpose. The first intention was to find a more specific reagent or by controlling the parameters of the procedure of an existing method to improve its specificity. The second purpose was to increase the number of existing reagents that are available for use in rhenium analysis with information on their limitations, so that for specific problems there would be more suitable procedures.

Approximately 50 organic reagents were briefly investigated. The reagents fitted the general pattern of having one or more of the following characteristics: they contained a nitrogen, sulfur, or phosphorus group; or had been used in the analysis of Tc, Ni, or Pd. A preliminary procedure was used to select 7 reagents for a more detailed investigation.

Preliminary procedure

To a set of 4 beakers, add 10 ml of hydrochloric acid to beakers A and B. To beakers C and D add 10 ml of a solution containing 50 μg Re/ml in 12 N hydrochloric acid. To beakers A and C add 5 ml of hydrochloric acid. To beakers B and D add 5 ml of 20% tin(II) chloride in hydrochloric acid. Add 5 ml of the organic reagent to each beaker and let stand for 30 min. Note the color change between the rhenium and blank solutions. Add 5 ml of water to each beaker and let stand for 30 min. The normality is now approximately 9 N. Repeat twice with 5 ml more water (7.2 N and 6.0 N). Then add 30 ml of water to bring the normality to approximately 3 N.

The following reagents were tried and selected for further investigations.

Group I (7 reagents): 1-phenyl-2-thiourea, furildioxime[4], toluene-3,4-dithiol[5], 5-methyl-1,2,3-cyclohexanetrione trioxime[6], 1,5-diphenylcarbohydrazide[7–10], nioxime[11], dimethylglyoxime (Na salt).

The following reagents were also tried.

Group II (41 reagents): 1-ethyl-3-phenyl-2-thiourea, *p*-toluenethiol, dichloro-

glyoxime, isobutyraldoxime, nitrosoresorcinol (Na salt), 2,4-pentanedione dioxime, 1,3-diethylthiourea, 5-amino-2-benzimidazothiol, methyl violet 2B, 4-phenyl-3-thiosemicarbazide, phenylglyoxaldoxime, 2,3-quinoxaline dithiol, rhodamine B, diphenylglyoxime, dithiooxamide, dithiooxalic acid (K salt), dithizone, thiocarbanilide, xylenol orange, thiosemicarbazide, diethyldithiocarbamic acid (Na salt)[12], fast sulphon black, phenyl-2-pyridyl ketoxime[13], 1,3-diethyl-2-thiourea, thioglycollic acid[14], 1-amino-2-naphthol-4-sulfonic acid, diphenylamine, mercaptosuccinic acid, 5-methyl-1,2,3-cyclohexanetrione-1,3-dioxime, 1-nitroso-2-naphthol, PAN, PAR, alizarin red S, benzohydroxamic acid (K salt), dicyclohexanone oxalyldihydrazide, 5-(*p*-dimethylaminobenzylidene)rhodanine, *s*-diphenylcarbazone, rhodanine, salicylaldoxime, salicylhydrazide, 1-nitroso-2-naphthol-3,6-disulfonic acid (disodium salt).

This second group of reagents did not appear as satisfactory under the conditions of the preliminary procedure. However, such reagents as phenyl-2-pyridylketoxime have been used in the determination of rhenium.

EXPERIMENTAL

Group I reagents

A. Procedure with 1-phenyl-2-thiourea

Reagents. 1-Phenyl-2-thiourea (2.5%)—dissolve 25 g of the reagent in 1 l of ethanol. Stannous chloride (10%)—dissolve 120 g of $SnCl_2 \cdot 2H_2O$ in 200 ml of hydrochloric acid and dilute to 1 l with water. Standard rhenium solutions—20 μg/ml and 4 μg/ml.

Preliminary procedure. Place 10 ml of rhenium solution (20 μg/ml) into small beakers and dilute with water to 20 ml. Then add 10 ml of 12 *N* hydrochloric acid, 5 ml of stannous chloride and 5 ml of 1-phenyl-2-thiourea solution. After 1.5–3.0 h, extract twice with 10-ml portions of chloroform. Dilute to 25 ml with absolute ethanol and determine the absorbance in 1-cm cells.

Results. Under the above conditions, the absorbance maximum occurred at 410 mμ.

Effect of HCl concentration. At 410 mμ, maximum absorbance (0.389) was found with 14.4–16.8 ml of 12 *N* HCl present.

Effect of 1-phenyl-2-thiourea concentration. With 15 ml of 12 *N* HCl present and varying amounts of 1-phenyl-2 thiourea solution, but otherwise under the above conditions, maximum absorbance (0.420) was found with 10–15 ml of reagent solution; the absorbance decreased if 5 or 20 ml of reagent solution was added.

Effect of stannous chloride concentration. Change in the concentration of the stannous chloride solution from 10% to 20% had a negligible effect.

Preparation of standard curves. Based on the above data, the optimum conditions were established and the following procedure was used subsequently. Dilute the aliquot containing rhenium to 20 ml with water. Add 15 ml of 12 *N* HCl, 5 ml of 10% stannous chloride solution and 10 ml of phenylthiourea solution by pipet or buret. Allow the solutions to stand for 1.5–3.0 h. Then make two 10-ml chloroform extractions each for 30 sec. Retain the extracts in a dry 25-ml volumetric flask. Make to volume with absolute ethanol. Read the absorbance in a spectrophotometer with chloroform in the reference cell at a wavelength of 410 mμ.

Conclusions. The procedure is reliable in the presence of at least 25-fold amounts of several important elements that are found with rhenium, notably copper and tungsten (see Table VII). The method should be useful in the determination of rhenium in rhenium–tungsten alloys.

B. Procedure with furildioxime

Reagents. Furildioxime (0.25%)—dissolve 1.25 g of the reagent in 500 ml of absolute ethanol. Standard rhenium solutions—5 μg/ml and 1 μg/ml.

Preliminary procedure. Place 10-ml aliquots of rhenium solution (5 μg/ml) into small beakers and add water to a total volume of 27 ml. Then add 3 ml of 12 *N* HCl, 5 ml of 10% stannous chloride solution and 15 ml of furildioxime solution. Extract with 10 ml and 5 ml of chloroform. Dilute to 25 ml with absolute ethanol and determine the absorbance in 1-cm cells.

Results. The wavelength of maximum absorbance was found to be 530 mμ.

Effect of HCl concentration. With no acid present, the solution was cloudy and almost colorless. Constant maximum absorbance (0.355) was obtained in presence of 2.5–3.75 ml of 12 *N* HCl.

Effect of time on color development. The absorbance reached a maximum after 2 h and remained constant for another 2 h, after which it gradually decreased.

Preparation of standard curve. Based on the optimum conditions noted above, the following procedure was used subsequently. Dilute aliquots containing rhenium to 27 ml with water. Add 3 ml of 12 *N* HCl, 5 ml of 10% stannous chloride solution and 15 ml of furildioxime solution. Allow to stand for 2 h. Transfer to a separatory funnel and complete transference with two 5-ml portions of chloroform. Extract the rhenium–furildioxime complex for 30 sec and reserve the chloroform layer in a dry 25-ml volumetric flask. Make a second extraction with 5 ml of chloroform and add this extract to the original. Dilute to volume with absolute ethanol. Read the absorbance with chloroform in the reference cell at a wavelength of 530 mμ.

Conclusions. The procedure is reliable in the presence of at least 10-fold amounts of most elements. The elements interfering seriously are palladium and molybdenum. The interference of molybdenum is serious because rhenium–molybdenum alloys are important, and because the two elements commonly occur together in nature.

C. Procedure with sodium dimethylglyoximate

Reagents. Sodium dimethylglyoximate solution (1%)—dissolve 1 g of reagent in 100 ml of water. Standard rhenium solutions—25 μg/ml and 5 μg/ml.

Preliminary procedure. Place 10 ml of rhenium solution (25 μg/ml) into 50-ml volumetric flasks. Adjust the volume to 30 ml with water. Add 5 ml of sodium dimethylglyoximate solution, acid and 5 ml of 10% stannous chloride solution. Adjust to volume with water and determine the absorbance in 1-cm cells.

Results

Effect of HCl concentration. Under the above conditions, varying amounts of 12 *N* HCl were added and the absorbance was determined at 2 wavelengths. The results are shown in Table I.

Effect of dimethylglyoximate concentration. With 2 ml of 12 *N* HCl and varying amounts of reagent under the above conditions, maximum absorbance at 445 mμ was found with 5 ml of reagent present. Addition of 10 ml caused salting out.

Effect of stannous chloride and HCl concentration. With 2 ml of HCl (12 *N*), and

varying amounts of stannous chloride solution in 20% HCl, the absorbance was determined at 3 wavelengths. The results are shown in Table II.

TABLE I

EFFECT OF HCl ON THE DMG METHOD

ml HCl (12 N)	*Absorbance at 445 mμ*	*Absorbance at 436 mμ*
1.0	0.392	0.408
1.5	0.457	0.473
2.0	0.428	0.447
2.5	0.417	0.427
3.0	0.408	0.408
3.5	0.401	0.394
4.0	0.398	0.387

TABLE II

EFFECT OF $SnCl_2$ AND HCl CONCENTRATIONS

ml 10% $SnCl_2$ in 20% HCl	*Total HCl*	*Absorbance in 1-cm cells*		
		At 436 mμ	*At 448 mμ*	*At 446 mμ*
3.0	2.6	0.391	0.374	0.381
		0.393	0.374	0.382
4.0	2.8	0.383	0.374	0.381
		0.383	0.374	0.381
5.0	3.0	0.376	0.374	0.379
		0.373	0.371	0.376

Effect of time on color development. With 2 ml of 12 *N* HCl and 4 ml of stannous chloride solution present the absorbance was found to be constant at 445 mμ for a period of at least 3 h.

Preparation of standard curves. Based on the above data, the following procedure was established and used subsequently. Place the aliquot containing rhenium in a 50-ml volumetric flask. Adjust the volume to 30 ml, then add 2 ml of 12 *N* HCl, 5 ml of sodium dimethylglyoximate and 4 ml of stannous chloride (10%) in 20% HCl. Let stand for 1 h. Dilute to volume with water. Read the absorbance in a spectrophotometer with water in the reference cell at a wavelength of 445 mμ.

Conclusions. The method appears to have a high level of precision in both 1- and 5-cm cells. However, there is a significant negative bias in the presence of molybdenum and a high positive bias in the presence of platinum and tungsten.

D. Procedure with 5-methyl-1,2,3-cyclohexanetrione trioxime

Reagents. 5-Methyl-1,2,3-cyclohexanetrione trioxime (MCHT) (1%)—dissolve 5.0 g of the reagent in 500 ml of ethanol. Standard rhenium solution—10 μg/ml.

Preliminary procedure. Place an aliquot of rhenium solution into a 25-ml volumetric flask and adjust the volume to 15 ml with water. Add HCl (12 *N*), MCHT and 1 ml of 20% stannous chloride solution in 20% HCl. Dilute to volume with water and determine the absorbance in 1-cm cells. If desired, the rhenium complex can be extracted into *n*-hexanol.

Results. With 20 ml of rhenium solution (10 μg/ml), 2 ml of MCHT and no additional HCl, the maximum absorbance occurred at 495 mμ.

Effects of HCl (6 N), MCHT concentration and time. With 10 ml of rhenium solution, varying amounts of HCl (6 *N*) and varying amounts of MCHT under the above conditions, the absorbance was measured at 495 mμ at various time intervals. The results are shown in Table III.

TABLE III

EFFECTS OF MCHT AND HCl CONCENTRATIONS AND TIME

ml MCHT (0.5%)	*ml HCl (6 N)*	*Absorbance*			*ml MCHT (1.0%)*	*ml HCl (6 N)*	*Absorbance*			
		45 min	*75 min*	*105 min*			*30 min*	*60 min*	*90 min*	*120 min*
2	1	0.524	0.518	0.504	5	1	0.521	0.516	0.520	0.518
5	1	0.555	0.554	0.548	5	2	0.531	0.539	0.542	0.546
10	1	0.565	0.583	0.593	5	3	0.522	0.526	0.519	0.526
2	2	0.428	0.400	0.362	5	4	0.512	0.501	0.500	0.497
5	2	0.510	0.496	0.477	5	5	0.483	0.475	0.478	0.465
10	2	0.584	0.590	0.602						
10	4	0.560	0.570	0.565						

Preparation of standard curve. Based on the above data, the optimum conditions were established and the following procedure was used subsequently. Place the aliquot containing rhenium in a 25-ml volumetric flask. Adjust the volume to 15 ml with water. Add 2 ml of 6 *N* HCl, 5 ml of 1% MCHT and 1 ml of 20% stannous chloride solution. Dilute to volume with water. Read the absorbance after 30 min with water in the reference cell at a wavelength of 495 mμ.

Extraction of rhenium complex with n-hexanol. The color was developed as above and the mixture was extracted, before the dilution, twice for 1 min with 10-ml portions of *n*-hexanol; the *n*-hexanol extracts were transfered to a dry 25-ml volumetric flask and diluted to volume with absolute ethanol. When the absorbances at 495 mμ in 1-cm cells were measured for 100 μg Re, a decrease from 0.574 after 45 min to 0.546 after 19 h occurred; however, the absorbances of blank mixtures decreased from 0.105 to 0.068 over the same time interval, so that the absorbance due to the rhenium complex actually increased from 0.469 to 0.478.

Conclusions. While the reagent shows moderate sensitivity and precision, it is both pH-sensitive and time-dependent. Even more important in assessment of this reagent is the multiplicity of serious interfering cations. The *n*-hexanol extraction procedure was not investigated for interferences.

E. Procedure with 1,5-diphenylcarbohydrazide

Reagents. 1,5-Diphenylcarbohydrazide in acetone—0.13 g/200 ml, 0.65 g/200 ml, and 1.30 g/200 ml. Standard rhenium solutions—20 μg/ml in HCl (8 *N*), 15 μg/ml in HCl (8 *N*), and 20 μg/ml in HCl (12 *N*).

Preliminary procedure. Place an aliquot of rhenium solution into a separatory funnel, adjust the volume to 30 ml with 12 *N* HCl and water, add 1,5-diphenylcarbohydrazide solution and extract twice for 45 sec with 8-ml portions of chloroform.

Transfer the extracts to a dry 25-ml volumetric flask, dilute to volume with absolute ethanol and determine the absorbance in 1-cm cells.

Results

Effect of HCl concentration. With 5 ml of rhenium solution (20 μg/ml) in 8 *N* HCl, 20 ml of 1,5-diphenylcarbohydrazide (0.13 g/200 ml), and varying concentrations of HCl, but otherwise under the above conditions, the absorbances were measured at 545 mμ. The absorbances changed from 0.075 through 0.292 to 0.237 as the normality of HCl in the solution before extraction was increased from 8 *N* through 10 *N* to 10.3 *N*.

Effect of 1,5-diphenylcarbohydrazide concentration. When 10 ml of rhenium solution (20 μg/ml) in 12 *N* HCl, 15 ml of 12 *N* HCl, 5 ml of water and varying amounts of 1,5-diphenylcarbohydrazide solution (1.30 g/200 ml) were mixed and the above procedure was followed, the results shown in Table V were found. A 1,5-diphenylcarbohydrazide concentration corresponding to 20 ml of the solution containing 0.65 g of reagent in 200 ml of acetone was accepted as optimum; this is equivalent to 10 ml of the stronger solution plus 10 ml of acetone.

TABLE V

EFFECT OF REAGENT CONCENTRATION

Reagent added (ml)	*Additional acetone (ml)*	*Absorbance*
5	15	0.772, 0.784
10	10	0.830, 0.836
15	5	0.835, 0.825
20	0	0.815, 0.805

Effect of time interval between addition of reagent and extraction. Exactly the same conditions were used as for the study of the effect of the reagent concentration, 20 ml of 0.325% reagent being added. A time interval of 2.0–3.5 min between the reagent addition and extraction had no effect on the absorbances, but with longer intervals the absorbances decreased significantly.

Preparation of standard curve. Based on the above data, the optimum conditions were established and the following procedure was used. Take aliquots containing about 15 μg Re in 8 *N* HCl. Adjust the volume to 15 ml with 8 *N* HCl, add 15 ml of 12 *N* HCl and transfer to a separatory funnel without rinsing the beakers. Add 20 ml of 1,5-diphenylcarbohydrazide solution (0.65 g in 200 ml of acetone) and let stand for 3.5 min. Rinse the original beakers with 8 ml of chloroform and add this to the separatory funnel. Shake for 45 sec and transfer the organic layer to a dry 25-ml volumetric flask. Repeat the extraction with a second 8-ml portion of chloroform. Combine the extracts and dilute to volume with absolute ethanol. Read the absorbance in a spectrophotometer with chloroform in the reference cell at a wavelength of 545 mμ within 2 h.

Conclusions. The 1,5-diphenylcarbohydrazide chloroform extraction procedure appears to be very promising, since of the cations checked at the 5000 μg level, only

molybdenum shows serious interference. However, the essential careful control of variables, especially the time interval, is a drawback.

F. Procedure with toluene-3,4-dithiol

Reagents. Toluene-3,4-dithiol (0.5%)—dissolve 5 g in 100 ml of water containing 20 g of sodium hydroxide; when completely dissolved, add 10 ml of thioglycollic acid and dilute to 1 l with water. Standard rhenium solution—5 μg/ml in 8 *N* HCl and 10 μg/ml in 8 *N* HCl.

Preliminary procedure. Place aliquots containing rhenium in small beakers. Add water and HCl (12 *N*) to give a volume of 35 ml. Then add 10 ml of toluene-3,4-dithiol solution and let stand for 2 h. Extract the rhenium complex with two 10-ml portions of chloroform, dilute to 25 ml with chloroform and determine the absorbance in 1-cm cells.

Results. With 10 ml of rhenium solution (5 μg/ml), 10 ml of 12 *N* HCl and 15 ml of water, taken through the above procedure, maximum absorbance was found at 692.5 mμ.

Effect of HCl concentration. When varying amounts of water and 12 *N* HCl totalling 25 ml were used, the absorbance increased as the amount of HCl increased from 5 to 15 ml; with 15–25 ml of HCl present, the absorbance remained constant (0.256), even when 10 drops of 48% hydrofluoric acid were also added. Accordingly, 8 *N* HCl was used for dilution in further work.

Effect of heating solutions. With varying amounts of rhenium solution (5 μg/ml) adjusted to 30 ml with 8 *N* HCl, the effect of heating the solution for 30 min at 80–90° before extracting with chloroform was examined. A comparison of the results obtained, with those obtained by a standing period of 2 h is shown in Table VI.

Preparation of standard curve. Based on the above data, the optimum condi-

TABLE VI

EFFECT OF HEATING ON DITHIOL COMPLEX FORMATION

Re (μg)	*Absorbance*	
	2-h standing	*30-min heating*
25	0.121, 0.121	0.132, 0.132
50	0.257, 0.259	0.266, 0.268
100	0.538, 0.540	0.540, 0.535
150	0.800, 0.810	0.805, 0.798

tions were established and the following procedure was used subsequently. Place the aliquots containing rhenium in small beakers and adjust the volume to 30 ml with 8 *N* HCl. Add 10 ml of 0.5% toluene-3,4-dithiol solution and place the beakers in a water bath at 80–90° for 30 min. Remove and cool to room temperature. Transfer the solutions to separatory funnels using two 5-ml portions of chloroform to make the transfer. Shake for 1 min and transfer the organic extract to a dry 25-ml volumetric flask. Make a second extraction with 10 ml of chloroform and combine with the original. Dilute to volume with chloroform and measure the absorbance with chloroform in the reference cell at a wavelength of 692.5 mμ.

Conclusions. While the method appears to have a reasonable degree of preci-

sion and sensitivity, there are many important interferences. Interferences beyond use of the method were found with as little as 1000 μg of Cu, Mo, Ni, Pt, and W.

G. Procedure with nioxime (1,2-cyclohexanedioxime)

Reagents. Nioxime—1.0% aqueous solution. Standard rhenium solution—50 μg/ml, and 20 μg/ml.

Preliminary procedure. Place 10 ml of rhenium solution (20 μg/ml) in a 50-ml volumetric flask, add HCl (6 *N*) and dilute to 30 ml with water. Add 5 ml of 10% stannous chloride solution in 20% HCl and 10 ml of nioxime solution. Let stand for 1 h, dilute to volume with water and determine the absorbance in 1-cm cells.

Results. In a preliminary inspection of the use of nioxime, it was observed that the maximum absorbance shifted wavelength with change in acid concentration. Maximum absorbance was observed at 438 and 452 mμ with 3, 4, 5, 6 or 7 ml of 6 *N* HCl under the above conditions. A wavelength of 440 mμ was selected for further work.

Effect of time on color development. When 5 ml of 6 *N* HCl was added in the above procedure, the absorbance reached a maximum (0.340) after 3 h and remained essentially constant after 5 h.

Preparation of standard curve. Based on the optimum conditions observed above, the following procedure was used subsequently. Place an aliquot of rhenium solution in a 50-ml volumetric flask. Add 5 ml of 6 *N* HCl and adjust the volume to 30 ml with water. Add 5 ml of 10% stannous chloride solution and 10 ml of 1% nioxime solution and let stand for 3 h. Then dilute to volume with water, and measure the absorbance with water in the reference cell at a wavelength of 440 mμ.

Conclusions. While the procedure is simple, it is very dependent on time and pH. The standard curve shows a negative deviation. In addition, many elements at the 1000 μg level interfere with the determination.

TABLE VII

AMOUNT OF ELEMENT CAUSING A DEVIATION OF $\pm$ 2% IN RECOVERY OF RHENIUM BY THE VARIOUS REAGENTS TESTED

Element	*Reagent*						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Al	5000	—	1000	5000	5000	5000	1000
Cr	100	1000	1000	CI	5000	5000	<1000
Co	5000	1000	1000	CI	5000	1000	<1000
Cu	5000	1000	1000	CI	5000	CI	CI
Fe	100	100	1000	CI	5000	1000	CI
Mn	500	5000	1000	<5000	5000	5000	<1000
Mo	500	CI	CI	CI	CI	CI	CI
Ni	500	500	1000	CI	5000	—	CI
Pt	CI	<1000	CI	—	5000	CI	CI
Ta	—	—	—	—	—	5000	—
Sn	—	—	—	—	—	5000	—
Ti	—	1000	—	—	—	5000	<1000
W	5000	1000	<1000	CI	5000	CI	1000
V	5000	1000	1000	<2500	5000	5000	<1000
Zn	5000	—	1000	—	5000	5000	—
Zr	—	—	1000	—	—	5000	1000

DISCUSSION

The program to evaluate some of the newer organic reagents used in the spectrophotometric determination of rhenium again indicates the lack of specificity of these reagents. For convenience, the interferences of various metals with the seven methods are summarized in Table VII. The investigation of approximately 50 reagents indicated a few new reagents that were of the caliber of the older reagents. The reagent 1-phenyl-2-thiourea looks especially promising for the determination of rhenium in rhenium–tungsten alloys. Under the proposed conditions, furildioxime, sodium dimethylglyoximate, and 1,5-diphenylcarbohydrazide also appear to be relatively free of interference.

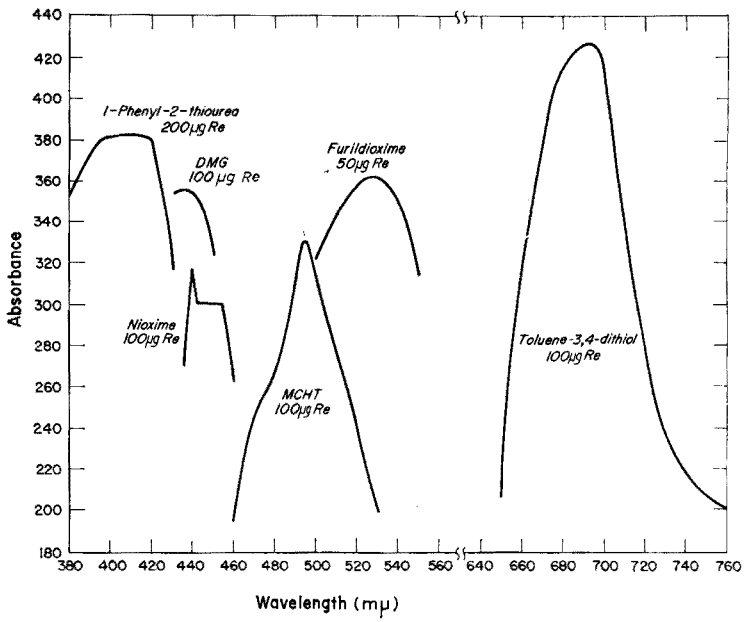

Fig. 1. Rhenium in a 25-ml volume.

TABLE VIII

WAVELENGTH MAXIMA AND MOLAR ABSORPTIVITIES

Reagent	*Maximum wavelength (mµ)*	*Molar absorptivity*
Phenyl-2-thiourea	410	9,550
Furildioxime	530	33,990
Sodium dimethylglyoximate	445	3,840
5-Methyl-1,2,3-cyclohexanetrione	495	23,250
1,5-Diphenylcarbohydrazide	545	20,460
Toluene-3,4-dithiol	692.5	23,250
Nioxime	440	3,930

The important portions of the various spectra are shown in Fig. 1, and the wavelengths of maximum absorbance and the molar absorptivities are summarized in Table VIII.

SUMMARY

Some 50 analytical reagents were briefly checked for use in the spectrophotometric determination of rhenium. Seven reagents were checked in detail and interferences were examined. The most promising reagents appeared to be 1-phenyl-2-thiourea and 1,5-diphenylcarbohydrazide.

RÉSUMÉ

Les auteurs ont essayé quelques 50 réactifs en vue de leur utilisation pour le dosage spectrophotométrique du rhénium. Sept d'entre eux ont été examinés en détail. Les deux réactifs les plus prometteurs semblent être la 1-phényl-2-thiourée et la 1,5-diphénylcarbohydrazide.

ZUSAMMENFASSUNG

Etwa 50 analytische Reagenzien wurden kurz auf ihre Eignung für die spektralphotometrische Bestimmung des Rheniums geprüft. 7 Reagenzien wurden genauer, einschliesslich der Störreaktionen untersucht. Die vielversprechendsten Reagenzien schienen der 1-Phenyl-2-thioharnstoff und das 1,5-Diphenylcarbazid zu sein.

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THE USE OF ANION-EXCHANGE RESIN FOR THE DETERMINATION OF TRACES OF GOLD IN COPPER

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In recent years, the ion-exchange separation of gold has been studied extensively by many workers. Gold adsorbs strongly on anion-exchange resin from chloride[1-5], iodide[5], cyanide[5,6] or nitrate[7] media. The chloride medium seems to be most useful for analytical purposes. In the present work, the anion exchange of gold from chloride–nitrate solutions is described and applied to the determination of 0.01 to 100 p.p.m. of gold in copper metal as a preliminary separation step for photometry or activation analysis.

