ANALYTICA CHIMICA ACTA

International monthly devoted to all branches of analytical chemistry
Revue mensuelle internationale consacrée à tous les domaines de la chimie analytique
Internationale Monatsschrift für alle Gebiete der analytischen Chemie

Editors

PHILIP W. WEST *(Baton Rouge, La., U.S.A.)*

A. M. G. MACDONALD *(Birmingham, Great Britain)*

Editorial Advisers

R. BELCHER, *Birmingham*
F. BURRIEL-MARTÍ, *Madrid*
G. CHARLOT, *Paris*
C. DUVAL, *Paris*
G. DUYCKAERTS, *Liége*
W. T. ELWELL, *Birmingham*
F. FEIGL, *Rio de Janeiro*
W. FISCHER, *Hannover*
G. GORBACH, *Graz*
M. HAISSINSKY, *Paris*
J. HEYROVSKY, *Prague*
M. ISHIDATE, *Tokyo*
M. JEAN, *Paris*
W. KIRSTEN, *Uppsala*
H. A. LAITINEN, *Urbana, Ill.*
M. LEDERER, *Rome*
H. MALISSA, *Vienna*
D. MONNIER, *Geneva*
H. A. J. PIETERS, *Geleen*
F. REIMERS, *Copenhagen*
A. RINGBOM, *Abo*
J. W. ROBINSON, *Baton Rouge, La.*
Y. RUSCONI, *Geneva*
E. B. SANDELL, *Minneapolis, Minn.*
W. SCHÖNIGER, *Basel*
A. A. SMALES, *Harwell*
W. I. STEPHEN, *Birmingham*
P. F. THOMASON, *Oak Ridge, Tenn.*
A. TISELIUS, *Uppsala*
H. WEISZ, *Freiburg i. Br.*
J. H. YOE, *Charlottesville, Va.*

ELSEVIER PUBLISHING COMPANY
AMSTERDAM

Anal. Chim. Acta, Vol. 31, No. 4, p. 301–404, October 1964

GENERAL INFORMATION

Languages

Papers will be published in English, French or German.

Submission of papers

Papers should be sent to: Prof. PHILIP W. WEST, Coates Chemical Laboratories, College of Chemistry and Physics, Louisiana State University, Baton Rouge 3, La. (U.S.A.)

or to

Dr. A. M. G. MACDONALD, Department of Chemistry, The University, Edgbaston, Birmingham 15 (Great Britain)

Authors should preferably submit two copies in double-spaced typing on pages of uniform size. Tables and legends for figures should be typed on a **separate** page. The figures should be in a form suitable for reproduction, drawn in Indian ink on drawing paper or tracing paper, with lettering etc. in **thin pencil.** The sheets of drawing or tracing paper should preferably be of the same dimensions as those on which the article is typed. Photographs should be submitted as clear black and white prints on glossy paper.

Standard symbols should be used in line drawings. The following are available to the printers:

All references should be given at the end of the paper. They should be numbered and the numbers should appear in the text at the appropriate places. The abbreviations of journals should conform to those adopted by the *Chemical Abstracts List of Periodicals*, 1961 Edition. A summary of 50 to 200 words should be included. Authors of papers in French or German are encouraged to supply also a translation of the summary in English.

Reprints

Twenty-five reprints will be supplied free of charge. Additional reprints can be ordered at quoted prices. They must be ordered on order forms which are sent together with the proofs.

Publication

Analytica Chimica Acta has six issues to the volume, approx. 600 pages per volume, two volumes per year.

Subscription prices (post free): $ 15.— or £ 5.7.6 or Dfl. 54.— per volume; $ 30.— or £ 10.15.- or Dfl. 108.— per year. Additional cost for copies by airmail available on request. For advertising rates apply to the publishers.

Subscriptions

Subscriptions should be sent to:

ELSEVIER PUBLISHING COMPANY, P.O. Box 211, Amsterdam, The Netherlands

SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 31, No. 4, October 1964

PRECAUTIONS IN THE UTILIZATION OF THERMOBALANCES

(in French)

Based on 25 years of experience of the pyrolysis of several thousands of inorganic and organic substances with reference to gravimetric analysis, studies of volumetric standards and studies of solid-state reactions, the author attempts to explain the disagreements in results obtained by users of various models of thermobalances. These differences are traced to 10 main causes: the type of apparatus, the thermocouple, the nature of the substance involved, the weight of this substance, the rate of heating, the nature of the atmosphere around the crucible, the gas flow-rate in the furnace, the nature and shape of the crucible, the sensitivity of the recorder trace, and the nature and weight of the residue.