EXPERIMENTAL

Apparatus

Hirama model II photoelectric filter photometer with 1-cm cells.

Nuclear reactor: Japan Atomic Energy Research Institute research reactor JRR-1.

Atomic scintillation counter with a 1.75 × 2 in. sodium iodide (thallium) scintillator (well-type) and a single-channel pulse-height analyser.

Reagents

Anion-exchange resin. Amberlite IRA-400, 50–100 mesh, chloride-form.

Standard gold solutions. Prepare a stock solution (100 μg of gold per ml) by dissolving pure gold foil in aqua regia and diluting with 1 *M* hydrochloric acid. Dilute this solution to 5 or 0.1 μg of gold per ml with 1 *M* hydrochloric acid.

Gold-198 solutions. Dissolve gold-198 in aqua regia, and dilute with 1 *M* hydrochloric acid or standard gold solutions. Determine the gold concentration photometrically if necessary.

All of the reagents used were of reagent grade. Water purified by ion exchange was used throughout the work.

Preparation of a resin column

Cut off the stem (internal diameter, 4 mm) of a funnel (diameter, 60 mm) to about 30 mm long. Pack a wad of cotton wool at the end of the stem, and add a slurry of resin (0.1 g at dry state) until a resin bed about 20 mm high is formed. Place

a wad of cotton wool on the resin bed. Condition the resin with dilute hydrochloric acid and ammonia solution alternately, and finally wash with 2 *M* hydrochloric acid.

Preliminary experiments

According to KRAUS AND NELSON[2,7], gold(III) is adsorbed strongly on anion-exchange resin from hydrochloric acid solutions at any hydrochloric acid concentration, but copper(II) is not adsorbed appreciably at hydrochloric acid concentrations below about 2.5 *M*. In nitric acid medium[7], gold(III) shows a moderately strong adsorption on anion-exchange resin, whereas copper(II) does not. The adsorption of gold on 0.1 g of Amberlite IRA-400 from 27 ml of solutions containing hydrochloric and nitric acids and copper was investigated by the use of 10 μg of gold-198 as a tracer. The results are shown in Table I. Under these conditions, copper is not adsorbed on the resin. As expected from the results of previous workers[7,8], the replacement of hydrogen ion by copper(II) seems to increase the distribution coefficient

TABLE I

ADSORPTION OF Au(III) ON ANION-EXCHANGE RESIN (0.10 g)

Solution	*Composition*				^{198}Au *remaining in the solution after 12h-equilibration (%)*
	Cu^{2+} *(g)*	H^+ *(M)*	Cl^- *(M)*	NO_3^- *(M)*	
1. 2 *M* HCl	—	2.0	2.0	—	0.4
2. 1 *M* $CuCl_2$–0.1 *M* HCl	1.7	0.1	2.1	—	<0.2
3. 1.25 *M* $Cu(NO_3)_2$–2 *M* HCl	2.1	2.0	2.0	2.5	2.1
4. 0.65 *M* $Cu(NO_3)_2$–1 *M* HCl	1.1	1.0	1.0	1.3	1.1
5. Cu (1.7 g) dissolved in HNO_3–HCl[a]	1.7	1.2[c]	2.4[c]	0.8[c]	0.8
6. Cu (2.1 g) dissolved in HNO_3–HCl[b]	2.1	2.4[c]	2.0[c]	2.9[c]	3.0

[a] Batch method.
[b] Column method.
[c] Estimated value.

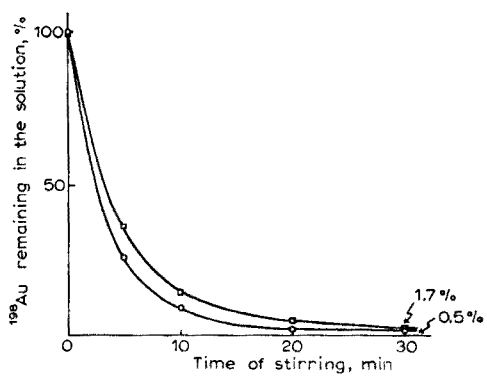

Fig. 1. Adsorption of gold on resin from stirred copper solutions. □ 30 ml of solution containing 10 μg of gold-198 and 2 g of copper (resin used: 0.1 g); ○ 75 ml of solution containing 10 μg of gold-198 and 5 g of copper (resin used: 0.2 g).

of gold in chloride solution. Figure 1 shows the adsorption rate of gold on resin from stirred copper solutions. A batch as well as a column operation with small amounts of resin can be used for the separation of gold from copper. As little as 0.01 μg of gold was separated by the column operation with a recovery of more than 99%.

Attempts to desorb gold perfectly from the resin column with 50 ml of the following eluents were unsuccessful: 1 *M* nitric acid, aqua regia, methyl isobutyl ketone, ethyl acetate, acetone–water–hydrochloric acid (90:5:5), and acetone–water–nitric acid (90:5:5). The last eluent desorbed 80 to 95% of gold, but bubbles were formed in the resin column.

Dry ashing of the resin followed by dissolution of the residue in aqua regia was therefore tried. Ashing temperatures above about 600° caused strong adhesion of gold to the crucible, which made the subsequent dissolution of the gold very difficult. At low ashing temperatures, an almost perfect recovery of gold was achieved with the use of an alumina crucible, but only about 93% of gold was recovered when a glazed porcelain crucible was used. Wet ashings of the resin with nitric or perchloric acid were incomplete.

From the above preliminary experiments, the following procedure was developed.

Procedure

Ion-exchange separation of gold from copper (Batch operation). Transfer a weighed sample (1–5 g) to a conical flask, add 8 *M* nitric acid (3 ml/g of copper) and concentrated hydrochloric acid (3 ml/g of copper), and heat to dissolve completely. Dilute the solution to about 15 ml/g of copper with water, and cool. If there is any insoluble matter such as silver chloride, filter it off on a filter paper, and wash several times with a few ml of 0.1 *M* hydrochloric acid. Transfer the combined filtrate and washings to a 500-ml conical beaker, add about 0.2 g of resin, and stir with a magnetic stirrer for about 40 min. Filter off the resin on a filter paper (No. 7, 5 cm diameter), and wash several times with a few ml of 0.1 *M* hydrochloric acid.

Ion-exchange separation of gold from copper (Column operation). Transfer a weighed sample (0.5–5 g) to a conical flask, add 8 *M* nitric acid (6–8 ml/g of copper), and heat to dissolve. Add concentrated hydrochloric acid (1.5–2 ml/g of copper), and continue heating to complete dissolution. Dilute the solution to 12–12.5 ml/g of copper with water, and cool. If there is any insoluble matter such as silver chloride, filter and wash as outlined above. Pass the combined filtrate and washings through a column of anion-exchange resin at a flow-rate of 1–1.5 ml/min. Wash the column with 10 ml of 0.1 *M* hydrochloric acid or 5 ml of 0.1 *M* nitric acid to remove copper. Remove the resin carefully together with wads of cotton wool from the funnel with the aid of a glass rod.

Photometric determination of gold. Wrap the resin containing gold (and wads of cotton wool) in a filter paper, transfer it to a 30-ml alumina crucible, and ash it at as low a temperature as possible. Cool, add a small amount of water and 1 ml of aqua regia, cover with a watch glass, and heat for a few min. Rub the interior of the crucible with a policeman. Filter the solution through a wad of cotton wool. Add a small amount of water and 1 ml of aqua regia to the crucible, and repeat the above manipulation twice more. Take an aliquot containing less than 6 μg of gold from the combined filtrates, and transfer it to a 50-ml beaker. Evaporate the solution to dryness on a

water bath, dissolve the residue in 1–5 drops of aqua regia and 0.5 ml of water, and evaporate just to dryness on a water bath (70°). Moisten the residue with 1 drop of concentrated hydrochloric acid, and again evaporate just to dryness on the water bath (70°). Add 2.5 ml of 0.12 *M* hydrochloric acid and 5.0 ml of 4% (w/v) sodium chloride solution, heat for a few min on the water bath, cool to room temperature, and add 0.50 ml of 1% (w/v) sodium fluoride solution. Swirl the solution gently and add 0.50 ml of ethanolic 0.003% *p*-dimethylaminobenzylidenerhodanine solution dropwise. Transfer the solution to a 10-ml volumetric flask, dilute to the mark with water, and mix the solution by inverting the flask 2 or 3 times. Do not shake vigorously. After 15–20 min, measure the extinction of the solution in a 1-cm cell at 562 mμ against water. Determine the gold concentration by reference to a calibration curve prepared with standard gold solutions.

Determination of gold by neutron activation. Transfer the resin containing gold and wads of cotton wool to a 50-ml beaker with the aid of a glass rod, and dry on a water bath. Remove the wads of cotton wool*, and wrap the resin in a sheet of paper. Prepare standards by adsorbing standard gold solutions on resin by the same procedure as for unknown samples. Pack the samples together with the standards into a polyethylene capsule (30 mm diam. $\times$ 50 mm), and irradiate in a reactor at a flux of $3–5 \cdot 10^{11}$ n/cm^2/sec for 4–5 h. After cooling for about 40 h, open the capsule, and transfer the resin to a folded filter paper. Ash the resin and paper in a 30-ml alumina crucible at as low a temperature as possible. Cool, add 0.3–0.4 ml of aqua regia, and heat to dissolve. Add 1 ml of standard gold solution (5.0 μg of gold per ml), and heat. Transfer the solution to a 100-ml separatory funnel. Add 0.3–0.4 ml of aqua regia to the crucible, and repeat the above manipulation twice more. Dilute the solution in the separatory funnel to 10 ml with water, add 10 ml of ethyl acetate, and shake for 2 min in a mechanical shaker. Discard the aqueous phase. Add 10 ml of 1.2 *M* hydrochloric acid saturated with ethyl acetate to the separatory funnel, and shake for 2 min. Discard the aqueous phase. After a few min, transfer a 1-ml aliquot of the organic phase to a polyethylene tube, and measure the gamma activity by means of a scintillation counter. Determine the amount of gold by reference to a calibration curve prepared with the standards. Check the radiochemical purity by measuring the gamma spectrum and the decay curve.

RESULTS AND DISCUSSION

Ion exchange–photometric method

The calibration curve in the photometric determination of gold was linear up to at least 0.6 p.p.m.; the error was about 0.02 p.p.m. in this range. No interference resulted from the presence of 100 p.p.m. each of copper, iron and mercury; 10 p.p.m. of silver; and 5 p.p.m. each of antimony, bismuth, cadmium, platinum and zinc. Even less than 0.5 p.p.m. of palladium, which is adsorbed strongly on the resin, interfered with the determination of gold. In this experiment, however, no palladium was found in all the samples. The copper remaining in the resin with gold was usually less than 1 mg.

Synthetic solutions prepared from electrolytic copper (containing 0.013 p.p.m.

* Gold does not adsorb on cotton wool.

TABLE II

SEPARATION AND DETERMINATION OF GOLD IN SYNTHETIC SAMPLES BY ION EXCHANGE–PHOTOMETRIC METHOD

Ion-exchange method	*Present*			*Au found*[a] *(μg)*	*^{198}Au recovered*[b] *(%)*
	Cu (g)	*Ag (mg)*	*Au (μg)*		
Batch	—	—	2.0	2.0	96
	1	—	5.0	4.7	97
	5	—	2.0	1.8	95
	1	3	110	103	100
Column	—	—	2.0	2.0	95
	1	—	2.0	1.8	98
	1	—	5.0	4.9	97
	5	—	2.0	2.0	97
	1	3	110	114	100

[a] By photometry.
[b] By radioactivity measurement (error, less than ±1%).

TABLE III

DETERMINATION OF GOLD IN CRUDE COPPER BY ION EXCHANGE(COLUMN OPERATION)–PHOTOMETRIC METHOD

Sample	*Sample taken (g)*	*Au found (p.p.m.)*	
		Proposed method	*Other methods*
Crude copper I	0.5 ~ 1.6	100, 102, 102 (av.) 101	95[a] 95[b] 101[c] (Ag 2762 p.p.m.)
Crude copper II	0.8 ~ 1.6	66, 64, 60 (av.) 63	69[a] 63[b] 66[c] (Ag 2269 p.p.m.)
Crude copper III	0.8 ~ 1.6	22, 22, 22 (av.) 22	23[a] 24[b] 22[c] (Ag 75 p.p.m.)

[a] Dry assay.
[b] Solvent extraction–photometric method[9].
[c] Amalgamation–photometric method[10].

of gold and 6 p.p.m. of silver), gold-198 and silver nitrate solutions, were analysed according to the ion exchange–photometric method just given. The results shown in Table II indicate that gold in copper can be determined by this method within an accuracy of 5–10% over the range 0.4 to 110 p.p.m. The blank value through the entire procedure was 0.0 μg of gold. The batch operation was found to be more difficult because of pulverisation of resin during stirring and strong adsorption of copper on filter paper; therefore, only the column operation was used for the analysis of crude copper. As shown in Table III, the results obtained by this method were in

good agreement with those obtained by other methods of analysis. The time required for a determination was 2–3 h.

Ion exchange–neutron activation method

The ion exchange(column operation)–neutron activation method was applied to the determination of gold in electrolytic copper. The analytical results and calibration curves are shown in Table IV and Fig. 2 respectively. About 0.013 p.p.m. of gold was found in electrolytic copper, in good agreement with the result obtained by the amalgamation–photometric method[10].

TABLE IV

DETERMINATION OF GOLD IN ELECTROLYTIC COPPER BY ION EXCHANGE(COLUMN OPERATION)–NEUTRON ACTIVATION METHOD

Experiment no.	*Sample taken (g)*	*Au found*[a] *(p.p.m.)*
	2	0.011[b]
1	4	0.010[b]
	2[c]	0.013
2	2	0.013
	4	0.013

[a] Analytical value obtained by amalgamation–photometric method[10]: 0.013 p.p.m.
[b] Less reliable value.
[c] 0.030 μg of gold was added before ion-exchange separation.

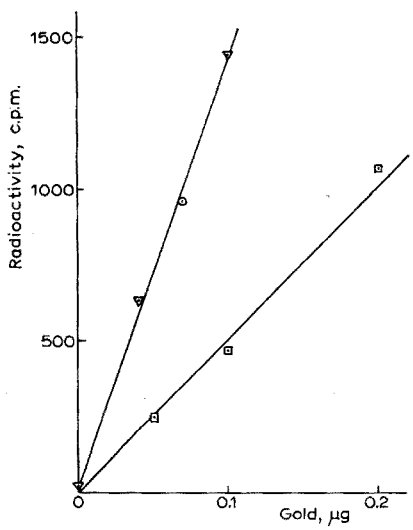

Fig. 2. Calibration curves in activation analysis. ⊡ ⊙ standard sample prepared by adsorbing gold on resin from 1 *M* hydrochloric acid; ▽ standard sample prepared by adsorbing gold on resin from a mixture of standard gold solution and gold-free copper solution (containing 4 g of copper) purified by ionexchange.

Since the loss of gold during the radiochemical separation was less than 5%, no correction was required actually. The gamma spectra (photopeak at 0.412 MeV) and decay curves (half-life, 2.7 days) showed that the radiochemical purity of the separated gold-198 was satisfactory.

The proposed method has two advantages. First, the radiochemical separation is very easy because of the weak radioactivity of the irradiated resin sample. A large amount of copper-64 is produced when the copper sample is irradiated directly[11,12]. Secondly, the self-shielding effect can be neglected. On the other hand, there is some possibility of contamination from reagents, vessels, etc. during the preliminary separation step. In this experiment, however, a blank value through the entire procedure corresponded to less than 0.0003 p.p.m. of gold in copper.

SUMMARY

The anion exchange of gold from chloride–nitrate medium is described and applied to the determination of 0.01 to 100 p.p.m. of gold in copper metal as a separation step before photometry or activation analysis.

RÉSUMÉ

Les auteurs proposent l'utilisation d'une résine (échangeur d'anions) pour la séparation de traces d'or dans le cuivre (0.01 à 100 p.p.m. d'or), avant de procéder à une analyse photométrique ou par activation.

ZUSAMMENFASSUNG

Die Abtrennung von Gold mit einem Anionenaustauscher aus einem Chlorid–Nitrat-Medium wird beschrieben und für die Bestimmung von 0.01 bis 100 p.p.m. Au in metallischem Kupfer angewandt. Das Verfahren eignet sich als vorhergehender Schritt für die Photometrie oder Aktivierungsanalyse.

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THE SPECIFICITY OF THE COLOUR REACTION BETWEEN AMMONIA AND 3-PHENYL-2-THIOHYDANTOIN

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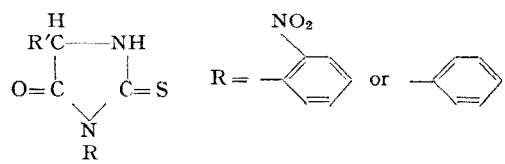
With the advent of automatic amino acid analyzers capable of handling the analysis of all constituents in a mixture, specific colour reactions permitting the determination of only one constituent, while valuable in qualitative analysis, find at best restricted use. However, in view of the precision and accuracy attainable in such reactions, it was considered profitable to examine carefully the specificity of a colour reaction reported to occur between 3-aryl-2-thiohydantoin (I, R = $C_6H_4NO_2$ or C_6H_5) and ammonia[1,2] and to establish conditions suitable for the determination



3-Phenyl-2-thiohydantoin (R = C_6H_5, R' = H: (PTH-glycine; I)

of 3-phenyl- (or 3-*o*-nitrophenyl-) 2-thiohydantoin. The relevance of this colour reaction for the determination of glycine will be obvious, since this amino acid like most others could be quantitatively converted to 3-phenyl-2-thiohydantoin (PTH-glycine)[3]. Relatively few methods are available for the colorimetric determination of glycine: the *o*-phthaldialdehyde reaction is unspecific, and the other micromethod based on the degradation of glycine to formaldehyde which is then distilled and determined colorimetrically with chromotropic acid is tedious, although it is both specific and yields generally reliable results[4].

EXPERIMENTAL

Special organic preparations

3-*o*-Nitrophenyl-2-thiohydantoin and the samples of 3-phenyl-2-thiohydantoins were those used in earlier investigations[1,5] with the following exceptions. *Δ*-PTH-threonine (R' = CH_3CH), PTH-threonine (R' = CH_3CHOH), and PTH-serine

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($R' = CH_2OH$) were made as described by LEVY[6]. The preparation of PTH-threonine[5,7] with m.p. 194° could not be reproduced; the samples used[6] had m.p. 208–212°. PTH-serine in a polymeric state[5] was also used in some experiments. 3-Acetyl-2-thiohydantoin (m.p. 174–176°) and 2-thiohydantoin (m.p. 225°) were prepared as described by NICOLET AND JOHNSON[8]. 3-Phenyl-hydantoin[9] (m.p. 154–158°) was prepared by reaction of glycine with phenylisocyanate.

Phenylthiocarbamyl (PTC) glycine ($C_6H_5NHCSNHCH_2COOH$) was prepared by reacting phenylisothiocyanate with glycine in aqueous pyridine (pH 8–9); after the completion of the reaction and removal of pyridine the solution was adjusted to pH 3–4. The separated solid was recrystallized from ethanol (m.p. 142–144°). PTC-glycine stored for several years at room temperature was found to be quantitatively transformed to PTH-glycine (m.p. 245–248°). The sample of hexaglycine used was from the Fischer collection and was made available by the kind courtesy of the late H.O.L. FISCHER.

Reagents and apparatus

3-Phenyl-2-thiohydantoin was dissolved in 40 parts by volume of warm 95% ethanol and diluted to 100 parts with water, to give a final concentration of the substance of 100–200 μg per ml. Solutions were prepared fresh.

Reagent A. 10 $\pm$ 0.1 *N* ammonium hydroxide was prepared by appropriate dilution of ammonia liquor and discarded when the concentration fell below 9.9 *N*.

Reagent B. A fresh 5% (w/v) solution of sodium nitroprusside.

Absorbances were recorded with a Klett-Summerson Colorimeter, with filter 540 or 490. Absorption spectra were recorded with a Beckman DU Spectrophotometer, and the infrared spectra with a Perkin-Elmer Infracord with the samples supported in a nujol phase.

Development of colour and stability

To 1 ml of a solution of PTH-glycine (0.26 μmole) in 40% ethanol was added 4 ml of reagent A. On mixing, a purplish-red colour developed immediately. To another tube containing the same amount of sample and 4 ml of reagent A, 0.1 ml of reagent B was added 3 min after the addition of reagent A and mixed. Suitable blanks were included in the set. Absorbances were recorded at intervals; the stability of the colour formed is shown in Fig. 1. Colour development was practically complete on addition of reagent A alone, but the colour decayed quickly with a loss of 25% in 60 min. This colour loss was effectively suppressed when reagent B was added 3 min after the addition of reagent A, and amounted to only 1–2% in the first 60 min and 5% at the end of 120 min. In all subsequent experiments both reagents A and B were used as above and colour measurements were made 20 min after the addition of reagent A. While the addition of reagent B had a stabilizing effect when used after reagent A, its addition before reagent A led to diminished colour formation.

Colour development with buffers (pH 10–11), or nearly saturated sodium carbonate solution in particular, could be achieved, but the intensity of colour was never more than a tenth of that given by 10 *N* ammonia although good stability without the addition of nitroprusside was observed for about 1 h. On the other hand, the use of a strong alkali such as dilute sodium hydroxide solution led only to a faint transient colour.

The highest colour intensities were obtained with ammonia liquor (28%). However, for the sake of reproducibility 10 *N* ammonia was chosen. The reagent was found satisfactory, with careful storage, for a week or longer. The variation in intensity of colour with ammonia concentration is shown in Fig. 2.

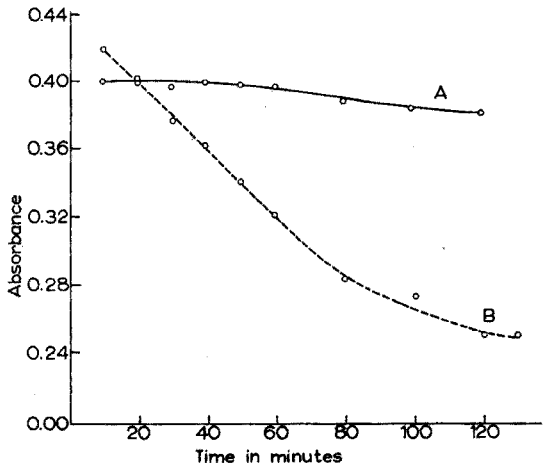


Fig. 1. Stability of colour formed by 0.26 μmole PTH-glycine, with reagent A (A — 540 filter total volume 5 ml) or with reagents A and B (B — 490 filter, total volume 5.1 ml).

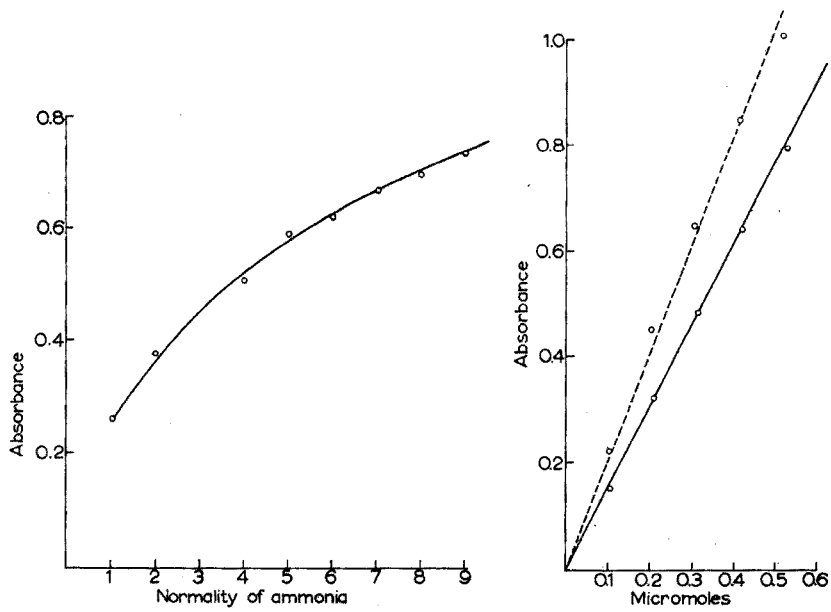


Fig. 2. Relation of intensity of colour formed to strength of reagent A. 0.52 μmole of PTH-glycine in 1 ml of 40% ethanol, with 4 ml of reagent A and 0.1 ml of reagent B.

Fig. 3. Calibration curves for 3-phenyl-2-thiohydantoin (——) and 3-*o*-nitrophenyl-2-thiohydantoin (---).

In an attempt to discover the reason for the instability of the colour, samples of PTH-glycine treated with reagent A were allowed to stand until all the colour had disappeared and then, after removal of excess ammonia, chromatographed on paper with 4 solvent systems. The dried sheets on staining with ninhydrin revealed one main spot which had the same R_F value as reference glycine.

Calibration curves

PTH-glycine (0.1–0.6 μmoles) was treated with reagent A and reagent B as described earlier and the colour measured after 20 min with a 490 filter against a water blank. A blank correction for reagent B was made after measuring the absorbance of 0.1 ml of reagent B mixed with 1 ml of 40% ethanol and 4 ml of reagent A against a water blank, within 10 min of mixing. The colour given by 3-*o*-nitrophenyl-2-thiohydantoin was also recorded for the same range of concentrations. The calibration curves are shown in Fig. 3. Beer's law was obeyed over the range of concentrations tested. At the 0.26 μmole level, measurements made with PTH-glycine indicated a precision close to 2%.

Conversion of microquantities of glycine to PTH-glycine[3]

The amino acid (0.5 to 5 μmole or an equivalent amount of peptide hydrolyzate) in 250 μl of a triethylamineacetate buffer (pH 10.1) was reacted with 250 μl of an acetone solution containing 0.15 to 1.5 μl of phenylisothiocyanate to form the phenylthiocarbamate which was subsequently cyclized, after removal of buffer salt and excess of reagent, in a solution containing 100 μl of water, and 200 μl of acetic acid saturated with hydrogen chloride. All solvent was removed *in vacuo* and the colour reaction carried out in the same test tube after dissolution of the sample in 1 (or more) ml of 40% ethanol, or in another test tube after transfer of an aliquot. Based on the colour formed in the reaction with ammonia, recoveries of 95.8 ± 2% were obtained.

Determination of the glycine content of hexaglycine

Samples of hexaglycine were hydrolyzed with 6 *N* hydrochloric acid (10 mg/ml) for 24 h at 108° in a sealed tube, dried and dissolved in the necessary volume of water. Aliquots were removed for the analysis of free glycine. Analysis for amino groups by the method of ROSEN[10] gave a value of 96.2 ± 3% of the theoretical amount of glycine expected. Other aliquots were converted to 3-phenyl-2-thiohydantoin and analyzed by the ammonia colour reaction. The actual yield was 96.4% of theory but, when corrected by the 95.8 ± 2% conversion factor, the yield amounted to 100.5% of theory, indicating a 100% weight purity of the peptide sample.

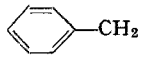
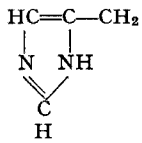
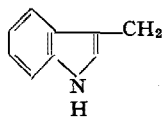
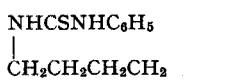
Specificity of the colour reaction

The observations on a variety of 2-thiohydantoins containing different substituents at the 5- and 3-positions are summarized in Table I. Neither 3-phenylhydantoin nor phenylthiocarbamyl glycine gave the colour reaction.

Of all the PTHs of the protein amino acids, derivatives of serine and threonine constitute a source of interference in the above method for PTH-glycine. The degree of interference is indicated in Table II. Of the derivatives tested, Δ-PTH-threonine interfered only slightly whereas PTH-threonine was the most active. PTH-serine[7]

TABLE I

SPECIFICITY OF COLOUR REACTION OF 2-THIOHYDANTOINS (I) WITH AMMONIA[a]

Substituent on carbon 5 R'	*Substituent on N at 3 position R*	*Colour formation*
	C_6H_5	None
	C_6H_5	None
	C_6H_5	None
	C_6H_5	None
H_2NOCCH_2	C_6H_5	None
$HOOCCH_2$	C_6H_5	None
$HOOCCH_2CH_2$	C_6H_5	None
$H_2NOCCH_2CH_2$	C_6H_5	None
	C_6H_5	None
	C_6H_5	None
$CH_3SCH_2CH_2$	C_6H_5	None
$CH_3CH(CH_3)CH_2$	C_6H_5	None
$CH_3CH(C_2H_5)$	C_6H_5	None
$CH_3CH(CH_3)$	C_6H_5	None
CH_3	C_6H_5	None
H	C_6H_5	+
H	$C_6H_4 \cdot NO_2$	+
H	$CH_3 \cdot CO$	±[b]
H	H	±[b]
CH_3CH	C_6H_5	None
$CH_3CH(OH)$	C_6H_5	+
$CH_2(OH)$	C_6H_5	±[c]
CH_2 (polymeric)	C_6H_5	+

[a] PTHs of proline and hydroxyproline, containing a fused ring system, do not give the reaction. Compounds other than those giving the test, were tested in amounts as high as 5 to 10 mg.

[b] No purplish-red colour with ammonia. With sodium nitroprusside, the colour formed fades in about 10 min.

[c] The ± signifies that while the compound does not react immediately, a slow colour formation occurs on the addition of sodium nitroprusside.

TABLE II

INTERFERENCE BY DERIVATIVES OF SERINE AND THREONINE

Compound	*Amount (μmole)*	*Amount colour formed as μmole PTH-glycine*	*% Colour yield on a molar basis*
PTH-serine (polymeric, m.p. 242–245°)	0.45	0.039	8.7
PTH-serine[a]	5.00	0.34	6.8
PTH-serine[b] (m.p. 174–178°)	23.80	0.65	2.7
PTH-threonine[a]	5.06	2.08	41.1
PTH-threonine[c] (m.p. 208–214°)	0.42	0.127	30.3
Δ-PTH-threonine[c] (m.p. 237–239°)	1.43	0.014	1.0

[a] Microsynthesized by the method of SJÖQUIST[3].
[b] No immediate reaction with ammonia, but colour after addition of reagent B. Colour measured at 20 min but the final colour on longer standing would be twice this value. On long standing (>1 h) with reagent A alone, colour slowly develops.
[c] Prepared by the method of LEVY[6].

was singular in forming no colour with reagent A only, but developing it after the addition of reagent B.

Isolation of product of reaction between PTH-glycine and ammonia

Ammonia vapor, from a slightly warmed bottle of ammonia liquor, was passed into a suspension of 1 g of PTH-glycine in 10–20 ml of 60% methanol or ethanol until all the sample had dissolved. The deeply coloured solution was taken to dryness at room temperature with the aid of a water pump and the process was repeated twice with the addition of a few ml of solvent. The sample was then triturated with ether (when it changed from a purplish-red to yellow-brown colour), filtered, washed with ether and dried in air. (Yield 85%. M.p. (d) 134–138°. Analysis: calculated for $C_9H_8ON_2S \cdot NH_3$, C 51.68, H 5.26, N 20.09; found, C 51.68, H 5.27, N 19.77%.) The

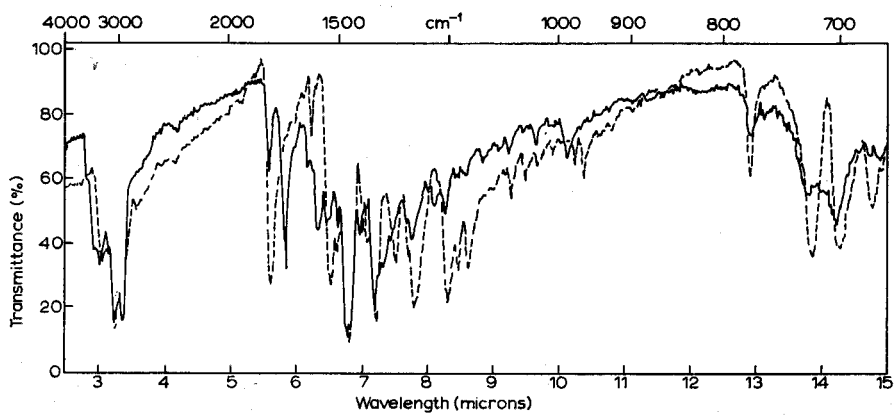


Fig. 4. Infrared spectra of 3-phenyl-2-thiohydantoin (– – – –) and of its ammonium salt (——). Nujol mull phase. Absorptions due to nujol below 700 cm^{-1}, at 720, 1370, 1460 and 2850–2900.

Rast method gave a molecular weight of 197 ± 7, while the isothermal distillation method with acetonitrile as the solvent, gave values in the range 180–200. The calculated molecular weight for the ammonium salt is 209.

The infrared spectra of this ammonium salt and that of PTH-glycine are shown in Fig. 4.

The ammonium salt was only sparingly soluble in ethanol and water at neutral pH values. Samples recrystallized from methanol-ether containing a few drops of ammonia liquor or from aqueous methanol or ethanol gave a product which was highly coloured like the original substance but contained no ammonia at all. (M.p. (d) 246–250°; reported[11] for 3-phenyl-2-thiohydantoin, 246–247°. Analysis: calculated for $C_9H_8ON_2S$, N 14.58; found, N 14.36%.) Keeping the samples in high vacuum for prolonged periods had the same effect. Also, the isolated ammonium salt on contact with dilute sodium hydroxide solution lost ammonia, which is evidence for the salt-like character of the isolated complex.

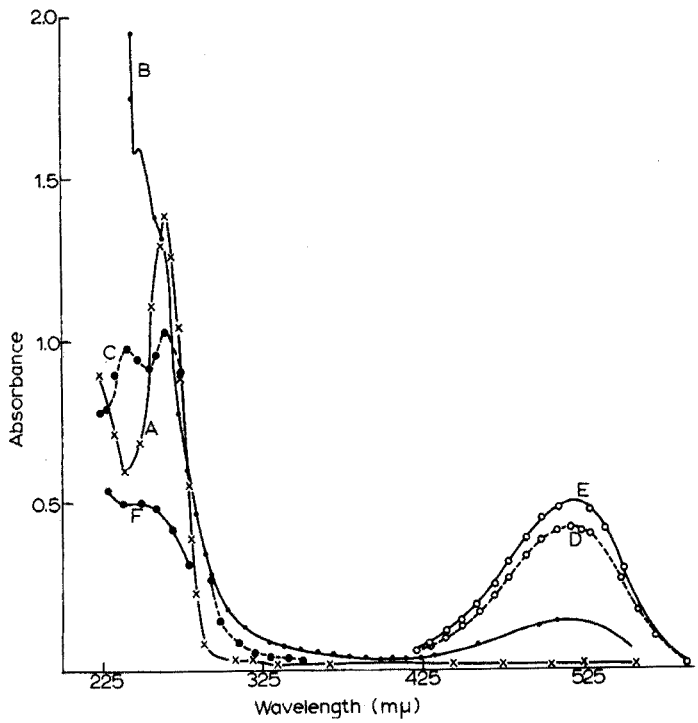


Fig. 5. Absorption spectra. (A) PTH-glycine in 95% ethanol, 0.096 μmole per ml. (B) NH_4^+ salt of PTH-glycine in 1% (w/v) ammonium solution. (C) NH_4^+ salt of PTH-glycine in 95% ethanol, 0.075 μmole per ml. (D) PTH-glycine, 0.26 μmole in 1 ml of 40% ethanol, with 4 ml of reagent A. (E) Same as D, with 0.1 ml of reagent B also present. (F) Absorption in the UV, as D.

Absorption spectra

PTH-glycine (52 μg) in 1 ml of 40% ethanol was treated with 4 ml of 10 *N* ammonia and the spectrum in the region 400–600 mμ was recorded, no correction being made for any loss of colour during the period of measurement (10–15 min). The spectrum for a similar sample to which 0.1 ml of reagent B was added 3 min after

addition of reagent A, was also recorded. The spectra of PTH-glycine and of its ammonium salt in 95% ethanol, of the ammonium salt in 1% (w/v) ammonia solution, and of the ammonium salt, from which ammonia had been removed in 95% ethanol were also recorded (Fig. 5).

Both the pigment formed with ammonia, and that formed in the presence of ammonia and sodium nitroprusside, by PTH-glycine had absorption peaks at 515–525 mμ. PTH-glycine in 95% ethanol showed the expected peak at 267 mμ (E_M = 14,500). The isolated ammonium salt in 95% ethanol showed peaks at 243 mμ (E_M = 13,050) and at 267 mμ (E_M = 13,750) and none in the visible region; with the solution in 1% (w/v) ammonia a peak at 250 mμ and a shoulder at 265 mμ were present. The spectrum of the ammonium salt freed of its ammonia, in high vacuum, showed only one absorption peak at 267 mμ.

The infrared spectrum of the ammonium salt differed from that of 3-phenyl-2-thiohydantoin in several important respects. The characteristic ring C=O absorption at 1770 cm^{-1} had all but disappeared and a new band around 1710 cm^{-1} appeared. The phenyl ring absorption was still present around 1600 cm^{-1}, while the bands in

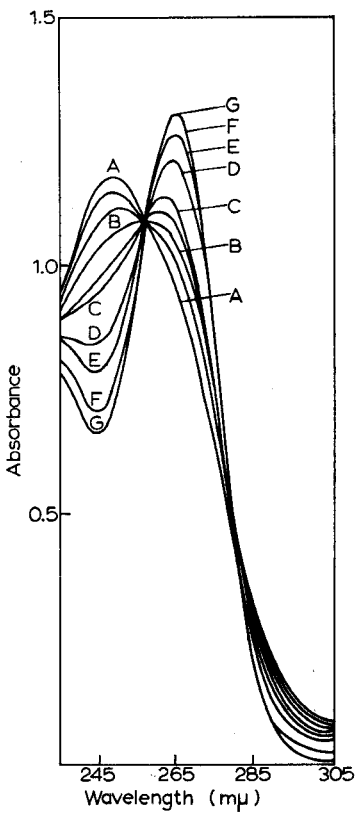


Fig. 6. Cyclization of 20 μg of PTC-glycine in 1.1 ml of 1.5 *N* HCl, at 37°. (A) PTC-glycine (o time), (G) PTH-glycine formed after completion of cyclization (630 min). Other curves (B), (C), (D), (E) and (F) represent intermediate stages of reaction at times 55, 96, 165, 240 and 450 min respectively.

the 940–1200, 1260–1420 and 1390–1570 cm^{-1} regions normally ascribed to the C=S and N—C=S groupings (thiocarbonyl group attached to one or more N atoms[5,12,13]) showed inappreciable intensity, though they were not entirely absent. Of the several new bands of both medium and weak intensity that appeared, some would correspond to NH_4^+, C=C, C=N and OH absorptions. The spectra are shown in Fig. 4.

DISCUSSION

The method of determination developed above for 3-phenyl- and 3-*o*-nitrophenyl-2-thiohydantoin appears quite satisfactory as a colorimetric method — with a precision close to 2%. This naturally suggested the possibility of determining glycine by conversion to PTH-glycine and applying the colour reaction. Free glycine was converted to PTH-glycine with a yield of 95.8 ± 2%, based on the colour yield of standard PTH-glycine. A slightly higher yield of 98%, has been reported in the literature[3] based on UV-absorption measurements, but these results might be slightly high because of absorbing impurities. While UV-absorption measurements in the 260–270 mμ region permit the determination of all 3-phenyl-2-thiohydantoins, determination of PTH-glycine in the presence of other PTH-amino acids is not possible without a prior separation by partition chromatography[14].

The application of a colour reaction for determination of glycine in a mixture of amino acids, as in a protein hydrolyzate, after conversion to the 3-phenyl-2-thiohydantoins would depend on the extreme specificity of the reaction. The observations on the specificity of the primary colour reaction between ammonia and thiohydantoins are therefore of particular interest. Since 3-phenyl-2-thiohydantoin answers the test while 3-phenylhydantoin does not, the presence of a thiocarbonyl group (>C=S) is essential. The nitrogen in the 3-position has to carry an aryl substituent, because neither 3-acetyl-2-thiohydantoin nor 2-thiohydantoin gives a colour with ammonia. Electronegative substituents on the 3-aryl moiety enhance the chromogenic property of the molecule — a 3-*o*-nitrophenyl group enhancing the colour yield by nearly 35% compared to the 3-phenyl substituent. The five-membered ring is essential, since phenylthiocarbamylglycine will react to the test only after ring closure. It should be noted also that substitution of the hydrogens on carbon 5 generally leads to a loss of chromogenicity. However, non-substitution of these hydrogens is not absolutely essential for the colour reaction with ammonia, as has been suggested earlier[15]. Thus PTH-threonine (I, R′ = CH_3CHOH) reacts to give about 30–40% of the colour which PTH-glycine yields. Conversion of the above compound (R′ = CH_3CHOH) to Δ-PTH-threonine (R′ = CH_3CH) leads to loss of the chromogen character. Further, PTH-serine (R′ = CH_2OH) is not a chromogen in the presence of 10 *N* ammonia, except on prolonged contact but is chromogenic in the presence of the added sodium nitroprusside. PTH-serine samples generally tend to polymerize, especially in the presence of ethanol following dehydration and all such samples are chromogenic, though only with an efficiency of about 10% compared to PTH-glycine. In this connection it may be noted that after microconversion of amino acids in a mixture to 3-phenyl-2-thiohydantoins, the determination of PTH-serine and PTH-threonine by UV spectrophotometry, subsequent to partition chromatography, is always unsatisfactory[3], perhaps owing to the dehydration and polymerization which readily occur with these two derivatives. In the application of the present method to glycine

in a mixture of amino acids after conversion to PTHs, the interference of derivatives of serine and threonine has to be taken into account. The possible elimination of this interference by destroying any serine and threonine present with alkaline periodate[16] remains to be explored.

The instability of the coloured complex formed by PTH-glycine with ammonia may be ascribed entirely to opening of the thiohydantoin ring by base catalysis, which is hindered somewhat by nitroprusside. The occurrence of glycine in the solution after the disappearance of the colour, confirms this view. That alkalies generally decompose phenylthiohydantoins to the parent amino acid at higher temperatures is well known[11]. It is worth noting that phenylthiocarbamylglycine, which is readily isolated in good yield compared to similar derivatives of the other amino acids in the acid form, reacts only sluggishly (630 min, Fig. 6) in acid solution (1.5 *N*, 37°) to form PTH-glycine; under these conditions sodium salts of other phenylthiocarbamylamino acids react to yield thiohydantoins within 30–60 min. Thus, difficult ring formation of 5-unsubstituted 3-phenyl-2-thiohydantoin in acid solutions is paralleled by extreme instability of the ring system in the presence of bases.

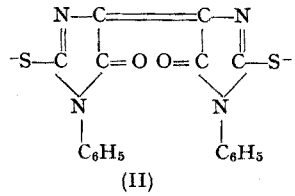
PTH-threonine yields glycine in yields as high as 67%, on treatment[17] with 0.1 *N* sodium hydroxide at 120° for 12 h. This hydrolytic reaction may proceed through initial elimination of the side chain at position 5 to yield as an intermediate 3-phenyl-2-thiohydantoin which then readily yields glycine in the presence of alkali. The high degree of interference (colour formation) by PTH-threonine on treatment with ammonia may arise similarly through formation of 3-phenyl-2-thiohydantoin in the presence of 10 *N* ammonia.

The absorption spectrum of the ammonium salt of 3-phenyl-2-thiohydantoin in 95% ethanol shows two peaks in the UV region (Fig. 5); the peak at 267 mμ can be assigned to the "dissociated" thiohydantoin form (I) and that at 242–243 mμ to the ammonium salt species (III, see later). The assignment of the 242–243 mμ peak to the ammonium salt species is confirmed by the fact that the ammonium salt (m.p. 134–138°) from which ammonia has been completely removed by recrystallization or high vacuum, yielding a product with the same m.p. (248–250°) as 3-phenyl-2-thiohydantoin, shows only one peak at 265–266 mμ (E_M = 14,800) which corresponds to that for 3-phenyl-2-thiohydantoin. However, in aqueous 1% ammonia the band at 243 mμ is shifted to around 249 mμ, while a hump at 265–267 mμ is also present, and the absorption in the visible region (515–525 mμ) begins to be prominent; this peak in the visible region has a molar absorptivity under the conditions of a determination of about 9,400. There is also one broad maximum in the UV region (240–255 mμ, E_M = 9,700) with no indication of any peak in the 267 mμ region.

The infrared spectrum of the isolated ammonium salt, which indicates loss of the ring thiocarbonyl and the ring carbonyl absorptions which are characteristic of 3-phenyl-2-thiohydantoin, and the presence of absorptions associated with the NH_4^+, C=C, C=N and OH groupings, along with the data on elementary analysis for the salt, would be consistent with the assignment of structure (III) to the pigment species.

SCHRAMM, SCHNEIDER AND ANDERER[2] were unsuccessful in isolating the pigment species and, based on analogy with the glyoxalin red of RUHEMANN AND STAPLETON[18], assigned structure (II) to the species. We consider structure (II) to be unsatisfactory for describing the coloured material, in view of the analyses of the ammonium salt which could be isolated quantitatively from the coloured solutions and the altera-

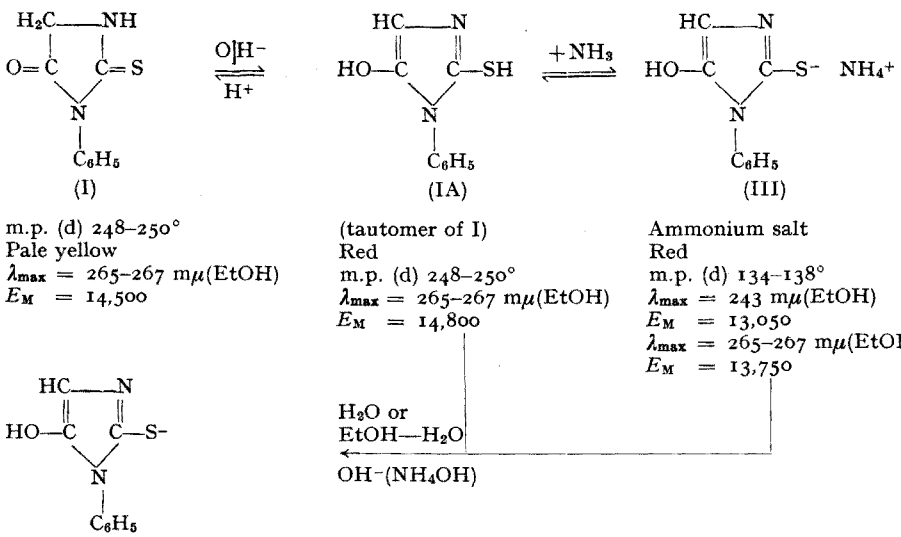
tions pertaining to the ring C=O absorption; also the C=O function in (II) would not be enolizable. Moreover, while bases catalyze (or participate in) salt and colour



(II)

formation with PTH-glycine, reaction conditions favouring oxidation are deleterious to colour stability, whereas reducing conditions have a stabilizing effect.

The observation that the isolated red pigment (III) has limited solubility in 95% ethanol or water may be due to a fairly covalent character of the salt linkage; however, the ammonia in the salt is completely removable by holding the sample in high vacuum for long periods or by attempting to recrystallize the material. Such samples without ammonia and with C, H and N contents, melting point, and UV spectra corresponding to those of 3-phenyl-2-thiohydantoin are still coloured red. This form is probably a tautomer (IA) of (I). (I), (IA) and (III) will in the presence of excess ammonia yield the characteristic purplish-red colour in solution, with a peak at 515–525 mμ. All these observations could be reconciled with the scheme outlined below:



(I) (IA) (III)

m.p. (d) 248–250°
Pale yellow
λ_{max} = 265–267 mμ(EtOH)
E_M = 14,500

(tautomer of I)
Red
m.p. (d) 248–250°
λ_{max} = 265–267 mμ(EtOH)
E_M = 14,800

Ammonium salt
Red
m.p. (d) 134–138°
λ_{max} = 243 mμ(EtOH)
E_M = 13,050
λ_{max} = 265–267 mμ(EtOH)
E_M = 13,750

H_2O or
EtOH—H_2O

$OH^-(NH_4OH)$

Fully dissociated ionic species
contributing to colour in solution
λ_{max} = 515–525 mμ (10 *N* NH_4OH)
E_M = 9,400
λ_{max} = 240–255 mμ (10 *N* NH_4OH)
E_M = 9,700

The validity of this scheme depends on the capability of (I) to isomerize to (IA) with the formation of a sulfhydryl function. This would be supported by the observation[19,20] that 3-*p*-tolyl-2-thiohydantoin can form a silver salt and that methylation of the thiohydantoin in alcoholic potassium hydroxide yields an S-methyl

compound which no longer forms a silver salt. Compounds such as DL-1-phenylacetyl-5-methyl-thiohydantoin[21] on treatment with alkali, are known to transform to structures containing new titratable groups.

This investigation was aided by a grant from the Rockefeller Foundation and by an Indian Institute of Science Scholarship to one of us (K.N.N.R.). The interest of Professor P. S. SARMA in this work is appreciated.

SUMMARY

The colour reaction between 3-phenyl-2-thiohydantoin and ammonia is studied quantitatively. Determinations of 0.1–0.6 μmoles of 3-phenyl-2-thiohydantoin are possible with a precision close to 2%. In analyses of amino acid mixtures for glycine after conversion to 3-phenyl-2-thiohydantoin, only derivatives ot serine and threonine interfere to a slight extent. The specificity of the primary colour reaction with ammonia and the structural requirements for it are discussed; a structure for the pigment species is proposed.

RÉSUMÉ

Les auteurs ont examiné la réaction colorée entre la 3-phényl-2-thiohydantoïne et l'ammoniaque, ainsi que sa spécificité. Il est possible de doser 0.1 à 0.6 μmoles de 3-phényl-2-thiohydantoïne avec une précision de 2%. Lors d'analyses de glycine, dans des mélanges d'acides aminés, seuls des dérivés de la sérine et de la thréonine gênent.

ZUSAMMENFASSUNG

Die Farbreaktion zwischen 3-Phenyl-2-thiohydantoin und Ammoniak wird quantitativ untersucht. Die Bestimmung von 0.1–0.6 μmol 3-Phenyl-2-thiohydantoin ist mit einer Reproduzierbarkeit von nahezu 2% möglich. Bei Analysen von Aminosäuremischungen auf Glycin nach Umwandlung in 3-Phenyl-2-thiohydantoin stören in geringem Ausmass nur die Derivate von Serin und Threonin. Die Spezifizität der primären Farbreaktion mit Ammoniak wird diskutiert. Eine Struktur für den Farbstoff wird vorgeschlagen.

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SUBSTITUTED HYDRAZONES AS TRIDENTATE CHELATING AGENTS

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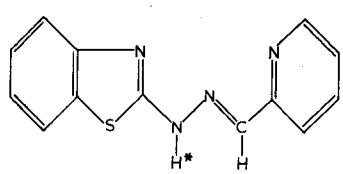
Compounds capable of behaving as tridentate chelating agents, considering the donor atoms involved and the steric restrictions imposed, can be selective in their reactions with metal ions; such ligands show significant possibilities as analytical reagents. The preparation of a number of substituted hydrazones (*e.g.* pyridine-2-aldehyde-2-pyridylhydrazone; PAPHY) and their use as tridentate donors have been described[1-3]; bis-complexes of these compounds with divalent metals gave cationic species containing two acidic hydrogens which could dissociate to yield new uncharged complexes. The formation and acid dissociation constants of the cationic complexes of PAPHY have been recently determined[4]; the possibilities of PAPHY as an analytical reagent had been previously outlined[5]. Several similar ligands currently under investigation in this laboratory show some differences in their reactions with metals; pyridine-2-aldehyde-2-quinolylhydrazone and pyridine-2-aldehyde-2-benzothiazolylhydrazone (I), for example, do not form bis-cationic complexes containing an acidic hydrogen but undergo chelation reactions involving proton replacement. This paper records the results of an investigation into the composition and stability of chelates with these ligands.



I

EXPERIMENTAL

Reagents

The hydrazones were prepared by heating together in ethanol equimolar proportions of 2-hydrazinobenzothiazole or 2-hydrazinoquinoline with pyridine-2-aldehyde. The pyridine-2-aldehyde-2-benzothiazolylhydrazone [1-(2′-benzothiazyl)-3-(2″-pyridyl)-1,2-diaza-2-propene] is pale green (m.p. 238°). The proton (marked H* in I) attached to the nitrogen of the hydrazine residue is lost during chelation; HT and T will be used to denote thiazole ligand and deprotonated reagent respectively throughout the paper. Analytical results: calculated for $C_{13}H_{10}N_4S$, C 61.4, H 4.0,

N 22.0, S 12.6%; found, C 60.9, H 3.8, N 22.4, S 13.2%.