C. DUVAL,

Anal. Chim. Acta, 31 (1964) 301–314.

IMPROVED GRAVIMETRIC DETERMINATION OF COBALT AS $K_3Co(NO_2)_6$

The gravimetric determination of cobalt based on the precipitation of $K_3Co(NO_2)_6$ becomes highly accurate when the precipitation is begun in a *boiling hot* solution. Under this condition the precipitate is pure $K_3Co(NO_2)_6$, and, correspondingly, quantities of cobalt from a few milligrams up to 175 mg are easily determinable with an error of less than 0.1 mg.

J. J. LINGANE,

Anal. Chim. Acta, 31 (1964) 315–317.

SPECTROPHOTOMETRIC STUDY OF N,N'-BIS(*m*-SULPHOBENZYL)DITHIOOXAMIDE AS A REAGENT FOR NICKEL(II)

N,N'-Bis(*m*-sulphobenzyl)dithiooxamide forms a water-soluble intensely red chelate with nickel(II) in buffered solutions at pH 10. A 1Ni : 1R complex is obtained. The absorption maximum of the complex lies at 500 mμ, where the absorbance of $(SB)_2DTO$ is negligible. The molar extinction coefficient is *ca.* 6962. Beer's law is obeyed over the range from 0.5 μg to 50 μg per ml.

A. A. JANSSENS, G. L. VAN DE CAPPELLE AND M. A. HERMAN,

Anal. Chim. Acta, 31 (1964) 325–330.

SPECTROPHOTOMETRIC DETERMINATION OF MANGANESE WITH N,N,N',N'-TETRAKIS(2-HYDROXYPROPYL)-ETHYLENEDIAMINE

A new spectrophotometric method for the determination of manganese with N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine has been developed. The sensitivity is 0.12 μg of Mn per cm^2 for log I_0/I = 0.001 and Beer's law is obeyed between 2 and 100 p.p.m. of manganese. An ion-exchange separation of interfering ions was satisfactory for the analysis of a variety of National Bureau of Standards samples with good accuracy. The standard deviation with a Mn-bronze was 1.25%.

L. PIKE AND J. H. YOE,

Anal. Chim. Acta, 31 (1964) 318–324.

A STUDY OF WINKLER'S METHOD FOR THE DETERMINATION OF AMMONIA

(in German)

A detailed study of WINKLER's method shows that the partial pressure of ammonia in a 0.1 or 0.01 *N* solution for analysis is significantly lowered only by a boric acid content of 4%; under such conditions there is no danger of loss of ammonia. However, the ammonia can be determined with greater certainty, speed and accuracy by direct titration without addition of boric acid. In the titration of boric acid-free ammonia solutions, the equivalence point lies within the pH range of methyl red; if boric acid (4%) is present, methyl orange should be used. Literature data on the choice of indicator are shown to be false.

E. SCHULEK, J. TROMPLER, H. E. ROKOSINYI, L. I. KONKOLY THEGE UND E. PUNGOR,

Anal. Chim. Acta, 31 (1964) 331–340.

2,3,5,6-TETRAKIS-(2'-PYRIDYL)-PYRAZINE AS A COLORIMETRIC REAGENT FOR IRON

The reactivity of the tridentate organic ligand, 2,3,5,6-tetrakis-(2'-pyridyl)-pyrazine, toward hydrogen ion and transition metal ions was investigated. The purple color of the bis-2,3,5,6-tetra-(2'-pyridyl)-pyrazine–iron(II) complex can be used in a colorimetric method for iron(II). The complex in aqueous solution exhibits a wavelength of maximum absorption at 575 mμ and a molar absorptivity of 19,800 in the pH range of 3.0–7.0. Beer's law is obeyed over the concentration range $5.0–100 \cdot 10^{-6}$ *M*. The perchlorate salt of the complex is extractable into chloroform and nitrobenzene with absorption maxima of 570 mμ and 573 mμ and molar absorptivities of 19,200 and 17,400, respectively. The effects of diverse ions and the results of determinations on selected samples are discussed.

R. T. PFLAUM, C. J. SMITH, JR., E. B. BUCHANAN, JR. AND R. E. JENSEN,

Anal. Chim. Acta, 31 (1964) 341–347.

THE COMPOSITION OF THE ZIRCONIUM–ARSENAZO COMPLEX

There is discordant information in the literature on the composition of the zirconium–arsenazo complex in solution. A detailed spectrophotometric study indicates that at pH 1.3, 1.7 and 3.5 the complex is formed by one mole of zirconium reacting with one mole of arsenazo (R), giving the empirical formula ZrR.