The pyridine-2-aldehyde-2-quinolylhydrazone [1-(2'-quinolyl-3-(2'-pyridyl)-1,2-diaza-2-propene] is pale yellow (m.p. 197°). HQ and Q will be used to denote quinoline ligand and deprotonated reagent respectively. Analytical results: calculated for $C_{15}H_{12}N_4$, C 72.6, H 4.9, N 22.6%; found, C 72.3, H 5.1, N 22.5%.

Reactions

The reactivity of the ligands with metal ions was observed by performing qualitative tests in which small amounts of metal ion solution (10 mg/ml) were added to 1% solutions of the reagents in alcohol. Similar color reactions were observed for all metals tested with the exception of iron. Iron(III) reacted with the benzothiazyl compound to give a blood red solution but iron(II) showed no reaction; the quinolyl compound, however, gave a blood red colour with iron(II), but no reaction was noted with iron(III). Both reagents developed colors varying from brown to red with Co^{2+}, Ni^{2+}, Cu^{2+}, and Pd^{2+} and less intense orange-yellow colors with Mn^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}. The remaining 23 metal ions tested, showed no color development when added to the test solutions. The composition and stability of the metal chelates were determined by spectrophotometric and potentiometric measurements.

Spectrophotometric study

A Bausch and Lomb Spectronic 505 recording spectrophotometer was used to measure the spectra of the chelates. The mole ratio method[6] was employed to

TABLE I

ABSORPTION PEAKS OF METAL COMPLEXES

Metal	*Ligand*	λ_{max}	*Mole ratio ligand : metal*	$E_{\lambda_{max}}$
Cu^{2+}	HQ	497	1:1	$2.28 \cdot 10^4$
	HQ	474	2:1 (pH 11)	$4.58 \cdot 10^4$
	HT	462	1:1	$1.57 \cdot 10^4$
	HT	436	2:1 (pH 11)	$3.46 \cdot 10^4$
Ni^{2+}	HQ	483	2:1	$1.96 \cdot 10^4$
	HT	447	2:1	$1.66 \cdot 10^4$
Co^{2+}	HQ	510	2:1	$1.80 \cdot 10^4$
	HT	473	2:1	$1.66 \cdot 10^4$
Pd^{2+}	HQ	563	1:1	$1.15 \cdot 10^4$
	HT	427	1:1	$9.41 \cdot 10^3$
Fe^{2+}	HQ	461	2:1	$1.02 \cdot 10^4$
Fe^{3+}	HT	425	2:1	$4.14 \cdot 10^3$

determine the composition of the chelates; a series of mixtures was prepared containing a fixed amount of metal ion but with increasing ratios of reagent to metal (or *vice versa*). The final solutions were 4:1 ethanol–water and a solution pH of 5–6 was maintained, except where otherwise specified, with dilute sodium hydroxide. Results obtained are given in Table I.

Metal ions, such as Ni^{2+}, Cu^{2+} and Pd^{2+}, which form square planar complexes, could react with the planar ligands to produce stable mono-chelates; however, under

the conditions employed, stable 1:1 chelates were detected only with copper and palladium. The high extinction coefficients of the chelates indicate their possible use for colorimetric determinations.

The compositions of the Mn^{2+} and Pb^{2+} chelates were not determined either potentiometrically or spectrophotometrically because of metal hydroxide formation. The composition of the zinc and cadmium chelates were not determined spectrophotometrically; neither Job plots nor mole ratio studies gave consistent results.

Potentiometric titrations

The chelates of HQ and HT were studied by performing a series of potentiometric titrations using standard 0.1 *N* sodium hydroxide as titrant. The limited solubility of the reagents in water necessitated the use of 1:1 dioxane–water in which the neutral chelates were soluble. The titration apparatus was essentially the same as that described by HAINES *et al.*[7].

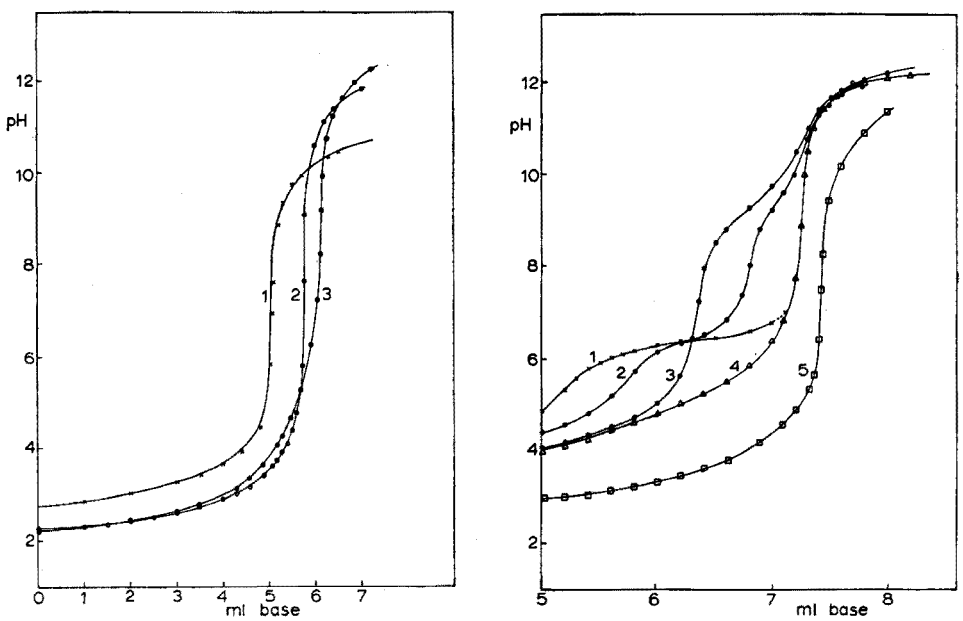


Fig. 1. HT titrations: 1, 50.0 ml of $1.309 \cdot 10^{-2}$ *N* perchloric acid, and $3.276 \cdot 10^{-4}$ moles HT; 2, perchloric acid, $5.205 \cdot 10^{-5}$ moles cobalt, and $10.41 \cdot 10^{-5}$ moles HT; 3, perchloric acid, $4.640 \cdot 10^{-5}$ moles zinc, and $9.280 \cdot 10^{-5}$ moles HT.

Fig. 2. HT titrations: 1, 50.0 ml of $1.134 \cdot 10^{-2}$ *N* perchloric acid, and $10.16 \cdot 10^{-5}$ moles copper; 2, perchloric acid, copper, and $5.080 \cdot 10^{-5}$ moles HT; 3, perchloric acid, copper, and $10.16 \cdot 10^{-5}$ moles HT; 4, perchloric acid, copper, and $20.32 \cdot 10^{-5}$ moles HT; 5, 50.0 ml of $1.738 \cdot 10^{-2}$ *N* perchloric acid, $6.300 \cdot 10^{-5}$ moles palladium, and $6.302 \cdot 10^{-5}$ moles reagent.

The acid dissociation constants were determined by titrating a mixture of the ligand and perchloric acid with standard base (curve 1, Fig. 1). The acid dissociation

constants of the cationic acid and ligand are defined by the equations

$$K_1 = \frac{[HR]\,[H^+]}{[H_2R^+]} \qquad K_2 = \frac{[H^+]\,[R^-]}{[HR]}$$

where $[H_2R^+]$, $[HR]$, $[R^-]$, and $[H^+]$ represent the concentrations of the protonated reagent, unprotonated ligand, reagent anion, and the hydrogen ion respectively. The pK_1 and pK_2 values so determined for HT were 3.00 and 10.81 respectively; for HQ these values were 5.26 and 12.91.

BJERRUM plots[8] of $\bar{n}$ *vs.* pR showed the existence of two species in which k_1 was much larger than k_2 for copper only; formation constants were read, therefore, directly from the curve. The formation constants of the palladium complexes were also determined by this method. For the remaining ions the differences in k_1 and k_2 were not large enough to give distinct breaks in the formation curves and accurate values of k_1 and k_2 could not be read directly from the curve at $\bar{n}$ equal to 0.5 and 1.5 respectively. The constants for these metal ions (Ni^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}) were calculated by plotting $\bar{n}/(1-\bar{n})R$ against $(\bar{n}-2)R/(1-\bar{n})$. The slope and intercept of this linear plot are equal to k_1k_2 and $-k_1$ respectively[9]. Results are listed in Table II. Hydrolysis of iron(III) prevented calculation of the formation constants for HT.

TABLE II

FORMATION CONSTANTS IN 1:1 DIOXANE–WATER AT 25°

Metal	*Ligand*	*log* k_1	*log* k_2	*log* k_1k_2
Cu^{2+}	HQ	11.60	8.65	19.52
	HT	10.48	8.59	18.94
Pd^{2+}	HQ	10.57		
	HT	10.33		
Ni^{2+}	HQ	10.46	9.39	19.85
	HT	9.86	9.37	19.23
Fe^{2+}	HQ	10.44	10.18	20.62
Zn^{2+}	HQ	10.24	9.26	19.50
	HT	9.33	9.05	18.38
Cd^{2+}	HQ	9.52	8.49	18.01
	HT	8.76	8.11	16.87

The ligands reacted with Fe^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+} to form octahedral complexes. The titration curves for these metal ions show only one break which corresponds to the neutralization of perchloric acid plus two equivalents of hydrogen per mole of metal added and is indicative of the formation of the bis-chelates.

A typical titration is shown in Fig. 1, curve 3. The interpretations of the remaining metal ion reactions were not the same and are discussed below.

Cobalt. No constants are reported for the formation of the cobalt complexes. Spectrophotometric measurements showed that a bis-chelate was formed, but potentiometric titrations indicated that only one proton was titrated per mole of metal during the reaction (curve 2, Fig. 1). The formation of a diamagnetic 2:1 chelate (ligand to metal ratio) was shown using the magnetic titration technique[10]; thus the metal was oxidized to the cobalt(III) state during reaction. The potentio-

metric and spectral results are explained on the basis that one equivalent of hydrogen ion is reduced by Co^{2+} resulting in the formation of a diamagnetic bis-chelate; only one equivalent of proton is therefore titrated per mole of metal ion added.

Palladium. The titration curves for palladium showed only one break which corresponds to the neutralization of added perchloric acid plus one equivalent of hydrogen ion titrated per mole of metal ion added (Fig. 2, curve 5). Titrations in which the metal:reagent ratio was 1:2 or greater also showed that only a mono-complex was formed; similar results were obtained spectrophotometrically.

Copper. Stable mono- and bis-chelates were formed in the reaction of copper with the ligands; however, the complexes present in solution were governed by the mole ratio of metal to ligand present.

The titration curves of copper with varying amounts of HT are shown in Fig. 2. One break was detected in the titration of the 2:1 (ligand:metal) mixture (curve 4); the equivalents of sodium hydroxide added corresponded to the neutralization of perchloric acid plus two equivalents of hydrogen liberated per mole of metal added and indicated the formation of a bis-chelate.

Two breaks were found in the titration of the 1:1 mixture (Fig. 2, curve 3).

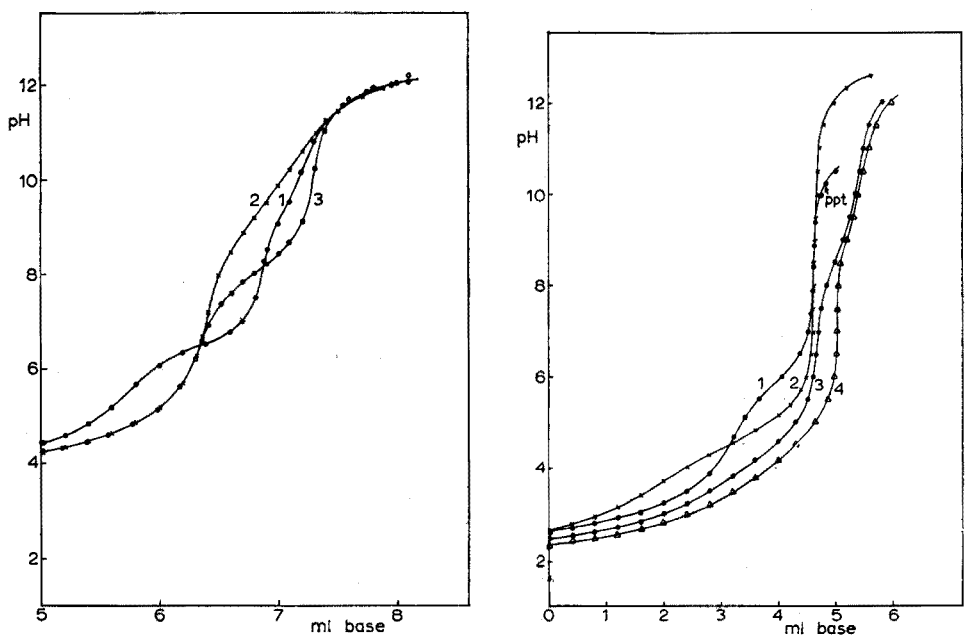


Fig. 3. HQ titrations: 1, 50.0 ml of $1.134 \cdot 10^{-2}$ *N* perchloric acid, $10.16 \cdot 10^{-5}$ moles copper, and $5.080 \cdot 10^{-5}$ moles HQ; 2, perchloric acid, copper, and $10.16 \cdot 10^{-5}$ moles reagent; 3, perchloric acid, copper, and $20.32 \cdot 10^{-5}$ moles reagent.

Fig. 4. PAPHY titrations: 1, 50.0 ml of $1.182 \cdot 10^{-2}$ *N* perchloric acid and $1.97 \cdot 10^{-4}$ moles reagent in water (reagent precipitated at pH 10.4); 2, as above, in 50% dioxane–water; 3, perchloric acid, $4.87 \cdot 10^{-5}$ moles cadmium, and $9.743 \cdot 10^{-5}$ moles reagent; 4, perchloric acid, $5.080 \cdot 10^{-5}$ moles copper, and $5.080 \cdot 10^{-5}$ moles reagent.

Copper itself was titrated at pH 6 (Fig. 2, curve 1) and the possibility that CuT_2 was first formed, followed by the titration of copper ion was excluded. The titration curve is explained on the basis that a mono-chelate is first formed and the second equivalence point, requiring the same amount of base to form the mono-chelate, is due to the dissociation of a water molecule attached to the coordination sphere of the complexed copper ion. Spectral measurements previously had shown the formation of this mono-chelate (Table I).

Three breaks were detected in the titration of the 1:2 mixture (Fig. 2, curve 2). The proton liberated during the formation of the mono-complex was first titrated, followed by the titration of excess copper ion to give the soluble hydroxide. The third break corresponded to the titration of the proton from the water molecule of the mono-complex.

A similar series of titrations was carried out with HQ and Cu^{2+} (Fig. 3). The reactions involved were the same as those for copper and HT when the ratio of ligand to metal was 1:1 or less (Fig. 3, curves 1 and 2). Two breaks, however, were observed in the titration of the 2:1 mixture (Fig. 3, curve 3). The first equivalence point, corresponding to the amount of perchloric acid present plus one equivalent of hydrogen ion per mole of metal added, showed the formation of the mono-complex. The second break requiring one further equivalent of base indicated the formation of the bis-chelate. Thus in the titration of HQ and copper both MQ and MQ_2 are stable entities when the ratio of reagent to metal is 2:1 or greater. Spectral measurements also confirmed the existence of the mono- and bis-chelates (Table I).

Metal–PAPHY titrations

LIONS *et al.*[1–4], with similar tridentate molecules, have identified two types of complexes with divalent metal ions; cationic complexes of the general formula $M(HR)_2X_2$ (where HR represents the uncharged ligand, and X is a monovalent anion), and the uncharged complex MR_2. Since such cationic complexes were not detected in this study, one of their ligands, pyridine-2-aldehyde-2-pyridylhydrazone (PAPHY), was prepared and potentiometric titrations were done in dioxane–water to discern the effects of this mixed solvent. Typical curves are shown in Fig. 4.

The formation of a protonated ligand is clearly indicated from the titration curves (Fig. 4, curves 1 and 2); the reagent is a stronger base, of course, in water than in the mixed solvent. The lowering of the pH along the titration curve in the titration of cadmium (curve 3) shows that chelate formation has occurred and two end-points are evident in the titration. The first end-point, corresponding to the number of equivalents of perchloric acid present shows that no protons are liberated in the initial chelation reaction and that the species formed must be either MHR^{2+} or $M(HR)_2^{2+}$; the second end-point, corresponding to the titration of 2 moles of hydrogen ion per mole of metal, shows the formation of the neutral bis-chelate, MR_2. Similar results were obtained with Mn^{2+}, Ni^{2+} and Zn^{2+} titrations.

The results obtained in dioxane–water for PAPHY are in agreement with those reported for water alone for the above metal ions[4]; the evidence for both cationic and neutral bis-chelates is indisputable. However, in the titration of Cu^{2+} and Pd^{2+} where mono-chelates are formed and with iron (bis-complex) the protons released in the chelation reaction are titrated together with the added perchloric acid. The absence of the intermediate perchloric acid equivalence point clearly

indicates that cationic complexes [$M(HR)^+$, $M(HR)_2^{2+}$] are not stable entities with these metal ions in dioxane–water solutions.

The titration curve for the 1:1 mixture of copper and PAPHY showed the liberation of 2 hydrogen ions in widely separated steps (Fig. 4, curve 4). As with the mono-complexes of Cu^{2+} with HQ and HT, the first of these protons was released during chelation and the second dissociated from a water molecule in the fourth coordination position of the copper. The titration curve of a 2:1 (PAPHY:Cu) mixture was identical to the equimolar titration, indicating that only a mono-complex of PAPHY and copper was formed.

CONCLUSIONS

Two types of complexes are formed between divalent metal ions and PAPHY, cationic bis-chelates and neutral bis-complexes. The failure therefore, to find cationic complexes with HQ and HT must be due to their differences in acidity. The trigonal nature of the ligand imposes steric restrictions on the metal acceptor ions, favouring planar or octahedral structures. Stable square planar 1:1 chelates were formed with copper and palladium; the former also formed a stable bis-chelate. The remaining metal ions, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Fe^{2+}, formed octahedral complexes only.

One of us (M.L.H.) gratefully acknowledges the financial aid given by the National Research Council in the form of a studentship.

SUMMARY

The compositions and stabilities of the metal chelates of two new tridentate ligands, pyridine-2-aldehyde-2-quinolylhydrazone and pyridine-2-aldehyde-2-thiazolylhydrazone are reported. Cationic bis-chelates, which have been reported with similar ligands, are not formed during chelation with these ligands. The copper reaction, in which both mono- and bis-chelates are formed, is discussed in detail.

RÉSUMÉ

Les auteurs ont examiné la composition et la stabilité de chélates métalliques obtenus avec deux nouveaux réactifs: pyridine-2-aldéhyde-2-quinolylhydrazone et pyridine-2-aldéhyde-2-thiazolylhydrazone. Alors que les bis-chélates cationiques ont été rapportés pour des réactifs identiques, ils n'ont pu être obtenus pendant la chélation avec les réactifs étudiés. Ils ont étudié en détail les réactions de ces deux agents de chélation avec le cuivre: formation de mono- et de bis-chélates.

ZUSAMMENFASSUNG

Über die Zusammensetzungen und Stabilitäten von Metallchelaten von 2 neuen dreizähnigen Liganden, Pyridin-2-aldehyd-2-chinolylhydrazon und Pyridin-2-aldehyd-2-thiazolylhydrazon wird berichtet. Kationische Bis-Chelate mit ähnlichen Liganden, über die bereits berichtet wurde, werden mit diesen Liganden nicht ge-

bildet. Die Kupferreaktion, bei der sowohl Mono- als auch Bis-chelate gebildet werden, wird im einzelnen diskutiert.

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A NEW METHOD OF RADIOACTIVATION ANALYSIS BASED ON THE QUANTITATIVE ISOTOPE DILUTION PRINCIPLE

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Quantitative isotope dilution analysis as proposed in 1958 by SUZUKI[1], is an interesting new analytical method for trace elements, and has been put in practice generally[2–5]. Later, RUZICKA AND STARY reported a similar method, and studied a number of applications[6].

Though radioactivation analysis is excellent for determination of trace elements, the non-destructive method has limitations: when a chemical separation is necessary, it must be done carefully to avoid radiochemical contamination; on the final stage the correction of yield should be made by an ordinary chemical method, *e.g.* a gravimetric method, etc.; accordingly, this method cannot avoid the introduction of many complicated processes. In ordinary activation analysis, moreover, the final determination is made by comparing the radioactivity with that of a standard sample, which makes it impossible to avoid errors from the fluctuation of neutron flux and the self-shielding of the sample. Especially in radioactivation analysis by charged particles, errors from these sources become greater than in the thermal neutron method, so that an accurate determination by comparison with a standard sample may be regarded as impossible at present.

In the quantitative isotope dilution method presented previously, after the addition of a known amount of radioactive isotope to the sample, the element in question is determined by measuring the change of specific activity. When this method is applied to an irradiated sample, the separation procedure becomes simple, the correction for yield is not necessarily taken into consideration, and the errors from flux fluctuation and self-shielding can be avoided completely. In the present work, the application of this principle to the determination of traces of copper and silver in pure metals such as tin and zinc, was studied.

PRINCIPLE OF THE METHOD

Method I. Extraction of a known amount

If the amount of the element in question in the sample is M_x and its radioactivity induced by irradiation is A, the specific activity is A/M_x. Accordingly, when

a portion of the element, m ($m < M_x$), is removed and its radioactivity, a, is measured, M_x is determined only by the measurement of radioactivity.

$$a = m\frac{A}{M_x} \tag{1}$$

In this method the strength of reagent used in the extraction of m must of course be known.

Method II. Variation in the amount of carrier

The specific activity of a radioactive isotope induced by irradiation is $A/M_x = S_1$, and, when a suitable amount of carrier, M, is added to a portion of this sample, the specific activity changes to $A/(M_x + M) = S_2$. If to each of these mixtures is added an equal amount of reagent, sufficient to react with an amount m which is less than the total amount of element, then after m has been removed from each mixture (the radioactivities being a and a', respectively), M_x is determined by the following equation:

$$\frac{S_1}{S_2} - 1 = \frac{M}{M_x} \text{ and } \frac{a}{a'} - 1 = \frac{M}{M_x} \tag{2}$$

This equation does not involve the absolute value of m, which therefore need not be known. When an equal amount is removed from each mixture, and the radioactivity is measured, M_x can be determined. If a chemical separation process is necessary and a determination by this method is carried out on the final solution, the yield cannot be corrected.

However, M_x can be determined easily without regard to the yield by the following procedure: the sample solution is first divided into two parts, to one of which M is added; then, after the minimum number of processes necessary to remove pure m from each part, a and a' are measured, and M_x is obtained from eqn. (2). Although this method looks complicated at first sight, since it requires double separation processes on a sample, in practice the separation process can be made more simply than in the ordinary case, because there is no need to recover the element completely.

*Method III. The comparison method**

In ordinary radioactivation analysis, after the standard, M_s, and the sample have been irradiated together, a greater amount of carrier, M, compared with M_x and M_s, is added to each mixture, and the same portion m ($m < M$) is removed from each to measure their radioactivities a_s and a. Where A_s is gross radioactivity of the standard caused by irradiation, M_x is determined by the following equation.

$$\frac{A_s}{M_s+M} = \frac{A_s}{M} = \frac{a_s}{m} \qquad \frac{A}{M_x+M} = \frac{A}{M} = \frac{a}{m} \tag{3}$$

$$\therefore\ M_x = \frac{a}{a_s} M_s \tag{4}$$

* Recently, RUZICKA AND STARY have reported the same principle[7], and determined zinc and copper in germanium[8].

Though this method is convenient, it is impossible to avoid completely the errors that arise in ordinary radioactivation analysis because the determination is carried out by comparison of the radioactivities of the standard and the sample.

Among these 3 methods the first and second method do not require comparison with standard samples, and therefore it is possible to avoid the various inevitable errors of ordinary radioactivation analysis. Furthermore, in the second method, it is also unnecessary to consider the chemical yield of the separation. If a chemical separation is not required, or if the chemical yield is almost 100%, the first method can be used, but in most cases the second method is the most reliable.

Because the objective nuclide may be generated occasionally by a secondary nuclear reaction of the base material or of a main component in a sample, serious error can be caused in ordinary radioactivation analysis; on the other hand, in Methods I and II, any secondary nuclear reaction does not produce errors but increases the specific activity, which is all the more favorable. However, the more minute the content of the element in question becomes, the more care is needed in the procedure of removing a portion; in this respect, Method III is superior.

EXPERIMENTAL

Reagents

^{64}Cu and ^{198}Au solutions used as tracers were prepared as follows. The pure metals (99.99% copper and 99.999% gold) were irradiated in JRR-1, and dissolved in nitric acid and aqua regia, respectively. ^{110m}Ag was imported from U.S.A.

Dithizone solution was prepared by dissolving dithizone of G.R. grade in carbon tetrachloride.

Diluted sulfuric acid and other solutions of reagents were prepared from special-grade materials and then shaken with dithizone solution to remove metallic impurities. Nitric acid and hydrochloric acid were purified by distillation of the special-grade acids. Organic solvents of C.P. grade were distilled twice. Redistilled water was used.

Apparatus

Radioactivity was measured mainly by means of a low background counter and a G-M counter (Japan Radiation and Medical Electronics Lab. Inc.); where necessary, a well-type single-channel γ-ray spectrometer (Hitachi Ltd.) was used.

Reaction of copper with dithizone

The determination of silver by quantitative isotope dilution analysis[2] and the extractive separation of silver[9] have already been investigated. The extraction of copper with dithizone is said to be quantitative at weak acidities[10], but to confirm this copper was extracted by shaking for 3 min with excess of dithizone solution in carbon tetrachloride; more than 99% of the copper was removed by a single extraction at acidities up to 0.5 N sulfuric acid.

The practical application of the method outlined above is possible if the reagent and the element required react with each other in a fixed ratio. To confirm this point, 10 ml of 0.1 N sulfuric acid containing 4–12 μg of copper and 1 ml of dithizone solution in carbon tetrachloride containing enough dithizone to react with 4 μg of copper were shaken for 40 sec. The radioactivity of the organic phase obtained is

shown in Table I, from which it is obvious that the reaction ratio is constant.

The relation between the carrier amount and the specific activity was examined. If the radioactivity of m taken out of one portion of ^{64}Cu is a, and, after the addition of the known amount of carrier, the radioactivity of m taken out of the mixed sample

TABLE I

REACTION RATIO BETWEEN COPPER AND DITHIZONE

Copper taken (μg)	*Activity of the extract (counts/min)*
4	1526 ± 18[a]
6	1602 ± 19
8	1554 ± 18
10	1542 ± 18
12	1583 ± 19

[a] Statistical error of counting rate.

TABLE II

REACTION RATIO BETWEEN COPPER AND DITHIZONE IN THE PRESENCE OF OTHER METALS

Copper taken (μg)	*Other metal present (μg)*	*Activity of the extract (counts/min)*
10	—	1521 ± 19
10	Zn^{2+} 11	1594 ± 19
10	Zn^{2+} 110	1549 ± 19
10	Sn^{2+} 10	1473 ± 19
10	Sn^{2+} 100	1534 ± 19
40	—	130.8 ± 5.2
40	Sn^{2+} 100	131.0 ± 5.2
40	Sn^{4+} 100	123.8 ± 5.0
40	Sn^{2+} 1000	130.0 ± 5.3
40	Sn^{4+} 1000	124.1 ± 5.0

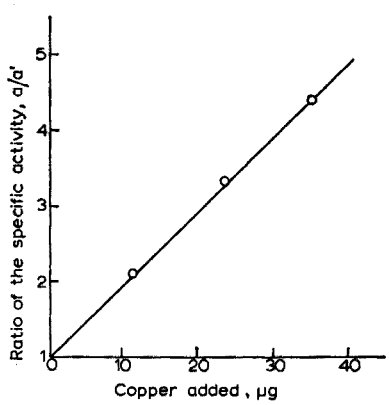

Fig. 1. Relation between the specific activity and the amounts of copper added.

is a', the relation between the added carrier amount and the ratio a/a' should be linear. It was shown in Fig. 1 that a plot of a/a' against the amount of copper added was a straight line passing through the origin at least over the range 10–40 μg of added copper; thus the reaction ratio of dithizone and copper is constant. The specific activity of a radioactive isotope can be determined easily by this method, as indicated by eqn. (2).

The coexistence of metals whose formation constants are smaller than that of copper dithizonate is presumed not to cause interference. As shown in Table II, the reaction with copper is not appreciably inhibited in 0.1 N sulfuric acid by the presence of zinc and tin up to about 1 mg, provided that the counting error is taken into consideration.

Extraction of copper and silver in the presence of a large amount of tin

In the determination of traces of heavy metals in the presence of a large amount of tin, it is better to carry out a prior separation of most of the tin. However, tin is liable to hydrolyse at lower acidities and the extraction of copper and silver with dithizone solution in carbon tetrachloride in 2 N sulfuric acid media was therefore studied. To 100–200 ml of 2 N sulfuric acid containing 0.5–1.6 g of tin*, a tracer was

TABLE III

EXTRACTION OF COPPER AND SILVER IN THE PRESENCE OF A LARGE AMOUNT OF TIN

Tracer	*Carrier (μg)*	*Tin (g)*	*Volume of the aqueous soln. (ml)*	*Tracer extracted[a] (%)*
^{64}Cu	13	0.5	100	84.0
^{64}Cu	8	0.6	100	87.0
^{110m}Ag	14	1.4	200	98.2
^{110m}Ag	14	1.6	200	91.7
^{110m}Ag	14	1.3	200	100.0

[a] Shaking time of 30 sec.

TABLE IV

EXTRACTION SEPARATION OF COPPER AND GOLD

Aqueous solution[a]	*Complexing agent[b]*	*Tracer back-extracted (%)*	
		^{64}Cu[c]	^{198}Au[c]
pH 5	0.1 M EDTA	0.1	0.3
NH_4OH(1:100)	0.1 M KCN	0.0	90.3
pH 2.7	0.1 M KCN	0.0	98.5

[a] 10 ml.
[b] 1 ml.
[c] Containing about 10 μg of carrier.

* Metallic tin was dissolved in sulfuric acid containing a small amount of nitric acid, followed by dilution to a prescribed acidity.

added, and the extraction with 5 ml of $2 \cdot 10^{-3}$ *M* dithizone solution in carbon tetrachloride was carried out. The results are shown in Table III; almost 90% of copper was extracted by a single extraction, and, practically 100% of copper was extracted by 3 successive extractions.

Separation of copper and gold

Although dithizone reacts with many heavy metals, those which react at comparatively high acidity and which required consideration in the present work were copper, silver and gold[10]. Of these, silver can be masked by chloride ion[9], so that only the separation of copper from gold needed investigation. Copper, silver or gold was extracted from an aqueous solution into 10^{-3} *M* dithizone solution. After separation, the organic layer was stripped with an aqueous solution containing various complexing agents. Results are shown in Table IV; gold and copper could be separated from each other by shaking with an aqueous solution of pH 2.7 containing potassium cyanide.

Analytical procedure

About 1 g of commercial tin and zinc was irradiated for one week in the nuclear reactor JRR-1*, or for 20 min in JRR-2*.

After being washed with water, the irradiated sample was dissolved in a little concentrated nitric acid and sulfuric acid, and the solution was then concentrated to a small volume by heating; this was followed by dilution to 1–2 *N* sulfuric acid. An excess of 10^{-4} *M* dithizone solution in carbon tetrachloride was added, and the mixture was shaken for 30 sec; the extraction was repeated. The organic phase (A) was shaken with 0.1 *N* hydrochloric acid, and silver was back-extracted. The aqueous solution was adjusted to pH 4–5 (solution B) and silver was extracted with dithizone solution in carbon tetrachloride. The organic phase thus obtained was evaporated gently with 12 *N* sulfuric acid containing a small amount of nitric acid; the acidic solution was diluted to 0.5–1 *N* sulfuric acid.

TABLE V

DETERMINATION OF COPPER IN METALLIC TIN AND ZINC

(11.7 μg of carrier added)

Sample no.	*Grade*	*Sample weight (g)*	*Activity of extract*[a] *(counts/min)*		*Copper found (μg)*	*Copper in sample*[b] *(μg)*	*Copper content (p.p.m.)*
			a	*a'*			
Sn–1	CP	0.9171	569.8	200.2	6.3[c]	11.2	12.2
Sn–2	CP	0.8210	1014.2	282.1	4.5[d]	9.9	12.1
Zn–1	GR	1.0399	2712.8	1428.8	13.0[c]	24.6	23.7
Zn–2	GR	1.0044	808.2	318.5	7.6[d]	17.6	17.5
Zn–3	99.999%	1.0195	4390.2	957.2	3.3[c]	5.2	5.1
Zn–4	99.999%	0.9037	6248.4	897.4	2.0[d]	3.6	4.0

[a] Corrected for background.
[b] Calibrated for the blank value (1.4 μg).
[c] In 25 ml of the sample solution (50 ml).
[d] In 20 ml of the sample solution (50 ml).

* The thermal neutron fluxes of JRR-1 and JRR-2 are about $3 \cdot 10^{11}$ n/cm²/sec and $4 \cdot 10^{13}$ n/cm²/sec, respectively.

The organic phase (A) obtained in the first extraction, from which silver had been removed, was shaken with dilute ammonia to remove most of the excess of dithizone, and then shaken with 10 ml of a buffer solution of pH 2.7 containing 1 ml of 0.1 *M* potassium cyanide to remove gold. This organic solution was evaporated gently with 12 *N* sulfuric acid containing a small amount of nitric acid, followed by dilution to 0.5–1 *N* (solution C).

To an aliquot of the mixed solutions B and C, dithizone solution in carbon tetrachloride in an amount less than that required to react completely with the element in question was added, followed by shaking. The radioactivity of the organic phase, *a*, was measured. To another aliquot a suitable amount of carrier, *M*, was added. The mixture was shaken with the same amount of dithizone solution in carbon

TABLE VI

DETERMINATION OF SILVER IN METALLIC TIN AND ZINC

Sample no.	*Grade*	*Sample weight (g)*	*Carrier added (μg)*	*Activity of extract*[a] *(counts/min)*		*Silver found (μg)*	*Silver in sample (μg)*	*Silver content (p.p.m.)*
				a	*a'*			
Sn–11	CP	1.3404	1.4	16.2	10.0	2.3[b]	5.8	4.3
Sn–12	CP	0.9171	3.0	0.6	0.2	1.5[c]	3.0	3.3
Zn–11	GR	1.0893	1.4	7.1	5.1	3.6[b]	9.0	8.3
Zn–12	GR	1.1778	1.4	6.8	4.2	2.3[d]	11.5	9.8
Zn–13	99.999%	1.0195	3.0	2.6	0.4	0.5[c]	1.0	1.0
Zn–14	99.999%	1.0583	3.0	1.3	0.3	0.9[c]	1.8	1.7

[a] Counting time for 100 ~ 180 min.
[b] In 10 ml of the sample solution (25 ml).
[c] In 12.5 ml of the sample solution (25 ml).
[d] In 5 ml of the sample solution (25 ml).

TABLE VII

COMPARISON OF THE RADIOACTIVITIES OF ^{64}Cu EXTRACTED FROM THE SAMPLE SOLUTION BY TWO SUCCESSIVE EXTRACTIONS

Expt. no.	*Radioactivity (counts/min)*	*Mean activity (counts/min)*
A_1	269.0 ± 7.4[a]	
		264.8 ± 4.2[b]
A_2	260.5 ± 7.3	
B_1	373.4 ± 8.7	
		372.4 ± 1.0
B_2	371.3 ± 8.7	
C_1	569.8 ± 10.7	
		569.0 ± 0.8
C_2	568.1 ± 10.7	
D_1	595.0 ± 11.0	
		599.1 ± 4.1
D_2	603.1 ± 11.0	
E_1	751.4 ± 12.3	
		765.4 ± 14.0
E_2	779.3 ± 12.7	

[a] Counting error.
[b] Relative error.

tetrachloride as before, followed by the measurement of the radioactivity a'. However, correction for yield was impossible by the above treatment, so that it was necessary to recover completely the element in question during the course of chemical separation. Therefore, for practical purposes, the sample solution in 1–2 N sulfuric acid obtained after irradiation was divided into two parts; to one part a known amount of carrier was added, and then the chemical treatment was carried out on both parts. Finally, the same amounts were extracted from the final solutions, and the radioactivities a and a' were measured.

RESULTS

The analytical results are shown in Tables V and VI. In the case of silver, measurements were carried out by the low background counter because the radioactivity based on ^{110m}Ag was weak. To ascertain the reproducibility of the final procedure, after an arbitrary amount of m had been extracted twice successively from the same aqueous final solution, the radioactivities of the two extracts were compared with one another. These values were in fairly good agreement, as shown in Table VII. This meant also that no contamination of heavy metals with large formation constants had occurred during the process. The half-life of the radioactivity of the extract agreed with that for ^{64}Cu. It is clear from the results that the amounts of copper and silver in 99.999% zinc are undoubtedly smaller than those in G.R. zinc.

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SUMMARY

The simple quantitative isotope dilution method proposed, allows the errors due to flux fluctuation and self-shielding, normal to ordinary radioactivation analysis, to be avoided. Three different methods are discussed. The most satisfactory method from a theoretical viewpoint involves division of the irradiated sample into two parts; in one part, an amount m is extracted and the radioactivity a is measured. An amount of carrier M is added to the other part, an amount m is again extracted and its radioactivity a' is measured. The unknown amount M_x is then calculated from $(a/a') - 1 = M/M_x$. A method for the determination of traces of copper and silver in metallic tin and zinc based on this principle is described.

RÉSUMÉ

Une nouvelle méthode d'analyse par radioactivation est proposée; elle est basée sur le principe de la dilution isotopique quantitative. Trois procédés ont été examinés; le plus satisfaisant du point de vue théorique consiste à partager l'échantillon irradié en deux parties. Sur l'une d'elle une quantité m est extraite et sa radioactivité a est mesurée. Sur l'autre partie, on ajoute un traceur M; on extrait à nouveau une quantité m et on mesure sa radioactivité a'. La quantité inconnue

M_x peut être ensuite calculée d'après la formule: $(a/a')-1 = M/M_x$. Les auteurs décrivent une méthode pour le dosage de traces de cuivre et d'argent dans l'étain et le zinc.

ZUSAMMENFASSUNG

Um Fehler zu vermeiden, wie sie unvermeidbar bei der gewöhnlichen Neutronenaktivierungsanalyse auftreten, wird die einfache quantitative Isotopenverdünnungsmethode angewandt. Von den drei diskutierten unterschiedlichen Methoden wird bei der vom theoretischen Gesichtspunkt zufriedenstellendsten die bestrahlte Probe in zwei Teile geteilt. In einem Teil wird die Menge m extrahiert und die Aktivität a bestimmt. Zum anderen Teil wird die Menge M eines Trägers hinzugegeben und wieder die Menge m extrahiert und die Aktivität a' gemessen. Die unbekannte Menge M_x wird dann aus der Gleichung $(a/a')-1 = M/M_x$ berechnet. Eine auf dieser Arbeitsweise beruhende Methode zur Bestimmung von Spuren Kupfer und Silber in metallischem Zinn und Zink wird beschrieben.

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DETERMINATION OF THE SULFHYDRYL AND DISULFIDE GROUPS IN PROTEINS BY METHYLATION, TREATMENT WITH RANEY NICKEL AND INFRARED SPECTROMETRY

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The role played by the sulfur-containing amino acids and cysteine in various metabolic processes is the basis of their great biological significance; numerous studies have been made on their determination. There are many different methods for the determination of sulfhydryl groups in protein hydrolysates and non-hydrolysed proteins; the more important depend on titration with silver and mercury ions[1–4], or on reaction with *p*-chloromercuribenzoate[5–8] or N-ethylmaleinimide[9]. Several photometric methods have also been suggested: for example, the reduction of cystine and cysteine to hydrogen sulfide, which is determined by Caro's reaction with dimethyl-*p*-phenylenediamine[10]; the changes in the colour of thiofluorescein in the presence of substances containing sulfhydryl groups[11]; and methods based on induced iodine-azide reaction[12].

The application of these methods to the same sample often leads to different results. For example, COLE, STEIN AND MOORE[13] examined the SH-group content of human haemoglobin by 4 known methods: amperometric titration with silver ions, reaction with N-ethylmaleinimide, oxidation, and in the form of S-carboxymethylcysteine. The results obtained for the number of SH-groups varied from 2 to 8. Since the highest number, 8, was obtained by amperometric titration with silver ions, it seems likely that this was due to a retention of silver ions by the sample studied. The differences in the results are undoubtedly caused also by the varying freedom of access of the different reagents used to the various SH-groups.

The development, therefore, of an accurate method for the determination of SH-groups, which could be applied both to hydrolysates and to non-hydrolysed proteins, would allow conclusions to be drawn about the number of total and accessible SH-groups, and about changes in the number of the accessible groups which occur as a result of certain processes involving the proteins. A method for the determination of the SH- and SS-groups in proteins is suggested in this paper. It is based on the methylation of the SH-groups of proteins and their hydrolysates with dimethyl sulfate, followed by treatment with Raney nickel. During this process, the sulfur from the SCH_3 group, produced by the methylation of the SH-groups, is removed by Raney nickel and methane is separated. The amount of methane is then determined by infrared spectrometry, as suggested previously[14]. Clearly, if the protein or its hydrolysate is subjected to reduction before methylation, the method may be applied also for the determination of disulfide groups of proteins.

EXPERIMENTAL

Reagents and apparatus

An alloy, containing 50% nickel and 50% aluminium (E. Merck, Germany) was used to obtain Raney nickel suspension. The catalyst was prepared as described previously (with prior boiling in water)[14].

The insulin was a crystalline product (Organon, Oss, Holland); it was stored at 2–3° in a vessel containing a saturated solution of calcium chloride (under these conditions the relative humidity is 40%). The moisture content of the insulin, stored in this manner, was 9.9%.

Trypsin was obtained by activation of crystalline trypsinogen in the presence of calcium salts[15]. The product was twice recrystallized and then dialyzed and lyophilized.

The egg albumin was a thrice recrystallized product (Serva, Entwicklungslabor, Germany).

Homogenates were obtained from the organs of a freshly killed Syrian golden hamster, after soaking them in physiological saline solution at 0° and then homogenizing them mechanically.

The reaction of the Raney nickel with methylated cysteine, protein hydrolysates or proteins was carried out in the apparatus described previously[14].

The methane determination was carried out with an infrared spectrophotometer (UR-10 Zeiss, 1958), which registered absorption curves in coordinate wave number/percentage transmittance. The 100-mm gas cell had potassium bromide windows.

Methylation of cysteine

Initially, diazomethane was tested as a methylation reagent to convert the SH-groups of cysteine to SCH_3, but the reaction was not quantitative. Dimethyl sulfate in an alkaline medium was therefore tried, and suitable conditions were easily found. Under the conditions selected, methylation of the other groups (NH_2 and COOH) did not occur to an appreciable extent; the product remained soluble in an alkaline medium and thus reacted smoothly further with the catalyst. The partial methylation of the other groups, of course, would not hinder the cysteine determination, because there would be no reaction with the Raney nickel.

Procedure (Method A). A sample, containing 0.2–10 mg cysteine was dissolved in 2–3 ml of distilled water in a small flask with a ground-glass joint, and *N* sodium hydroxide solution was added dropwise to give pH 9–10 (a small crystal of phenolphthalein was added). Dimethyl sulfate (1–5 mg) was added and the mixture was stirred for 15 min at room temperature. If the solution became colourless owing to the neutralization of hydroxide, further drops of alkali were added. Dimethyl sulfate interfered with the spectrophotometric determination, and the excess had to be completely eliminated after the methylation. For this purpose, a 5–10-fold excess (with respect to dimethyl sulfate) of salicylic acid was added to the alkaline solution of the methylated cysteine. If the solution became colourless, several drops of the hydroxide solution were added. The solution was stirred again for 15 min, and then made slightly acidic with several drops of 6 *N* hydrochloric acid. A 20-cm glass tube on a ground-glass joint was then placed on the flask and the solution was boiled for 20–25 min.

Under these conditions, the methanol produced in the saponification of the esters, escaped from the reaction medium through the short tube. After cooling, the tube was carefully flushed, the solution was made alkaline again and then treated with Raney nickel; the apparatus and the method described previously[14] were used for the desulfurization.

Methylation of reduced cystine

Reduction of cystine to cysteine (Method B). Zinc in an acid or alkaline medium was initially tried for the reduction of cystine. Poor results were obtained, possibly because of hydrogenation of cystine to hydrogen sulfide or because of formation of zinc sulfide on the fresh metal surfaces produced during dissolution. The second explanation seems more probable; if the hydrogenation resulted in hydrogen sulfide, then in alkaline medium, methylmercaptan should have been formed in the subsequent methylation, but was not detected. No better results were obtained by modifications of the method and it was therefore necessary to find milder conditions for the reduction of the disulfide group. Good results were obtained by reduction with tin in hydrochloric acid[16].

A sample of 0.2–10 mg of cystine was dissolved in 1 ml of 1 : 1 hydrochloric acid and 10–100 mg of sheet tin was added. About 15 min were required for the reduction; during this interval the small flask with the solution was immersed twice for 2–3 min in a water bath at 60–70°. The acid used for hydrolysis was employed in the reduction of the cystine for its determination in protein hydrolysates. The completion of the reduction was determined by a discoloration of the solution lasting for 15–20 min.

Methylation of the reduced cystine (Method C). Immediately after the reduction of cystine in the acid medium, half the necessary amount of dimethyl sulfate was added to the mixture. Any undissolved tin sheet was eliminated by transferring the solution to another flask and, after careful rinsing with distilled water, a small phenolphthalein crystal was added. The solution was then adjusted to pH 9–10, and the remainder of the dimethyl sulfate was added. The mixture was stirred for 10–15 min, and then dimethyl sulfate was eliminated and the determination completed as for cysteine.

Methylation of the accessible SH-groups of proteins (Method D)

A sample of protein (10–50 mg, according to the sulfur content) was dissolved in 1–2 ml of water in a 10–15-ml flask and the pH was adjusted with *N* sodium hydroxide to 9–9.5, with a small crystal of phenolphthalein as indicator; 5–20 mg of dimethyl sulfate were then added and the solution was stirred for 40–45 min. An excess of salicylic acid was then added and stirred for another 10–15 min. The protein was then hydrolyzed by refluxing with 6 *N* hydrochloric acid at 135°. The subsequent procedure was the same as in the determination of cysteine.

Methylation of the total SH-groups of proteins (Method E)

The same procedure was followed as for methylation of accessible SH-groups, but after the hydrolysis, half of the necessary amount of dimethyl sulfate was added to the solution, which was carefully neutralized with a concentrated solution of sodium hydroxide under cooling. Then the remainder of the dimethyl sulfate was added,

and the solution was stirred for 10–15 min. After elimination of the dimethyl sulfate, the subsequent procedure was the same as for cysteine.

Methylation of accessible and total SH-groups in organ homogenates

Organs of a freshly killed animal (Syrian golden hamster) were soaked for 1 h in physiological saline solution at 0° and then mechanically homogenized. A sample (200–300 mg) of the homogenate was adjusted to pH 9–9.5 (phenolphthalein) and stirred with 20–30 mg of dimethyl sulfate for 1 h. The elimination of the excess with salicylic acid and the remaining procedure were as in the determination of cysteine. The accessible SH-groups in the homogenate were determined in this way. When the determination of the total SH-groups was required, the procedure outlined in the paragraph above was applied.

The hydrolysis of proteins and homogenates was carried out by the following 3 methods:

(*a*) with 6 *N* hydrochloric acid in a sealed ampoule at 105° for 16 h;

(*b*) with 6 *N* hydrochloric acid in an open flask under reflux at 135° for 20 h;

(*c*) with formic and hydrochloric acid in order to prevent decomposition of the cystine[17].

The moisture content in the samples examined was determined in all cases in parallel samples.

In all cases the final part of the determination followed the procedure described in a previous paper on the determination of methionine by infrared spectrometry of the methane formed with Raney nickel[14]. It should not be forgotten that in this treatment with Raney nickel, methane is formed not only from methylated cysteine but also from the methionine present in the proteins. Each determination of SH-groups or SS-groups in proteins or their hydrolysates should therefore be accompanied by a parallel determination of methionine; the amount of cysteine or the number of SH-groups is then determined by subtracting the amount of methane obtained from the methionine alone.

The results from numerous determinations of cysteine and cystine made it possible to construct a calibration graph. The experimental plot of the extinction *vs.* mg of cysteine or cystine/2 was parallel to the plot obtained previously for methionine. This indicates that the reduction and methylation proceed quantitatively. Moreover, it is possible to prepare the methionine experimental plot only and then read the cysteine and cystine from it merely by multiplying them by the corresponding factor, which is 0.81192 for cysteine and 0.80523 for cystine/2.

RESULTS AND DISCUSSION

After the preliminary investigations on cysteine and cystine, the method was applied to proteins (Table I).

The residues of cysteine in organ homogenates of the Syrian golden hamster, available for methylation, were also determined (Table II).

As is seen from Table I, the method allows the determination of the amount of cysteine, and the number of free SH-groups in proteins. The relative error does not exceed 3%. Considering the ready reactivity of the SH-groups in proteins (oxidation, bonding with metal ions, etc.), as well as the difficulties of purifying proteins, this

TABLE I

CYSTEINE CONTENT IN PROTEINS

Protein	*Residues of cysteine*	*Method used*	*Literature data*
Insulin (after reduction)	5.99	B, E	6[18]
Trypsin (after reduction)	5.80	B, E	6[19]
Egg albumin	2.94 (accessible)	D	3[20,21]
	4.86 (total)	E	5[22]

TABLE II

CONTENT OF SH-GROUPS, ACCESSIBLE FOR METHYLATION, CALCULATED AS CYSTEINE RESIDUES

Organ	*% Cysteine/100 g dry tissue*
Heart	0.78
Kidney	0.44
Liver	0.88

accuracy appears to be excellent. The time required for a single determination is about 2 h.

A check of the results obtained for the number of the accessible SH-groups was of particular importance, but proved very difficult because the literature data are not comparable in many cases; the number of SH-groups found in particular samples depends largely on the reagent used. For instance, in a study of the rate of interaction of the SH-groups of β-lactoglobulin with *p*-chloromercuribenzoate or N-ethylmaleinimide, it was found that the reaction with N-ethylmaleinimide was considerably slower in native protein, whereas the rate of reaction with both reagents was the same when the protein was denatured[23]. KATZ AND MOMMAERTZ[24] obtained analogous results in studying the behaviour of the SH-groups of actine with various reagents; the six SH-groups reacted readily with *p*-chloromercuribenzoate in the native and denatured state, but N-ethylmaleinimide reacted with only two SH-groups in G and F actine, whereas reaction with silver ions indicated 5 SH-groups in G actine and 4 SH-groups in F actine. These results make it possible to grade the SH-groups of actine as follows: 2 accessible SH-groups, 2 intermediate reacting groups and 2 slowly reacting groups which are needed for the G $\rightarrow$ F transformation.

A study of the reactivity of the SH-groups of a protein with respect to dimethyl sulfate was therefore of interest. Finding a suitable sample of protein was impeded, however, by the lack of reliable and reproducible literature data. Egg albumin has been shown, by oxidation with iodine and by titration with *p*-chloromercuribenzoate, to have 3 accessible SH-groups out of a total of five[20-22]. The results obtained in the present work (Table I) show that when the protein is in the native state, 3 SH-groups are readily accessible to methylation; the results on the total number of SH-groups also agree with literature values[22]. These results were naturally corrected for the methane released from the methionine present.

The possibility of determining not only the total free SH-groups but also the number of readily accessible groups, *i.e.* groups which react with dimethyl sulfate under mild conditions, seems an important advantage of this method. Under the mild con-

ditions (room temperature, pH 9), it may be assumed that most proteins are not denatured, so that the method allows the determination of the accessible SH-groups of the native protein.

Since the determination of the total SH-groups is done in two stages and in the first stage the readily accessible SH-groups are immediately methylated and made unavailable for further reaction, it seems probable that the total number of SH-groups determined in this way is closest to the actual total number in the native protein.

To check the application of the suggested method for studying the metabolism of the sulfur-containing amino acids in animal organs, organs of the Syrian golden hamster were analysed. The results for the number of accessible SH-groups, calculated as residues of cysteine and corrected for methionine, are shown in Table II. A fuller statistical study of the cysteine and methionine contents will be published elsewhere.

The results obtained for insulin and trypsin (Table I) show that if the protein hydrolysate is reduced before methylation, the method is satisfactory for the direct determination of the total disulfide groups of the protein. The use of formic acid hydrolysis to prevent decomposition of cystine in the case of insulin[17], was also satisfactory for trypsin. However, this possibility for the direct determination of the total disulfide groups is unlikely to be used in practice, because disulfide groups are more readily determined from the difference between the total sulfur and the sulfur bonded as methionine and cysteine.

Nevertheless, the method provides a means of determining the accessible SS-groups of the protein (after a correction for free SH-groups and methionine). For this purpose the non-hydrolysed protein must be reduced under mild conditions, methylated with dimethyl sulfate and only then hydrolysed.

The results from the determination of the readily accessible SH- and SS-groups of non-hydrolysed proteins should be very important, because, in combination with results for methionine and results obtained by desulfurization, as well as microstructural investigations, they should provide considerable evidence on the macrostructure of the protein.

SUMMARY

A method is described for the determination of the total and accessible free SH-groups in proteins, based on the methylation of the SH-groups in non-hydrolysed proteins with dimethyl sulfate, followed by hydrolysis, additional methylation (for the total free SH-groups) and treatment with Raney nickel. The methane released is measured by infrared spectroscopy. Methionine must be determined separately and a correction made. The relative error is 3%, and the time necessary for one determination is about 2 h (excluding the time for hydrolysis). The method may also be applied to the determination of accessible SH-groups of homogenates of internal organs. If the protein hydrolysate and the non-hydrolysed protein are reduced before methylation, the total and accessible disulfide groups can also be determined.

RÉSUMÉ

Une méthode est décrite pour le dosage des groupes SH– de protéines. On procède par méthylation des groupes SH– des protéines non hydrolysées, à l'aide de sulfate

de méthyle, puis par hydrolyse, méthylation (pour obtenir SH- total) et traitement avec nickel Raney. Le méthane libéré est mesuré par spectroscopie infra-rouge. La méthionine doit être dosée séparément. On peut doser également les groupes -S-S- par réduction de l'hydrolysat de protéine et de la protéine non-hydrolysée avant méthylation.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben zur Bestimmung der totalen und zugänglichen freien SH-Gruppen in Proteinen, die auf der Methylation der SH-Gruppen in nichthydrolisierten Proteinen mit Dimethylsulfat beruht mit folgender Hydrolyse, zusätzlicher Methylation für die totalen freien SH-Gruppen und der Behandlung mit Raney-Nickel. Das entstandene Methan wird infrarotspektroskopisch gemessen. Methionin muss getrennt bestimmt und eine Korrektur durchgeführt werden. Der relative Fehler beträgt 3%. Für eine Bestimmung werden etwa 2 h ausgenommen der Zeit für die Hydrolyse benötigt. Die Methode kann ebenso für die Bestimmung von zugänglichen SH-Gruppen von Homogenaten innerer Organe angewandt werden. Falls das Protein hydrolisiert und das nichthydrolisierte Protein vor der Methylation reduziert werden, können die totalen und zugänglichen Disulfidgruppen ebenso bestimmt werden.

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AN AUTOMATIC TITRATION CONTROL UNIT

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(Received August 20th, 1964)

The fundamental requirement for automatic termination of a chemical titration is a means of controlling and eventually stopping the addition of titrant as the end-point is approached. The first techniques added titrant continuously until the end-point was reached[1,2]; this usually resulted in over-titration, due to slowness of reaction rate[3]. A slow addition of titrant overcame this problem but involved a lengthy titration time and reduced any advantage to be gained from automation. Later devices incorporated a combination of 2 titration rates where the determination proceeded rapidly up to a point immediately prior to the end-point; the apparatus then automatically switched to a slower titration rate to complete the operation[4]. The various types of titrator have been reviewed[5]. The unit to be described[6] is based on the latter system; the reduced titration rate is achieved by pulsing the titrant electronically in a manner similar to that adopted in a manual titration. The practical effect is a dropwise addition of the titrant with a variable time between drops of up to 20 sec if desired. The drop size or pulse length can also be varied as required.

EXPERIMENTAL

Apparatus

The switching unit (Fig. 1) consists of a galvanometer relay (Pantam Messcontactor Unit, Gossen Ltd.) with 2 independent contact settings, which are actuated by a lamp and photocell arrangement, which is situated within the meter housing. The first contact actuates a relay with a preset time cycle, *e.g.* 0.1–1.0 sec *on*, 3–15 sec *off*, independently and continuously variable, until the second meter contact is reached, when the relay is switched off. The relay has double-pole, double-throw contacts, for which connections are brought out to the front panel.

The galvanometer movement is intended to be used either directly or in conjunction with other equipment, such as a pH meter, valve millivoltmeter, DC amplifier, etc. The contactor unit meter movement is simply placed in series with the internal meter, an output socket usually being provided on the equipment for this purpose. A 100-μA movement is fitted to the contactor unit; this is readily adaptable to other current ranges by the addition of a shunt resistor.

The output relay contacts are used to control the equipment used in a chemical determination.

Circuit description

The photocell outputs from the Pantam Messcontactor Unit, at terminals 12 and 17 respectively (Fig. 2) actuate relays RLA and RLB after suitable DC amplification. Switch S.1 is used to reverse the connections to RLA, RLB, dependent on whether the potential is rising or falling. Relay RLB can be optionally locked on, after energising, in order to eliminate the risk of the titration apparatus being re-energised due to mishandling, once the end-point has been reached. The switching sequence is automatically reset by switching S.2 to the "standby" position.

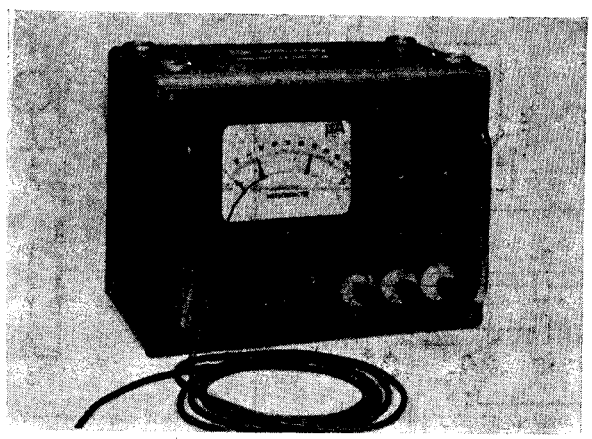

Fig. 1. Auto-titration switching unit Type SP 8/1.

The pulsing circuit is brought into operation by relay RLA, connecting the output relay RLD to contact RLC 1 by releasing RLA 1. The astable oscillator stage (VT 1, VT 2) of suitable pulse repetition frequency feeds into a monostable oscillator stage (VT 4, VT 5) which is of short time constant in the unstable state compared to the astable oscillator. The time constants of both oscillators are independently adjustable by variable resistors RV 3 and RV 1, which are mounted on the front panel of the instrument.

Application

The switching unit can be used to terminate a chemical determination automatically where volumetric addition or coulometric generation (constant current) of titrant is employed. The end-point must be detected by a method giving, or capable of conversion to, an electrical signal. The meter movement is connected to the equipment which is monitoring the progress of the titration and the output contacts are connected to a constant current source and timer in the case of a coulometric titration, or a motor-operated buret for a volumetric titration.

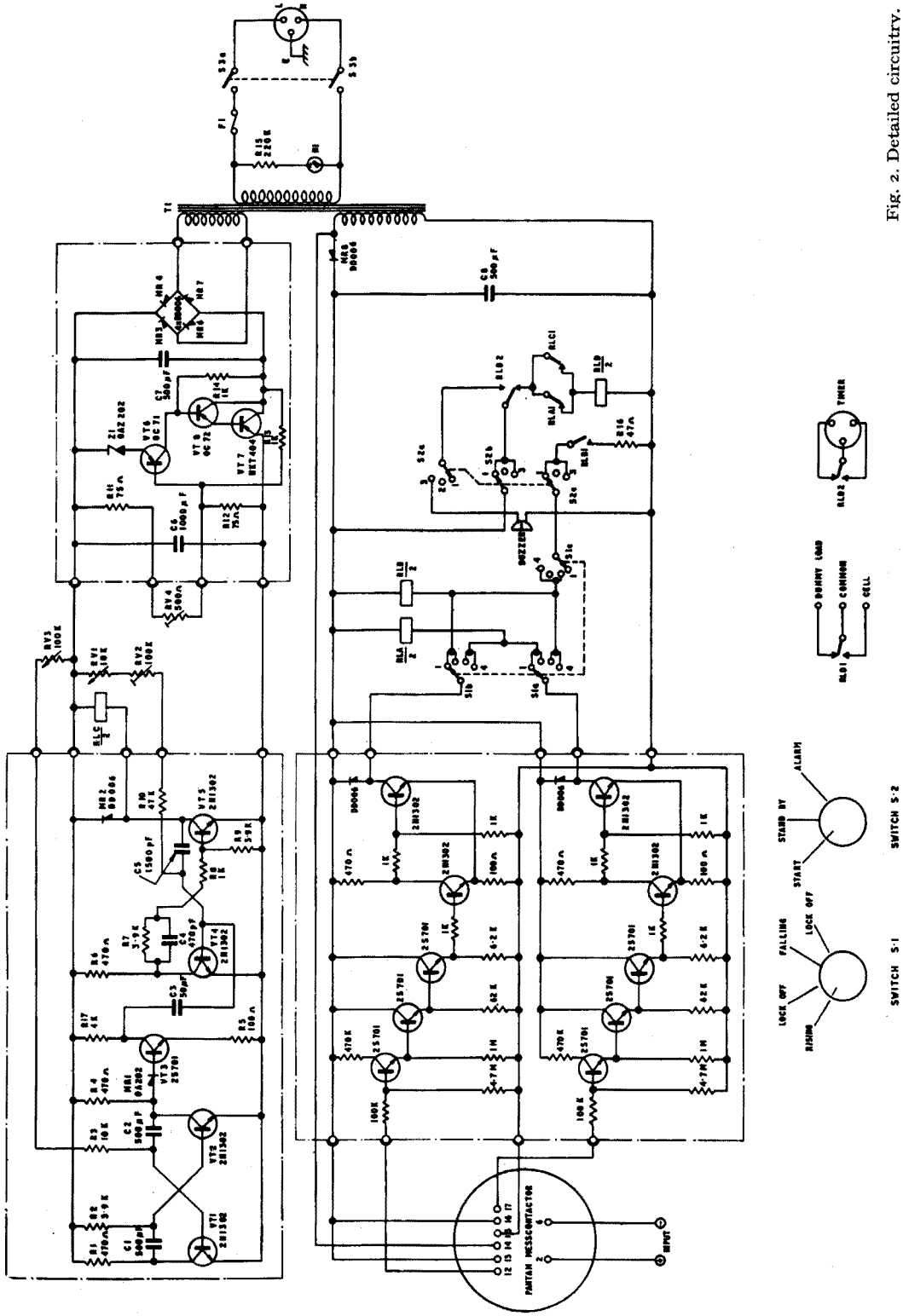

Fig. 2. Detailed circuitry.

The determination proceeds continuously until the first contact is reached, at a point which precedes the end-point. The unit then adds small increments of titrant, at a predetermined time interval, and allows the solution or electrodes to reach equilibrium between each addition. When the second contact is reached the titration is automatically terminated.

The equipment can be used to follow either a rising or a falling signal.

We have used the contactor unit as a slave to a millivoltmeter in terminating a constant current titration automatically (Fig. 3). The titrations are of about 1000-sec

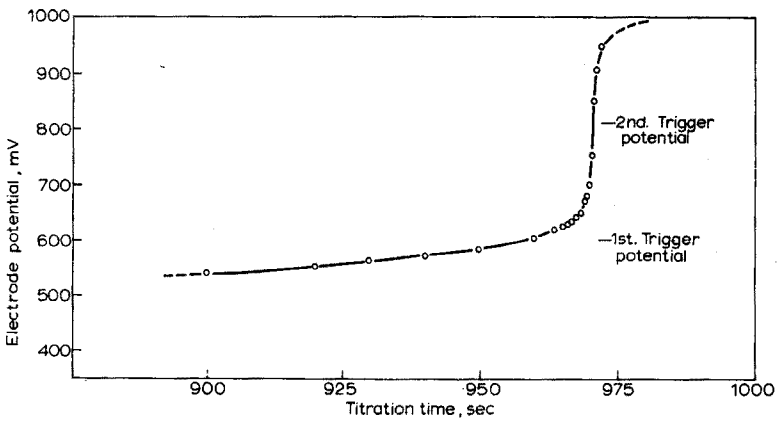

Fig. 3. Titration curve with Pt/S.C.E. indicator electrodes and electrogenerated cerium(IV).

duration and the switching unit will control the determination to $\pm$ 0.1 sec; the theoretical precision is thus $\pm$ 0.01%. The precision is more dependent on the particular titration and end-point system employed than on the switching unit, but the Fig. illustrates the potentialities of the equipment. In order to set up the apparatus a manual titration was conducted first. The resulting curve was inspected and the control meter contacts were set up. An automatic titration was set in progress and near the end-point slight adjustment was made to the triggering potential and rate of addition/generation of titrant, in order to optimise the conditions.

As a module in an automatic titration system the applications of this equipment are numerous. Whilst we have only used the apparatus for a coulometric titration, it could be used to control a motorised peristaltic or syringe buret or a solenoid valve for a conventional volumetric titration. End-point detection is not limited to the potentiometric method and the unit could easily be used to detect a colorimetric end-point, monitoring the output from a phototransistor or photocell in conjunction with an appropriate colour filter. It could also be used with amperometric titrations; the accuracy would compare favourably with the equivalent manual titration.

DISCUSSION

The switching unit can be used to approach a titration end-point in a manner similar to that of a human operator. If the first trigger potential is correctly set then

the end-point will be reached in the minimum of time and with negligible overshoot. All determinations will be carried out in an exactly similar way, eliminating personal variations. The progress of the titration can be observed and, if necessary, adjustments can be made to trigger potentials, etc. As the end-point is approached in a coulometric titration, the current remains at the same value, thus obviating any risk of change in current efficiency and avoiding complex current integration, the only parameters being time and current. Since the contacts are mechanical settings, no drift can result from day to day such as could occur with, for example, an electronic trigger type of circuit.

SUMMARY

A unit is described for the automatic termination of coulometric or volumetric titrations in conjunction with a suitable valve millivoltmeter and motorized buret or constant current generator. Two values of potential can be preset on the instrument, in either rising or falling sequence. On reaching the first potential, the end-point is approached by pulsed addition of the titrant; the titration is terminated at the second potential. The *on* and *off* time cycles for the pulsing circuit are independently variable from 0.1 to 1.0 sec *on* and 3 to 15 sec *off*.

RÉSUMÉ

Un système est décrit pour le contrôle automatique de titrages coulométriques ou volumétriques. Il peut être utilisé pour effectuer des dosages chimiques automatiques avec addition volumétrique ou génération coulométrique (à courant constant) du réactif de titration. Le point final est décelé grâce au fonctionnement d'un signal électrique.

ZUSAMMENFASSUNG

Es wird eine automatische Endpunktsanzeige für coulorimetrische oder volumetrische Titrationen beschrieben in Verbindung mit einem geeigneten Röhren-Millivoltmeter und einer automatischen Bürette oder einem Konstantstromgenerator. Zwei Potentialwerte können mit dem Instrument in steigender oder fallender Folge vorgewählt werden. Nach dem Erreichen des ersten Potentials nähert man sich dem Endpunkt durch pulsierende Zugabe des Titrationsmittels; die Titration wird beendet beim zweiten Potential. Die Ein- und Auszeitzyklen für den pulsierenden Stromkreis sind unabhängig voneinander variierbar von 0.1–1 Sek. für "Ein" und 3–15 Sek. für "Aus".

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THE NATURE OF LIGHT

PART II. THE RELATIONSHIP BETWEEN PHOTONS, ELECTRONS AND POSITRONS

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In a previous paper[1] a model of a photon was proposed which would satisfy the mathematical behaviour of light when it is acting either as a wave or as a particle. A tangible physical model was also envisaged. It was hoped that this model would help us to understand the nature of light and provide new approaches to the solution of the difficult enigma of its true nature.

In the model proposed, a photon consisted of two charges: one + and one −. Each charge was equivalent to half the charge of a positron and an electron respectively. Such half-charges have never been observed experimentally, but this does not preclude their existence. It may be that they are too unstable to exist alone and need to be in pairs of equal or opposite charge. The existence of such half-charges can be deduced as follows.

It is known that electrons and positrons react to form two rays. This suggests a relationship between electrons, positrons and photons. It is proposed that this relationship is as follows.

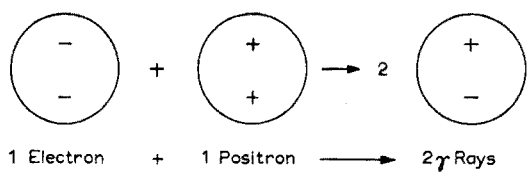

Fig. 1. Formation of two γ-rays from one electron and one position.

Since two γ-rays are produced from one electron and one positron, we have produced two − and two + charges from the one electron and one positron. In order to maintain constancy of electrical charge, it is suggested that the electron and positron are each composed of two charges rotating around the center of mass in the same fashion as the photon.

It must be pointed out that the twin charge and size of the electron are such as to produce tremendous repulsive forces at this close proximity. This would normally preclude the stable existence of such a particle. However, it may be that the two

halves of the electron have different magnetic properties towards each other, than the combined pair has towards other bodies. It is also conceivable that these halves are not actually separated from each other in the electron but provide two centers of negative charge rotating about the center of mass. In this case no strong mutual repulsion would take place within the electron—only a distortion of its shape and the formation of a "duo-pole"—a phenomenon which can be described as being similar to a dipole but with equal, similar charges.

With this structure the electron should exhibit wave motion similar to the photon. Some experimental evidence supporting this view is listed below.

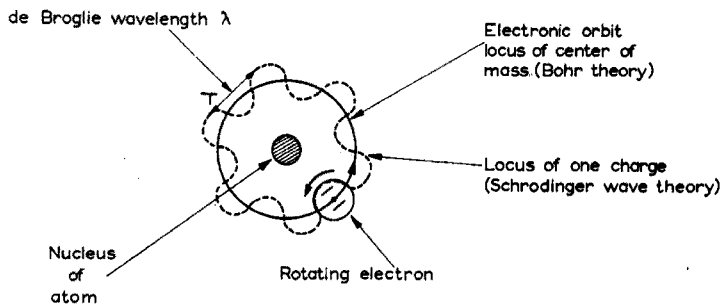

Fig. 2. Locus of the charges of an orbiting electron.

According to Bohr theory,

$$2\pi rmv = nh \tag{1}$$

de Broglie suggested that the electron had wave motion, and further stated that it was inconceivable that the orbital path was anything but a whole number of wavelengths, *i.e.*

$$n\lambda = 2\pi r \tag{2}$$

hence

$$mvn\lambda = 2\pi rmv = nh \tag{3}$$

or

$$\lambda = \frac{h}{mv} = \frac{h}{p} \text{ where } p = \text{the momentum of the electron} \tag{4}$$

It will be remembered that Compton's value for the momentum of a photon was $hv/c = h/\lambda$, hence:

The momentum of a photon $= p = \frac{h}{\lambda}$ (Compton effect). λ = photon wavelength.

The momentum of an electron $= p = \frac{h}{\lambda}$. λ = de Broglie electron wavelength.

The similarity in these expressions indicates a strong relationship between the mass, energy, and physical characteristics of photons and electrons.

The model proposed for an electron would have a center of mass, and two rotat-

ing negative charges. Movement of the center of mass would give it translational energy. Rotation of the charges while the mass was moving would produce two charges whose loci move like a sine wave.

When an electron with this structure is in orbit around an atom it would be expected to exhibit a de Broglie wavelength. The locus of the center of mass would be an electronic orbit in the style of the Bohr atom. The locus of either of the rotating charges would have wave-like properties, with a wavelength equal to the distance travelled by the center of mass during one half of a complete revolution of the electron.

This suggests an atomic structure with a pulsating electronic field set up by each electron; this pulsing must be physically and mathematically reproduced during each revolution of each electron. For stability of the atom or molecule, each electron in each orbit must reproduce the same effective field on the other electrons during each revolution of the various electrons. If this is not so, the atom would be unstable and electrons would be spontaneously ejected. This stability would require the de Broglie relationship $n\lambda = 2\pi r$. With the proposed model, the rotating electron would provide a pulsating magnetic field, which would be a function of the "duo-pole" charge of the electron, its wave number and the velocity of the center of mass. The rotation of the "duo-pole" would produce a magnetic pulse perpendicular to the axis of rotation with wave-like characteristics and would be in sympathy with the classical work of de Broglie.

In contrast to the Bohr atom, the Schrödinger wave equation is based on harmonic or wave-like motion of the electron. This can be considered to be based on the properties of the rotating charge of the electron and a physical interpretation of the process can be obtained. With this model, it is possible to understand the physical reasons for the similarities and differences between the Bohr atom and the Schrödinger atom.

Other supporting evidence for the wave-like character of electrons is found in electron microscopy where many of the properties of light are found, including diffraction phenomena. This demonstrates clearly the close similarity between electrons and photons.

Properties of the proposed model of the photon

The proposed model of the photon is basically a Newtonian particle, composed of two rotating electrical charges. From this it should be possible to deduce its properties. Examples of some of these deductions are given below.

It should be mentioned at this point that the mathematical interpretations of wave mechanics are well established and should be upheld by this model. This thesis is intended only to present a physical picture of light particles which may have particulate and wave-like properties. The sustaining of wave mechanical mathematics however is necessary if the model is valid. Happily no contradictions have yet been noted.

The presence of the rotating charges would set up intense local magnetic fields perpendicular to its axis of rotation. This may be a possible explanation for the intense absorption of light exerted by very low concentrations of atoms and molecules, the basis of many analytical techniques such as colorimetric analysis, UV and IR absorption, etc. An attractive magnetic field is suggested between orbiting electrons and nearby photons. Of course the photons and electrons would have to

be in phase and in the same frequency or some order of frequency, in order to interact and be absorbed by the atom. The absorption itself may be due to the temporary formation of a combined electron–photon particle, which moves to a higher energy state giving an "excited" atom. Emission spectrography suggests that the life time of this combined particle is between 10^{-6} and 10^{-8} sec. After this time the photon is re-emitted and the electron descends to the ground state. If, however, the photon is transferred to another electron, the original electron would descend to the ground state without emission, but the photon would be emitted from another part of the molecule. It may have lost rotational energy in the process and therefore appear at a different frequency. This is the basis of fluorescence and phosphorescence.

The intense local field of the photon would also cause mutual attraction between synchronized photons and repulsion between unsynchronized photons. Bunches of photons would result, coherent in themselves, but not coherent with other bunches. Such bunches may explain the blinking of stars, where the great distance travelled may enable substantial separation of these bunches from each other. Also, the size of the bunches may increase by picking up stray photons in space.

It is observed with interference fringes that the rings are not sharp and that the total energy involved remains constant. This suggests that some degree of unsynchronization between bunches of photons and approaching photons can be tolerated. If the phase difference is small enough, the phases of the photons concerned may be mutually adjusted to become compatible.

Information on this point may be obtained by superimposing two parallel laser beams to produce interference fringes. The two beams should then be made increasingly non-parallel until no interference fringes are obtained. The angle between the beams should be related to the lack of synchronization of the photons.

Although there are intense local magnetic fields near the photon, the effective radius would only be of the same order of magnitude as atoms. External magnetic fields would have little effect because both the positive and the negative charges involved are oscillating around each other and would produce a net zero charge to a remote superimposed magnetic field. This would explain the apparent lack of response of light to magnetic and electrical fields, although the Zeeman effect indicates that some interaction takes place, possibly between bunches of photons and the superimposed magnetic field. These bunches of photons would be synchronized within the bunch but not between each bunch. The effect of a superimposed magnetic field would therefore vary slightly from one bunch to the next. Some proof of the existence of charged photons should be forthcoming using continuous coherent light—as emitted from a laser. Coherent light should be light in which the photons are rotating in phase. If such a light source was impinging on a small metal surface, as in Fig. 3, light from the laser should arrive at the metal surface in a steady state of orientation between the charges on the photon and the metal surface. If the distance between the metal surface and the laser source is kept constant and the coherency of the light maintained, the metal surface would be struck continuously by one charge, *e.g.*, the positive charge of the photon. If now the metal is charged negatively, the photon should be attracted and absorption should take place. If the surface is positively charged repulsion, or reflection, should occur. Similarly alternate absorption and reflection should occur if the charge on the metal surface is kept constant, but the distance from the coherent source is varied. These alternate reflections and absorp-

tions should be a function of the wavelength of the light. One problem may arise with this experiment. The effective radius of the magnetic field of each charge on the photon is limited, since it is counterbalanced by the opposite charge at remote points; hence, interaction is only over a small distance. A negative charge on the metal surface is in effect an excess of electrons. In this case the reaction should be as predicted. However, a positive charge may be only a depletion of electrons on the surface. This reaction may not be as predicted. The photons may interact with residual electrons rather than the shielded positive nuclei of the metal and a result other than that predicted would result.

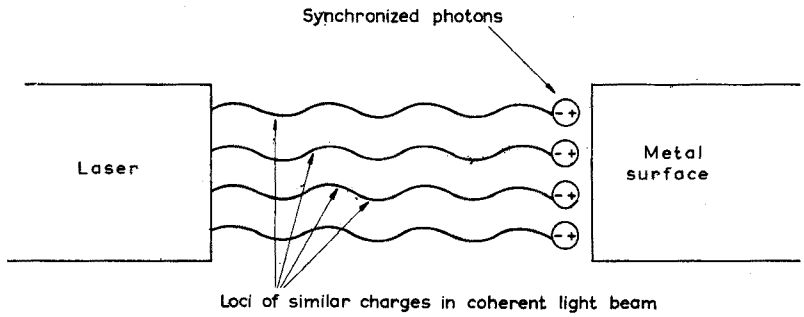

Fig. 3. Reflection of coherent light beams.

It will be noted that the model of the photon has *mass*. This conclusion was also implicit in calculations derived from Compton scattering. It will be noted that these calculations lead to the momentum hv/c being a function of the wavelength of the particle. As mentioned earlier, when hv/c is equated to mc, Einstein's equation $E = mc^2$ is derived. An alternative proof of Einstein's equation may be presented at this point.

Supposing an unstable atom is about to emit a γ-ray from its nucleus. The γ-ray is at rest before emission, but travels at the speed of light after emission. Its gain in kinetic energy is therefore $\frac{1}{2}mv^2$ or $\frac{1}{2}mc^2$. Simultaneously the recoil experienced by the residual atom is also $\frac{1}{2}mc^2$. The total energy produced is therefore mc^2. Hence the amount of energy produced on the loss of mass m from the nucleus is E and is equal to mc^2, *i.e.* $E = mc^2$. This calculation of course assumes that the photon has virtually no kinetic energy when in the nucleus of the atom. There is of course no proof for this assumption.

Coherent light from lasers.

The formation of coherent light in lasers can be physically interpreted as follows.

Light is pumped into an atomic or molecular system and photons are absorbed. These photons promote electrons to an excited state.

A light pulse is then injected into the system. This pulse is a single cloud of photons which are coherent to each other. As this cloud passes various excited elec-

tron–photon pairs, an intense local field is exerted on them by the light cloud. Those photons which are coherent with the light cloud or train are attracted to it and increase its size. During each passage through the excited atom, the light train is augmented by these photons.

Since they are coherent with the train, a coherent light train grows, which after several reflections finally emerges from the system.

When the emerging beam impinges on a surface, the roughness of the surface will cause some randomness in the reflection. This randomness will break up the coherence of the light train. Since each photon produces a local magnetic field, intense, local, repelling and attracting fields will be set up. These may be responsible for the exotic interference fringes observed when laser beams are reflected at plane surfaces.

The Doppler effect

If light is a particle as proposed in this thesis, it is necessary to provide an alternative explanation for the "Doppler" effect. This effect—originally observed with sound—states that the velocity of the wave through the transmitting medium is constant, the waves are emitted at constant frequence, but to an observer in the path of the approaching emitter, the waves appear to be bunched closer together and hence arrive at increased regularity, *i.e.*, at greater frequency. With the proposed particle the same apparent change in frequency will be observed if the velocity of the emitter is superimposed on the velocity of the photon. If the frequency of the particle at the point of emission remains constant, but the velocity relative to the receiver is increased, then the number of pulses passing the receiver per unit time will increase. This again results in an apparent increase in frequency, a result similar in all respects to the Doppler effect, but caused by a change in velocity rather than wavelength. Use of this phenomenon has been made in measuring the relative speed of the stars by the wavelength shift in their spectrum. The spectral shifts could be interpreted as a difference in the speed of light relative to the observer. The relative speed of the stars would be superimposed on the speed c of the emitted photons. The net result would be a similar shift in the spectrum of the stars from which the relative velocity of the stars could be calculated as before.

Relationship with relativity theory

The development of the Relativity Theory and in particular the Special Relativity Theory, is intimately concerned with the speed of light. It is based on the observation that the speed of light is always c and is independent of the relative motion of the emitter and the receiver. The current views of light, *i.e.* as waves or photons, give no reason why this should or should not be so. The model proposed here would also give no reason to expect this observation. The particle is Newtonian in nature and should travel at a constant velocity unless acted upon by a superimpressed force. It is conceivable that there is an interaction between the magnetic field of the photon and its environment, giving rise to an interaction akin to Fresnel Drag. However, movement of the observer should still make a difference to the relative velocity of the photon contrary to observed accepted data. This problem has frustrated physicists for a long period of time. Although Einstein's treatment mathematically resolves the problem, it is difficult to accept as a physical reality.

Einstein's relativity theory and the special relativity theory are based on two axioms, *i.e.*, if two observers are moving in a straight line relative to each other, they cannot tell who is moving; and, if both measure the speed of light, the same answer is obtained. From this is evolved the clock paradox that time is different for these observers, and that $\tau = \sqrt{1 - (v/c)}$.

The proposed model says nothing about relativity. Being Newtonian in nature, it should obey classical laws. Its velocity should not necessarily be c but $c + V$, where V is the relative velocity of the emitting particle and c is the velocity of emission relative to the source.

To suppose that the speed of light is always c defines an absolute velocity. By difference c—c is absolute rest. Since it is not possible to identify absolute rest it must be equally impossible to identify an absolute velocity.

Recently DINGLE[2] pointed out an apparent inconsistency in the special theory of relativity. This of course met with a storm of protest, but no concrete evidence that he was wrong. Other attempts to confirm the inequivocability of c[3,4] have been made. It was concluded from each of these that the speed of light was confirmed as c. However, there was a weakness in each case. In the former[3], two γ-ray sources were used, one recoiling and one not recoiling. It was hoped to demonstrate that the difference in velocity of the source was superimposed on the velocity of the emitted γ-ray. No such difference was observed. However, it was assumed that one source was in a state of recoil and the other was not. This is tantamount of assuming the result, since it can be argued equally successfully that the results indicate no differences in velocity between the sources.

The second experiment uses a source of white light which passes through a rotating semi-circular glass disc. It is claimed that this gives rise to light from a source with varying velocity. However, since the actual source is not moved, only the varying Fresnel drag of the rotating semi-circular disc is superimposed on the light rays. As indicated in the article, this difference would not be detectable. However, we would not expect any other difference to be obtained since the actual source itself is not moved.

Conclusion

The model of a photon presented in this thesis provides a tangible understanding of many of the contradictory properties of light. The usefulness of any model is to provide a starting point which may be successively tested and improved until an ultimate truth is developed. It is hoped that in some way, this model will be useful in this respect.

SUMMARY

It is proposed that a photon is composed of a positive and a negative charge. The relationship between photons, electrons and positrons is discussed. A mechanism of the production of coherent light by lasers is also proposed.

The nature of light is intimately concerned with the Special Case of the Theory of Relativity. However the model proposed provides no clue to explain why this theory should hold.

RÉSUMÉ

Dans le modèle proposé, le photon est composé d'une charge positive et d'une charge négative. La relation entre photons, électrons et positrons est examinée. Un mécanisme de production de lumière cohérente par lasers est également proposé. La nature de la lumière est intimement liée au "Cas Spécial de la Théorie de la Relativité".

ZUSAMMENFASSUNG

Es wird vorgeschlagen, dass ein Photon aus einer positiven und einer negativen Ladung zusammengesetzt ist. Die Beziehung zwischen Photonen, Elektronen und Positronen wird diskutiert und ein Mechanismus zur Herstellung von kohärentem Licht durch Laser vorgeschlagen. Die Natur des Lichtes ist eng verbunden mit der speziellen Relativitätstheorie. Das vorgeschlagene Modell gibt jedoch keinen Aufschluss, warum diese Theorie gehalten werden sollte.

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STEAM DISTILLATION METHODS FOR DETERMINATION OF AMMONIUM, NITRATE AND NITRITE*

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Numerous colorimetric and distillation methods of determining ammonium, nitrate, and nitrite have been described[1-7], but most of these methods are not suitable for analysis of biological materials. The colorimetric methods are subject to interference by various substances which occur in extracts of biological materials, and are not readily applicable to turbid or colored extracts. The chief disadvantages of the distillation methods are that they are generally tedious and time-consuming, require a relatively large amount of nitrogen for accurate analysis, and are subject to interference by amides and other alkali-labile organic nitrogen compounds.

For studies in our laboratory of nitrogen transformations in soils, it was necessary to have methods of determining ammonium, nitrate, and nitrite in soil extracts that were rapid, specific, and precise and would permit accurate determination of 0.1–2 mg of these forms of nitrogen. Additional requirements were that the methods should be readily applicable to turbid or colored extracts, and should permit nitrogen isotope-ratio analysis of ammonium, nitrate, and nitrite in tracer studies of the fate of ^{15}N-enriched compounds in soils. The methods to be described have been found to meet these requirements. In these methods, the form of nitrogen under analysis is converted to, and determined as, ammonium, which is readily oxidized to nitrogen gas for mass spectrometer assay of nitrogen-15[8]. The ammonia is separated by steam distillation, collected in boric acid–indicator solution, and determined by titration with standard acid. Magnesium oxide is used for distillation of ammonia and finely divided Devarda alloy is used for reduction of nitrate and nitrite to ammonium. The methods are based on the findings (*a*) that interference by glutamine and other alkali-labile organic nitrogen compounds in alkali-distillation methods of determining ammonium ion can be eliminated by performing the distillation with steam using a small amount of magnesium oxide and a very short period of distillation and (*b*) that both the amount of magnesium oxide and the time required for quantitative reduction of nitrate and nitrite to ammonium by steam distillation with magnesium oxide and Devarda alloy can be reduced by use of ball-milled alloy. The method for determination of nitrate in the presence of nitrite depends upon the fact that sulfamic acid decomposes nitrite rapidly and quantitatively to nitrogen at room temperature, but does not react with ammonium or nitrate or interfere with their determination by steam distillation with magnesium oxide and Devarda alloy.

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EXPERIMENTAL

Apparatus

Steam distillation apparatus. The apparatus used (Fig. 1) is designed so that flasks fitted with standard-taper (19/38) ground-glass joints can be used as distillation chambers. The steam required for distillation is generated by heating distilled water in a 5-l flask that contains pumice or glass beads (to promote smooth boiling) and

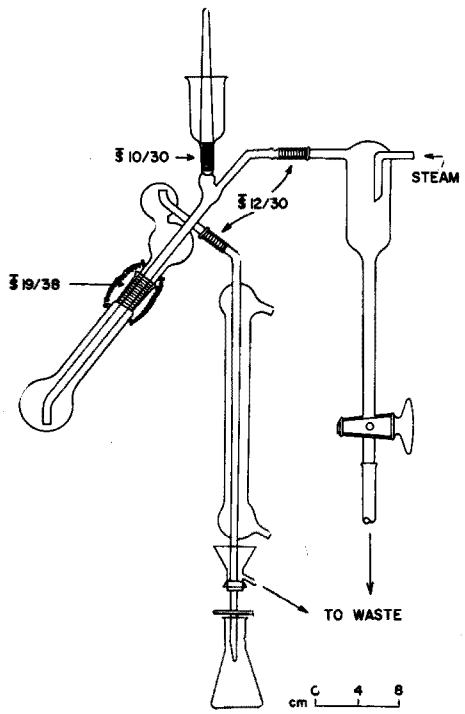

Fig. 1. Steam distillation apparatus.

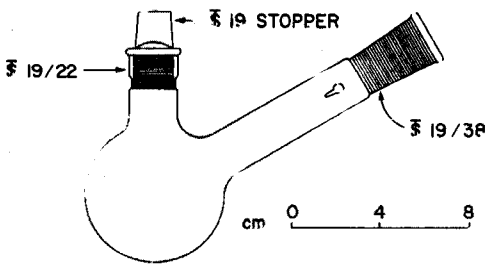

Fig. 2. Distillation flask with side-arm for addition of Devarda alloy.

about 3 ml of concentrated sulfuric acid (to trap any ammonium in the distilled water). Before use, the apparatus should be steamed out for about 10 min to remove traces of ammonia, and the rate of steam generation should be adjusted so that 7–8 ml of distillate are collected per minute. The flow of cold water through the condenser of the apparatus should be such that the temperature of the distillate obtained by using this rate of distillation does not exceed 22°. The desired rate of distillation is readily obtained if the steam generator flask is heated by an electric heating mantle and the power supply to the mantle is controlled by a variable transformer. The trap at the base of the water jacket on the condenser is to prevent water condensing on the external surface of the condenser from entering the flask used to collect the distillate.

Distillation flasks. The flasks used are 100-ml Kjeldahl flasks fitted with a side-arm, standard-taper ground-glass joints, and glass hooks, as shown in Fig. 2.

The neck of the side-arm is fitted with a standard-taper polyethylene stopper. The flask dimensions should be such that, when the flasks are connected to the distillation apparatus, the distance between the tip of the steam inlet tube and the bottom of the flask is approximately 4 mm.

Reagents

Magnesium oxide. Heat heavy magnesium oxide (U.S.P.) in an electric muffle furnace at 600–700° for 2 h. Cool the product in a desiccator containing KOH pellets and store it in a tightly stoppered bottle.

Boric acid–indicator solution. Dissolve 20 g of reagent-grade boric acid in about 700 ml of hot water and transfer the cooled solution to a 1-l volumetric flask containing 200 ml of 95% ethanol and 20 ml of mixed indicator solution prepared by dissolving 0.330 g of bromocresol green and 0.165 g of methyl red in 500 ml of 95% ethanol. After mixing the contents of the flask, add *ca.* 0.05 *N* sodium hydroxide cautiously until a color change from pink to pale green is just detectable when 1 ml of the solution is treated with 1 ml of water. Then dilute the solution to volume with water and mix it thoroughly.

Devarda alloy. Ball-mill reagent-grade alloy (Fisher Scientific Co., Chicago, Illinois; J. T. Baker Chemical Co., Phillipsburg, New Jersey) until the product will pass a 100-mesh screen and at least 75% of it will pass a 300-mesh screen. Store the finely divided alloy in a tightly stoppered bottle.

Sulfamic acid. Purify reagent-grade sulfamic acid by crystallization from hot water[9,10], and dissolve 2 g of the purified reagent in 100 ml of water. Store the solution in a refrigerator.

Sulfuric acid. 0.005 *N* standard.

Standard (ammonium + nitrate)-N solution. Dissolve 0.236 g of ammonium sulfate and 0.361 g of potassium nitrate in water, dilute the solution to 2 l in a volumetric flask, and mix thoroughly. If pure dry reagents are used, this solution contains 25 μg of ammonium-N and 25 μg of nitrate-N per ml. Store in a refrigerator.

Standard (ammonium + nitrate + nitrite)-N solution. Dissolve 0.236 g of ammonium sulfate, 0.246 g of sodium nitrite, and 0.361 g of potassium nitrate in water, dilute the solution to 2 l in a volumetric flask, and mix thoroughly. If pure dry reagents are used, this solution contains 25 μg of ammonium-N, 25 μg of nitrite-N, and 25 μg of nitrate-N per ml. Store in a refrigerator.

Procedures in the presence of nitrite

Ammonium-N. Add 5 ml of boric acid–indicator solution to a 50-ml Erlenmeyer flask marked to indicate a volume of 30 ml and place the flask under the condenser of the steam distillation apparatus so that the end of the condenser is about 4 cm above the surface of the boric acid. Pipet an aliquot of sample containing up to 2 mg of inorganic-N into a distillation flask, add water until the total volume is *ca.* 20 ml, and add 0.2 g of magnesium oxide through a dry powder funnel with a long stem reaching into the bulb of the flask. Attach the flask by springs as shown in Fig. 1 and commence distillation by closing the stopcock on the steam by-pass tube of the distillation apparatus. When the distillate reaches the 30-ml mark on the receiver flask, stop the distillation by opening the stopcock on the steam by-pass tube, rinse the end of the condenser, and determine ammonium -N in the distillate by titration with

0.005 *N* sulfuric acid from a 5-ml microburet graduated at 0.01-ml intervals. The color change at the end-point is from green to a permanent, faint pink.

(Nitrate + nitrite)-N. After removal of ammonium-N from the sample as described in the previous section, remove the stopper from the side-arm of the flask, add 0.2 g of Devarda alloy through a dry powder funnel reaching into the flask about 1 cm below the base of the ground joint on the side-arm, and immediately replace the stopper in the neck of the side-arm. Then determine the ammonium-N liberated by steam distillation as described in the previous section.

(Ammonium + nitrate + nitrite)-N. Follow the procedure described for determination of ammonium-N, but add 0.2 g of Devarda alloy to the distillation flask immediately after addition of magnesium oxide and before connection of the flask to the distillation apparatus.

(Ammonium + nitrate)-N. Follow the procedure described for determination of (ammonium + nitrate + nitrite)-N, but, before addition of magnesium oxide and Devarda alloy, treat the sample in the distillation flask with 1 ml of sulfamic acid solution, and swirl the flask for a few seconds to destroy nitrite.

Nitrate-N. Follow the procedure described for determination of (nitrate + nitrite)-N, but perform the analysis on a sample that has been treated with sulfamic acid to destroy nitrite as in the procedure for determination of (ammonium+nitrate)-N.

Procedures in absence of nitrite

Ammonium-N. Follow the procedure described for determination of ammonium-N in the presence of nitrite.

Nitrate-N. Follow the procedure described for determination of (nitrate + nitrite)-N in the presence of nitrite.

(Ammonium + nitrate)-N. Follow the procedure described for determination of (ammonium + nitrate + nitrite)-N in the presence of nitrite.

DISCUSSION

Apparatus

The steam distillation apparatus was designed so that it can be rapidly disassembled and washed between distillations to prevent cross contamination of samples when it is used for distillation of ammonia in ^{15}N analysis. It is very convenient for the methods described, but a similar type of apparatus in which the parts are connected by rubber or plastic tubing instead of ground-glass joints can be used if ^{15}N analysis of the ammonia liberated by distillation is not required. It is not necessary (or desirable) that the ground-glass joints of the distillation apparatus be greased, but the stopcock on the steam by-pass tube should be lubricated. The Teflon (DuPont fluorocarbon resin) dry-film stopcock lubricant sold under the trade name of Fluoro-Glide is satisfactory.

Reagents

The magnesium oxide employed is ignited before use to remove the magnesium carbonate usually present in the magnesium oxide supplied commercially, and it is stored in an air-tight container to protect it from atmospheric carbon dioxide. Magnesium oxide containing carbonate could liberate carbon dioxide during the distilla-

tion (particularly when the sample under analysis is acidic), and carbon dioxide interferes with the titration of ammonia. When standard hydrochloric or sulfuric acid is used for collection of ammonia, it is customary to boil the distillate for a few minutes to remove carbon dioxide before titration with alkali. This is not permissible when boric acid is used for collection of ammonia, because ammonia would be lost[11,12]. The use of heavy magnesium oxide is recommended, because the light variety tends to creep up the walls of the distillation flask and enter the spray trap of the distillation apparatus.

Finely divided Devarda alloy can be readily prepared from the alloy supplied commercially by ball-milling it for several hours using a ceramic vial and ceramic balls.

It is not necessary to use accurately weighed amounts of magnesium oxide or Devarda alloy, and calibrated glass spoons[13] can be used for addition of these reagents.

Reagent-grade sulfamic acid sometimes contains an appreciable quantity of ammonium and usually requires purification. The method of purification recommended is simple and effective, and only traces of ammonium were detected in sulfamic acid purified by this method, dried thoroughly and stored for 2 years in an air-tight bottle. Aqueous solutions of sulfamic acid are not completely stable, because sulfamic acid is slowly hydrolyzed to form ammonium bisulfate. However, this hydrolysis is extremely slow at 5°, and only traces of ammonium were detected in sulfamic acid solutions prepared as recommended and stored for 6 months in a refrigerator. The 2% sulfamic acid solution used for destruction of nitrite does not liberate measurable amounts of ammonium under the conditions employed.

The sharpness of the end-point obtained in titration with 0.005 *N* sulfuric acid to determine ammonia in distillates collected in boric acid–indicator solution depends upon the source of the indicators, and it may be necessary to alter the recommended proportions of bromocresol green and methyl red to obtain a satisfactory end-point. The sharpness of the end-point also depends upon the quality of the boric acid[14]. The 5 ml of 2% boric acid solution used can effectively absorb about 5 mg of ammonium-N[14,15]. It is recommended that the 0.005 *N* sulfuric acid be standardized against tris(hydroxymethyl)aminomethane [2-amino-2-(hydroxymethyl)-1,3-propanediol], and that titrations be performed using a variable-speed magnetic stirrer and a teflon or glass-coated bar.

Procedures

The Devarda method[16] has been widely adopted for nitrate analysis of fertilizers[1], but its use for determination of nitrate in soils and other biological materials has been limited because of the interference by organic nitrogen compounds which decompose with formation of ammonia when distilled with sodium hydroxide. Attempts have been made to modify the Devarda procedure for determination of nitrate in solutions containing alkali-labile organic nitrogen compounds, and several modifications involving distillation with magnesium oxide instead of sodium hydroxide have been described[17–20]. However, like the original Devarda procedure, these methods are tedious and time-consuming and are subject to interference by amides and other organic nitrogen compounds.

The procedures described developed from the finding that interference by alkali-labile organic nitrogen compounds in distillation methods for ammonia can be

eliminated by performing a short steam distillation with a small amount of magnesium oxide. This is illustrated by Table I, which shows the amounts of ammonia-N liberated by steam distillation of 20-ml aliquots of solutions containing 10 μg of nitrogen per ml in the form of glucosamine or galactosamine with different amounts of magnesium oxide and sodium hydroxide. These two compounds were selected because they are exceptionally labile towards alkaline reagents and cause serious interference in most distillation methods. The data in Table I show that both compounds are extensively

TABLE I

AMOUNTS OF AMMONIA-N LIBERATED BY STEAM DISTILLATION OF HEXOSAMINES WITH ALKALINE REAGENTS

Hexosamine	*Reagent*	*Amount of reagent (g)*	*Ammonia-N liberated (% of total N) Period of distillation (min)*[a]			
			2	*R*	*5*	*10*
Glucosamine	MgO	0.1	0	0	0.5	1.2
	MgO	0.2	0	0.6	0.9	2.0
	MgO	1.0	1.0	2.5	5.0	18.0
	NaOH	0.1	80.5	82.2	84.7	85.0
	NaOH	1.0	86.4	88.5	90.2	90.8
Galactosamine	MgO	0.1	0	0	1.4	9.1
	MgO	0.2	0	0.8	2.5	10.5
	MgO	1.0	1.8	2.9	6.3	21.0
	NaOH	0.1	68.4	73.0	74.6	75.2
	NaOH	1.0	74.2	76.5	80.4	80.9

[a] Rate of distillation, *ca.* 7.5 ml/min; *R*, period used in methods described (*ca.* 3.3 min).

decomposed with formation of ammonia when steam-distilled with sodium hydroxide, but do not yield ammonia when steam-distilled with 0.1–0.2 g of magnesium oxide for 2 min, and release very little ammonia when steam-distilled with 0.2 g of magnesium oxide for 3.3 min. Tests showed that quantitative recovery of ammonium was readily obtained by steam distillation of 20 ml of ammonium sulfate solution containing 2 mg of ammonium-N with 0.1 or 0.2 g of magnesium oxide for 2 min; this indicated that it might be possible to determine nitrate and nitrite without significant interference by alkali-labile organic nitrogen compounds by steam distillation in the presence of Devarda alloy and 0.1–0.2 g of magnesium oxide, if the period of distillation required for quantitative reduction of nitrate and nitrite did not exceed 3.3 min. It was found that quantitative reduction of nitrate or nitrite could not be achieved within this period when reagent-grade Devarda alloy or the $<$ 300-mesh fraction of this alloy was used, but was readily obtained if the reagent-grade alloy was ball-milled before use. The effect of the particle size is illustrated by Table II, which also shows the effects of varying the amounts of magnesium oxide and Devarda alloy on the recoveries obtained in analysis of 20-ml aliquots of potassium nitrate solution containing 100 μg of nitrate-N per ml. Similar results were obtained using 20-ml aliquots of sodium nitrite solution containing 100 μg of nitrite-N per ml. It was found that 3.3 min were required for quantitative recovery of nitrate and nitrite using 0.2 g of magnesium oxide and 0.2 g of ball-milled Devarda alloy as recommended in the procedures described. The amount of magnesium oxide used has a marked effect on the

rate of reduction of nitrate and nitrite by reagent-grade alloy, and quantitative reduction can be obtained within 3.3 min if 1.0 g of magnesium oxide and 1.0 g of reagent-grade alloy are used. However, distillation with 1.0 g of magnesium oxide can lead to significant interference by alkali-labile organic nitrogen compounds (Table I). Quantitative recovery of nitrate and nitrite can be achieved within 3.3 min with only 0.1 g of magnesium oxide if the amount of ball-milled alloy is increased to 0.5 g (Table II). Use of 0.2 g of magnesium oxide is recommended because tests using 20-ml aliquots of soil and plant extracts showed that methods involving distillation with less than 0.2 g of magnesium oxide for 3.3 min gave low recoveries of nitrate and nitrite, even when 1.0 g of ball-milled alloy was used.

TABLE II

EFFECTS OF AMOUNTS OF MAGNESIUM OXIDE AND DEVARDA ALLOY AND OF PARTICLE SIZE OF ALLOY ON RECOVERY OF NITRATE

Amount (g)		*Particle size of alloy*[b]	*Recovery of nitrate (%)*[a] *Period of distillation (min)*[c]			
Magnesium oxide	*Devarda alloy*		*2*	*R*	*5*	*10*
0.1	0.2	RG	6	12	21	39
0.2	0.2	RG	23	40	57	86
0.5	0.2	RG	43	69	91	100
1.0	0.2	RG	63	94	100	100
0.2	0.5	RG	24	49	64	100
1.0	0.5	RG	56	97	100	100
0.2	1.0	RG	46	79	96	100
1.0	1.0	RG	92	100	100	100
0.2	0.2	10–100 mesh[d]	7	11	20	50
0.2	0.2	100–150 mesh[d]	23	39	63	97
0.2	0.2	150–300 mesh[d]	45	71	88	100
0.2	0.2	< 300 mesh[d]	68	92	100	100
0.1	0.2	BM	69	87	100	100
0.2	0.2	BM	91	100	100	100
1.0	0.2	BM	92	100	100	100
0.1	0.5	BM	91	100	100	100
0.2	1.0	BM	92	100	100	100

[a] Recoveries between 99.7 and 100.3% are reported as 100%.
[b] RG, reagent-grade alloy (100% < 10 mesh, 29% < 100 mesh, 22% < 150 mesh, 7% < 300 mesh); BM, ball-milled alloy (100% < 100 mesh, 91% < 150 mesh, 76% < 300 mesh).
[c] Rate of distillation, *ca.* 7.5 ml/min; *R*, period used in methods described (*ca.* 3.3 min).
[d] Material obtained by sieving reagent-grade alloy.

No difficulty due to foaming was experienced in analysis of soil extracts by the steam distillation methods described, but extensive frothing does occur when some plant extracts are steam-distilled with magnesium oxide and Devarda alloy. This problem can be alleviated by addition of a small amount of mineral oil or silicone spray, but this can lead to low or erratic results in determination of nitrate or nitrite. It is recommended, therefore, that use of these anti-foam reagents be avoided, and that larger flasks (150- to 250-ml capacity) be used for distillation when foaming is a problem.

Controls should be performed in each series of analyses to allow for ammonium-

N derived from the reagents used. Also, the methods should be checked at intervals by analyzing 10-ml aliquots of the standard (ammonium + nitrate)-N and (ammonium + nitrate + nitrite)-N solutions. Both solutions are stable for several months if stored in a refrigerator.

Accuracy and precision

The methods give quantitative results with 20-ml aliquots of solutions containing up to 100 μg per ml of nitrogen in the form of ammonium, nitrate, or nitrite. Their accuracy and precision are illustrated by Table III, which gives the results of 6 analyses by each method of 20-ml aliquots of a solution containing 10 μg per ml of ammonium-N [as $(NH_4)_2SO_4$], nitrate-N (as KNO_3), and nitrite-N (as $NaNO_2$).

TABLE III

ACCURACY AND PRECISION OF METHODS

Method	*μg N*			*Relative error (%)*
	Added	*Found*[a]	*Standard deviation*	
Ammonium-N	200	199	1.3	−0.5
Nitrate-N	200	199	1.5	−0.5
(Ammonium + nitrate)-N	400	399	1.6	−0.3
(Nitrate + nitrite)-N	400	398	1.9	−0.5
(Ammonium + nitrate + nitrite)-N	600	598	2.2	−0.3

[a] Average of 6 determinations.

Specificity

The specificity of the methods is illustrated by Table IV, which shows that only 2 of 69 organic nitrogen compounds tested yielded ammonia under the conditions of the procedures described (glucosamine and galactosamine in methods 1, 2, and 4). The methods were applied to an amount of compound containing 200 μg of nitrogen dissolved or suspended in 20 ml of water. If glucosamine or galactosamine are present in the sample under analysis, interference by these compounds in determination of ammonium, nitrate, and nitrite can be eliminated by use of methods 1, 3, and 5 if the period of distillation in method 1 is reduced to 2 min.

Effect of various ions

Table V shows the results obtained in studies of the effect of various cations and anions on determination of nitrate by the method recommended for determination of (ammonium + nitrate + nitrite)-N. The tolerance limit recorded for each ion represents the concentration at which the ion did not interfere with determination of nitrate in 20 ml of potassium nitrate solution containing 2 mg of nitrate-N. Similar results were obtained in studies of the effect of these ions on the determination of nitrite in 20 ml of sodium nitrite solution containing 2 mg of nitrite-N by the method for determination of (ammonium + nitrate + nitrite)-N. None of the ions listed in Table V interfered with the method for determination of ammonium, even when the concentrations of these ions greatly exceeded the tolerance limits for determination of nitrate. For example, this method was not affected when the concentration of

TABLE IV

AMOUNTS OF AMMONIA-N LIBERATED FROM VARIOUS ORGANIC NITROGEN COMPOUNDS UNDER CONDITIONS OF METHODS DESCRIBED

Compounds	*Ammonia-N liberated (% of total N) Method*[a]				
	1	*2*	*3*	*4*	*5*
Amino acids (29)[b]	0	0	0	0	0
Hexosamines:					
Glucosamine	1	1	0	3	0
Galactosamine	1	3	0	5	0
Purines (5)[c]	0	0	0	0	0
Pyrimidines (4)[d]	0	0	0	0	0
Amides (6)[e]	0	0	0	0	0
Miscellaneous (23)[f]	0	0	0	0	0

[a] Methods: 1, ammonium-N; 2, nitrate-N; 3, (ammonium + nitrate)-N; 4, (nitrate + nitrite)-N; 5, (ammonium + nitrate + nitrite)-N.
[b] Aspartic acid, glutamic acid, glycine, alanine, valine, norvaline, leucine, isoleucine, norleucine, α-amino-*n*-butyric acid, phenylalanine, tyrosine, tryptophan, cysteic acid, methionine, serine, threonine, phenylserine, hydroxylysine, arginine, lysine, histidine, ornithine, citrulline, proline, hydroxyproline, β-alanine, γ-amino-*n*-butyric acid, glucosaminic acid.
[c] Adenine, guanine, xanthine, hypoxanthine, uric acid.
[d] Thymine, uracil, cytosine, alloxan.
[e] Urea, biuret, asparagine, glutamine, nicotinamide, glycinamide.
[f] Creatine, creatinine, hydantoin, allantoin, betaine, choline, ethanolamine, dicyandiamide, nicotinic acid, glucosaminic acid, chitin, N-acetylglucosamine, hippuric acid, sarcosine, β-lactoglobulin, gelatin, casein, gliadin, glycylglycine, glycinol, alaninol, *o*-aminophenol, nucleic acid (yeast).

TABLE V

EFFECT OF VARIOUS IONS ON REDUCTION OF NITRATE

Ion	*Source*	*Molarity permitted*	*Ion*	*Source*	*Molarity permitted*
K^+	KCl	4.0[a]	Zn^{2+}	$ZnSO_4 \cdot 7H_2O$[b]	10^{-3}
Na^+	NaCl	4.0[a]	Fe^{2+}	$FeSO_4 \cdot 7H_2O$[b]	10^{-2}
Li^+	$Li_2SO_4 \cdot H_2O$	3.0[a]	Al^{3+}	$Al_2(SO_4)_3 \cdot 18H_2O$[b]	10^{-1a}
Ca^{2+}	$CaCl_2 \cdot 2H_2O$	10^{-1}	Fe^{3+}	$Fe_2(SO_4)_3$[b]	10^{-1a}
Ba^{2+}	$BaCl_2 \cdot 2H_2O$	10^{-1}	Cl^-	NaCl	4.0[a]
Mg^{2+}	$MgCl_2 \cdot 6H_2O$	10^{-2}	SO_4^{2-}	$Li_2SO_4 \cdot H_2O$	3.0[a]
Co^{2+}	$CoCl_2 \cdot 6H_2O$[b]	10^{-1a}	PO_4^{3-}	Na_2HPO_4	10^{-4}
Mn^{2+}	$MnSO_4 \cdot H_2O$[b]	10^{-4}	SiO_3^{2-}	$Na_2SiO_3 \cdot 9H_2O$	10^{-6}
Cu^{2+}	$CuSO_4$	10^{-2}	$B_2O_7^{4-}$	$Na_4B_2O_7 \cdot 10H_2O$	10^{-1a}

[a] Highest molarity tested.
[b] Used neutralized solution.

silicate or phosphate was 0.1 *M*, whereas both of these anions interfered at much lower concentrations with determination of nitrate or nitrite (Table V). The effect of phosphate on reduction of nitrate and nitrite by Devarda alloy has apparently not been noted previously, but BRABSON AND KARCHMER[17] and DROUINEAU AND GOUNY[19] found that Devarda methods involving prolonged distillation with strong alkali were affected by silica dissolved by this alkali from the glass Kjeldahl flasks used.

Application

The methods have been used mainly for analysis of extracts obtained by shaking soil samples with water or neutral salt solutions (*e.g.*, *M* K_2SO_4, 2 *M* NaCl). Their applicability to extracts of other biological materials has not been studied in detail, but they appear satisfactory for analysis of plant extracts. This is illustrated by Table VI, which shows that the methods gave quantitative recovery of ammonium, nitrate, and nitrite added to soil and plant extracts. The extracts used were prepared by shaking 10 g of soil or 0.5 g of plant leaf material with 200 ml of *M* potassium chloride, and 20-ml aliquots of extract were analyzed before and after addition of 5 ml of a solution containing 20 μg per ml of ammonium-N [as $(NH_4)_2SO_4$], nitrate-N (as KNO_3), and nitrite-N (as $NaNO_2$).

TABLE VI

RECOVERY OF AMMONIUM-N, NITRATE-N, AND NITRITE-N ADDED TO SOIL AND PLANT EXTRACTS

Extract	*Recovery of added N (%)*		
	NH_4-N	*NO_3-N*	*NO_2-N*
Soil			
Marshall silt loam	99.9	100.0	100.1
Tama silt loam	100.2	99.8	99.8
Webster clay loam	100.0	99.6	100.5
Nicollet loam	99.9	100.0	99.7
Glencoe clay loam	100.1	99.8	100.0
Plant			
Alfalfa	99.6	100.2	100.0
Soybean	100.1	99.8	99.9
Bromegrass	100.0	99.9	100.0
Corn	100.1	100.1	99.7
Oat	99.8	100.0	100.1

SUMMARY

Steam distillation methods of determining ammonium, nitrate, and nitrite in the presence of alkali-labile organic nitrogen compounds are described. They involve the use of magnesium oxide for distillation of ammonium, ball-milled Devarda alloy for reduction of nitrate and nitrite to ammonium, and sulfamic acid for destruction of nitrite. The methods are rapid, accurate, and precise, and they permit nitrogen isotope-ratio analysis of ammonium, nitrate, and nitrite in tracer studies using ^{15}N-enriched compounds. They give quantitative recovery of ammonium, nitrate and nitrite added to soil and plant extracts, and appear suitable for analysis of biological materials.

RÉSUMÉ

Les auteurs décrivent des méthodes de distillation à la vapeur pour le dosage de l'ammonium, des nitrates et des nitrites, en présence de composés organiques

azotés. On utilise l'oxyde de magnésium pour le dosage de l'ammonium, l'alliage Devarda pour la réduction des nitrates et des nitrites en ammonium, et l'acide sulfamique pour la destruction des nitrites. Ces méthodes sont rapides, exactes et précises et elles permettent des analyses lors d'études de traceurs, utilisant des composés enrichis en ^{15}N. Elles donnent des résultats quantitatifs en ammonium, nitrate et nitrite, additionnés à des extraits de sols et de plantes et semblent applicables à l'analyse de substances biologiques.

ZUSAMMENFASSUNG

Es werden Dampfdestillationsmethoden beschrieben zur Bestimmung von Ammonium, Nitrat und Nitrit in Gegenwart von alkaliumbeständigen organischen Stickstoffverbindungen. Sie verwenden Magnesiumoxyd für die Destillation des Ammoniums, Devardasche Legierung für die Reduktion von Nitrat und Nitrit zu Ammonium und Aminosulfonsäure für die Zerstörung des Nitrits. Die Methoden sind schnell, genau und reproduzierbar und sind verwendbar bei der Analyse des Stickstoffisotopenverhältnisses von Ammonium, Nitrat und Nitrit bei Tracer-Untersuchungen mit ^{15}N angereicherten Verbindungen. Das zu Boden- und Pflanzenextrakten gegebene Ammonium, Nitrat und Nitrit wurde quantitativ wiedergewonnen. Die Methoden scheinen für die Analyse von biologischem Material geeignet zu sein.

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SHORT COMMUNICATION

o-Aminobenzenethiol: an oxidimetric indicator for hydrogen ion and reagent for metals

Many organic reagents are used in analytical chemistry, but few can be used directly as titrimetric reagents. *o*-Aminobenzenethiol has been shown to react with several different metal ions[1-4] and produces with its oxidation product, bis(2-aminophenyl)-disulfide, a reversible electrode system which permits its application to titrimetric analysis. The study reported here has shown that the thiol can serve as a hydrogen ion indicator in potentiometric acid–base titrations in a manner similar to the quinhydrone system, as a precipitant in the potentiometric titration of metals and also as a reducing agent in oxidation–reduction titrations.

Experimental

The potential measurements were made with a Beckman Zeromatic pH meter using conventional glass and calomel electrodes. All potential measurements were made in a water bath at 20 ± 0.2°. Potentiometric titrations were made using a Metrohm E 336 Potentiograph with a Beckman platinum electrode as the indicator and an asbestos-fiber calomel electrode as the reference. The electrode system was standardized by comparison to the quinhydrone electrode in standard buffer solutions. *o*-Aminobenzenethiol (henceforth thiol) and bis(2-aminophenyl) disulfide (henceforth disulfide) were donated by the American Cyanamid Co.[5]; solutions of the thiol were stored in an oxygen-free reservoir system which consisted of a large Erlenmeyer flask (as reservoir) and a 20-l carboy (as pressure tank) connected with glass tubing to the automatic buret of the potentiograph. The entire system was flushed and closed with a slight positive pressure of nitrogen. When in use the thiol solution could be transferred to the reaction vessel without exposure to the atmosphere. The sensitivity of the thiol to atmospheric oxidation required this system and the titer of the thiol remained constant for a month when protected in this manner. Thiol solutions were standardized by titration to the starch end-point with triiodide[5]. The concentration of thiol used for all of the potentiometric titrations was 0.03224 ± 0.00003 *M*.

In the case of potential measurements, solutions were prepared which were saturated with respect to the thiol and the disulfide and allowed to equilibrate at 20° before measuring. Oxygen was removed by flushing with nitrogen before the pH or potential was measured. The pH was changed by adding solutions of hydrochloric acid or sodium hydroxide. Both increasing and decreasing changes in pH were made. Figure 1 contains the data obtained. In addition to the study on saturated solutions, potential and pH measurements were made with solutions which were unsaturated with respect to the thiol, although, because of its extreme insolubility they were always saturated with the disulfide as a minute atmospheric oxidation would produce a sufficient quantity for the purpose. In order to hold the ionic strength approximately constant for these studies an aliquot of an acidic solution of the thiol was diluted with 1 *M*

potassium chloride. When the pH fell within the region where the Nernst equation was valid, the thiol concentration could be calculated from the measured potential. The pH was then increased by gradual addition of 1 *M* sodium hydroxide. The data are given in Fig. 2.

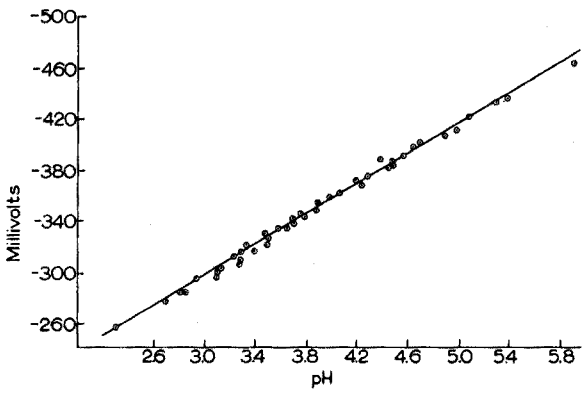

Fig. 1.

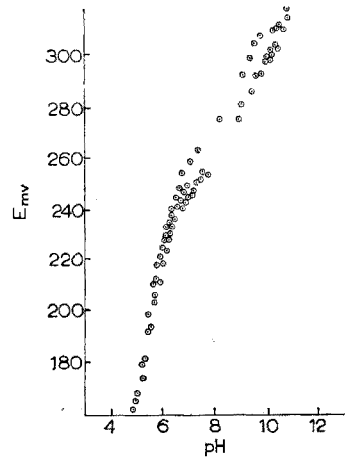

Fig. 2.

Numerous metal ions are either precipitated or reduced by the thiol; of these silver, copper, lead, zinc, cadmium and nickel were determined successfully by potentiometric titration (Table I).

The thiol can also be used in place of sodium thiosulfate in indirect iodimetric titrations and a standard brass sample (NBS 37d) was analyzed for copper. The solution containing the brass sample was buffered with ammonium bifluoride to pH 3.5. Excess potassium iodide was added and the triiodide produced was titrated with standard thiol. The results of 6 determinations gave an average value of 70.5% copper with a standard deviation of 0.4% compared to the reported value of 70.78%.

TABLE I

POTENTIOMETRIC TITRATION DATA FOR METAL IONS

Metal	*No. of detns.*	*Av. found (mg)*	*Std. dev.*	*Reference analysis*	
				Metal found (mg)	*Method*
Ag^{+}	14	12.1	0.1	12.0	Primary standard silver nitrate
Cu^{2+}	5	88.5	0.3	88.2	Primary standard electrolytic Cu
Ni^{2+}	4	46.0	0.2	46.0	Dimethylglyoxime
Pb^{2+}	6	323.2	0.6	324.4	Sulfate
Cd^{2+}	5	96.2	0.2	96.7	Pyrophosphate
Zn^{2+}	6	58.5	0.2	58.4	Ammonium phosphate

Discussion

The plot of pH *vs.* potential (Fig. 1) shows that the Nernst equation is valid for the thiol–disulfide couple through the pH range 2.5–5.5. As the acid dissociation constant of the thiol[6] is $1.6 \cdot 10^{-7}$, the extent of ionization above pH 5.5 becomes important and the line deviates significantly from that predicted by the Nernst equation. At pH values below 2 the amine group of the thiol becomes protonated and the measured potential becomes a function of ionic strength which also results in a deviation from the straight line. A least squares fit of all the data from pH 2.2 to 5.8 gave a line with an intercept of +0.119 V. This corresponds to an $E°$ of +0.127 V for the reaction:

$$NH_2C_6H_4S\text{–}SC_6H_4NH_2(\text{solid}) + H_2 = 2NH_2C_6H_4SH(\text{solid})$$

The slope of the line is 0.0587 V per equivalent at 20°. The solutions were saturated with the thiol and the disulfide and an excess of both substances was always present. As the pH could be varied at will within the limits 2.2 to 5.5 without causing a deviation from the straight line, the electrode couple can be considered reversible when the solid thiol and disulfide are the reference compounds. Above pH 5.5 the solubility of the thiol becomes too great for the convenient use of saturated solutions. Consequently, potential measurements in alkaline solutions are necessarily functions of ionic strength and concentration. Figure 2, which shows the data obtained from potential measurements at the higher pH values, shows the deviation from the straight line, but it also demonstrates the reproducibility of the potential measurements when the pH is changed while the total thiol concentration is kept constant. Because of this behavior, titrations can be made with either acids or bases, where the thiol–disulfide couple acts as a hydrogen ion indicator; and titrations are possible where the thiol acts as a reagent for metal ions and the couple serves as a redox indicator, even in pH regions where the couple cannot be considered to be reversible.

Acid–base titrations were made with the couple and a platinum wire as the indicator electrode. A small amount of the thiol was added and in each case sufficient disulfide was present to activate the electrode couple. For example, 5 titrations of 5-ml portions of *ca.* 0.1 *M* hydrochloric acid with standard sodium hydroxide gave a value of 0.0975 ± 0.0005 *M* for the acid. Also, approximately 0.1 *M* ammonium hydroxide and acetic acid were successfully titrated with standard hydrochloric acid and sodium hydroxide. In all cases well formed titration curves were obtained with potential breaks of 200 to 250 mV.

When the thiol was used as a reagent for metals it was necessary to buffer the solutions at some predetermined pH value. For example, it was found that silver ion could be titrated quantitatively at pH values of 3 to 10. In the region 3 to 8 the titration curve showed a double inflection but above 8 only a single inflection point was found, coinciding with the equivalence point of the titration. In the more acid region the second inflection point coincided with the equivalence point. Table I contains the analytical data and shows the accuracy and precision of the results of the silver titration made at pH 9.7 in an ammonium borate buffer. Table I also gives the data for the titration of copper, nickel, lead, cadmium and zinc, all of which were made in an ammonium acetate buffer at pH 7. The results of a referee analysis of each metal are also given and the two methods are seen to be in good agreement.

Many metal ions react with the thiol and studies are being carried out to ex-

tend the number of substances which can be determined, either by direct or indirect means.

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BUCHBESPRECHUNG

HANS TOLLERT, *Analytik des Kaliums. Die chemische Analyse*, Bd. 51, Ferdinand Enke Verlag, Stuttgart, 1962, 416 S., Geheftet DM 75.—. Ganzl. DM 79.—.

Der Verfasser hat es unternommen, die umfangreiche Literatur über die Analyse des Kaliums im Zusammenhang darzustellen, ein schwieriges und mühsames Unterfangen, weil die zahlreichen zu berücksichtigenden Veröffentlichungen (etwa 100 Literaturstellen werden zitiert) im Schrifttum weit verstreut sind und weil ferner die Meinung verschiedener Autoren über bestimmte Verfahren keineswegs immer übereinstimmt. Die grosse Bedeutung des Kaliums in verschiedenen Bereichen und seine besonderen analytischen Eigenschaften sind Ursache gewesen, dass zahlreiche verschiedene Methoden recht unterschiedlicher Bedeutung vorgeschlagen worden sind. Neben exakten Verfahren existierten andere, die richtige Werte nur unter bestimmten Arbeitsbedingungen infolge Fehlerkompensation liefern, die aber trotzdem in besonderen Fällen Bedeutung haben können. Neben den klassischen Verfahren, Gravimetrie und Massanalyse, haben in den letzten Jahren zunehmend Methoden physikalischer Natur Bedeutung gewonnen, wie Flammenphotometrie, Röntgenfluoreszenzanalyse oder Messung der Radioaktivität. Der Verfasser hat versucht, alle diese verschiedenartigen Verfahren hinsichtlich ihrer Bedeutung, Genauigkeit und Empfindlichkeit zu charakterisieren. Für den Benutzer ist dies ohne Zweifel von grossem Nutzen, selbst wenn er schliesslich der Meinung des Verfassers nicht in allen Fällen zustimmen wird.

Der 1. Teil des Buches enthält auf 96 S. eine Besprechung der physikalischen und physikalisch–chemischen Bestimmungsmethoden. Im 2. Teil sind die nasschemischen Fällungs- und Trennungsverfahren auf etwa 150 S. besprochen worden, entsprechend der Bedeutung dieser Verfahren bis in Einzelheiten. Für den Praktiker wird der 3. Teil, Anwendungen auf bestimmte Problemstellungen, von besonderem Interesse sein.

Die Beschreibung der einzelnen Methoden ist meist so vollständig, dass auf ein Heranziehen der Originalliteratur verzichtet werden kann. Es ist dies zweifellos ein besonderer Vorteil des Buches, doch hätte es nicht an Wert verloren, wenn manche Dinge kürzer gefasst worden wären. Hierzu gehört die relativ breite Behandlung von Verfahren, die nur vorübergehend von Interesse zu sein schienen und heute nur noch historische Bedeutung besitzen (wie z.B. die Bestimmung des Kaliums als K-perrhenat). Zu den Verfahren, die kaum praktische Bedeutung für die Analyse des Kaliums besitzen dürften, zählen auch die Papierchromatographie und die Ringkolorimetrie. Nach Meinung des Referenten genügt hier eine Erwähnung im Interesse der Vollständigkeit. Selbst eine Beschränkung der Darstellung auf wenige Seiten scheint noch zuviel zu sein. Bei der Beschreibung von Verfahren physikalischer Natur geht der Verfasser für ein Werk, das der Analyse nur eines Elementes gewidmet ist, oft zu sehr in Einzelheiten ein. Bemerkungen zur Geschichte der Flammenphotometrie u.a. hätten sich mit einem Hinweis auf entsprechende Spezialwerke erledigen lassen. Leider findet man eine nicht unerhebliche Zahl von Unexaktheiten des Ausdrucks und unsauberen Formulierungen; Wortbildungen wie "Äthanolüberchlorsäuren" für eine Lösung von Perchlorsäure in Äthanol sind unschön. Der Verfasser spricht von "Austauscherharz, das mit Wasserstoff gesättigt ist" und meint Wasserstoff-Ionen. Bei Angabe von molarem Extinktionsmoduls fehlt mehrfach die Angabe der zugehörigen Wellenlänge. Diese und weitere ähnliche Ungereimtheiten sowie eine Reihe von Druckfehlern, hätten bei sorgfältiger Korrektur des Manuskriptes vermieden werden müssen. Gerade im Zusammenhang mit diesen kritischen Bemerkungen muss aber ausdrücklich betont werden, dass das Buch in seiner Gesamtheit jedem an der Analytik des Kaliums interessierten Chemiker nur nachdrücklich empfohlen werden kann, denn es ist hier die bekannte Literatur bis zum Jahre 1962 praktisch vollständig erfasst und nach sachlichen Gesichtspunkten geordnet, wobei auch die im Ostraum erschienenen Veröffentlichungen, die oft schon aus sprachlichen Gründen schwer zugänglich sind, berücksichtigt wurden.

H. BODE (Hannover)

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ANNOUNCEMENT

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For further information apply to the Chairman of the Meeting, Dr. G. PARISSAKIS, Professor of the Technical University of Athens, 42 Patission street, Athens, Greece, or to: G.A.M.S., 1 Rue Gaston Boissier, Paris 15e, France.

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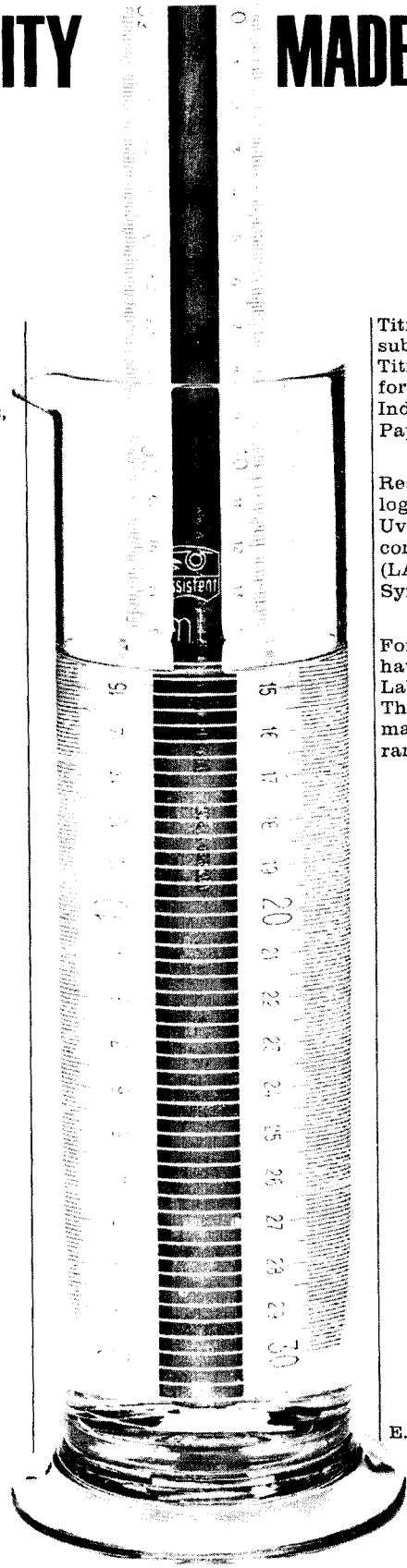

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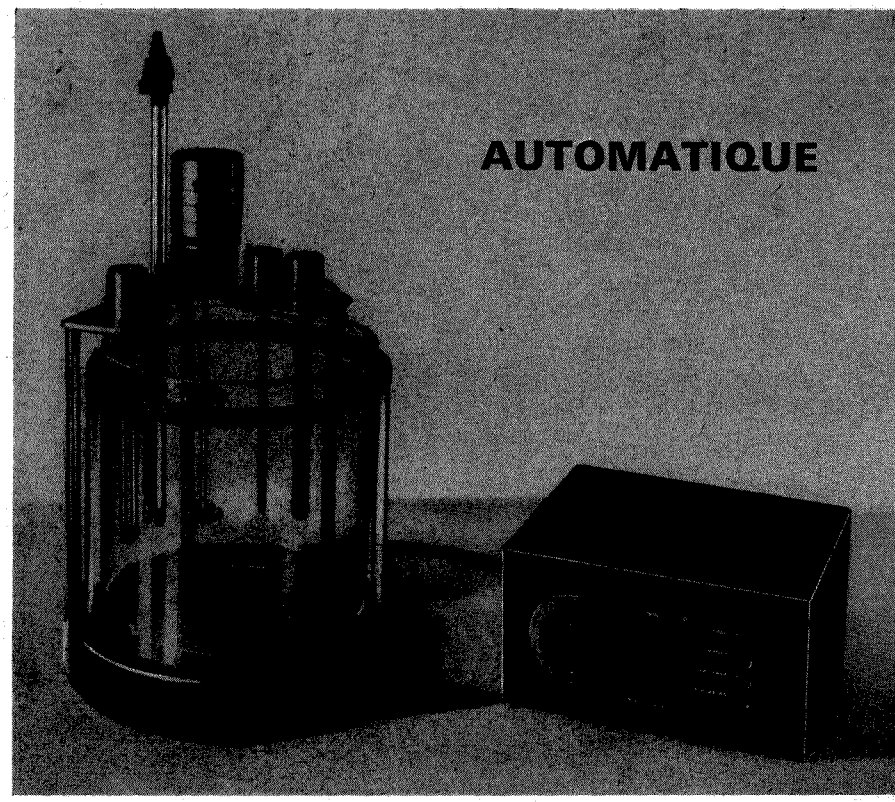

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