H. ONISHI AND H. NAGAI,

Anal. Chim. Acta, 31 (1964) 348–351.

CHELATES OF NITROSYLRUTHENIUM(III) IONS WITH NITROSONAPHTHOLS

(in German)

A spectrophotometric study of the reactions of nitrosylruthenium(III)-ion with nitrosonaphthols in dilute nitric and hydrochloric acid media is described. The complexes formed contain Ru:R ratios of 1:1 to 1:3, depending on the hydrogen ion concentration and on the molar ratios of the complex-forming species. Mixtures are usually obtained.

C. KONEČNÝ,

Anal. Chim. Acta, 31 (1964) 352–358.

THIN-LAYER CHROMATOGRAPHIC SEPARATION AND ANALYSIS OF POLYNUCLEAR AZA HETEROCYCLIC COMPOUNDS

Many thin-layer chromatographic systems can be used for the general separation of aza heterocyclic compounds from polynuclear aromatic hydrocarbons. Several can be used for the separation of the aza compounds from each other. With alumina as the adsorbent the sterically hindered aza heterocyclic compounds can be readily separated from the non-hindered aza compounds.

The use of the trifluoroacetic acid spot test on the thin-layer plate is discussed. Spectral data for the aza compounds are presented and discussed.

E. SAWICKI, T. W. STANLEY, J. D. PFAFF AND W. C. ELBERT,

Anal. Chim. Acta, 31 (1964) 359–375.

THE PAPER CHROMATOGRAPHIC ISOLATION OF NUCLIDES IN AIR SAMPLES

A method is outlined for the quantitative transfer of nuclides of strontium, barium, cerium, zirconium, lanthanum, yttrium, and niobium from solution to a paper for chromatographic separation. Carrier quantities of iron are precipitated as the hydroxide from a carbonate solution and filtered directly onto the chromatographic paper. A two-dimensional development is utilized to isolate the individual nuclides.

This separation procedure is applied to air dust samples containing nuclear debris. Radiometric determinations may be conveniently made by γ-spectrometric or conventional low-background β-measurements.

G. A. WELFORD AND E. L. CHIOTIS,

Anal. Chim. Acta, 31 (1964) 376–381.

PRECIPITATION OF ZINC SULFIDE BY HYDROLYSIS OF THIOACETAMIDE IN THE PRESENCE OF HYDRAZINE HYDROCHLORIDE

Zinc sulfide can be precipitated quantitatively from solutions buffered at pH 2 using thioacetamide–hydrazine hydrochloride mixtures as precipitants. Optimum results are obtained using equimolar quantities of thioacetamide and hydrazine hydrochloride. Coprecipitation of cobalt sulfide was studied using citrate–citric acid, and sulfate–bisulfate buffers. Optimum separations are achieved using a sulfate–bisulfate buffer containing ammonium thiocyanate.

R. B. HAHN AND D. L. PRINGLE,
Anal. Chim. Acta, 31 (1964) 382–385.

DETERMINATION OF SERUM LACTIC DEHYDROGENASE BY AN AUTOMATIC REACTION-RATE METHOD

An automatic spectrophotometric reaction-rate method is described for the determination of lactic dehydrogenase (LDH) in blood serum. The method is based on the oxidation of lactic acid in the presence of LDH and diphosphopyridine nucleotide (DPN) to form an absorbing species DPNH. The time required for a small fixed (about 0.06 unit) change in absorbance is measured automatically and related to the LDH concentration. Automatic results obtained on samples containing 0.05 to 0.2 ml of serum show good proportionality and a coefficient of variation of 1.3%. Serum samples (0.2 ml) are analyzed at a rate of 15 to 20 per hour.

T. P. HADJIIOANNOU AND P. L. SANTOS,
Anal. Chim. Acta, 31 (1964) 386–393.

A SIMPLE CELL ATTACHMENT FOR SPECTROPHOTOMETRIC TITRATIONS

(Short Communication; in French)

Y. LE DUIGOU AND A. BRÜCK,
Anal. Chim. Acta, 31 (1964) 394–395.

ELECTROGRAVIMETRIC DETERMINATION OF LEAD AS LEAD DIOXIDE

(Short Communication)

R. L. CARMAN III AND J. J. MARKHAM,
Anal. Chim. Acta, 31 (1964) 395–396.

LOW-TEMPERATURE DIFFERENTIAL THERMAL ANALYSIS

(Short Communication)

M. M. MARKOWITZ AND D. A. BORYTA,
Anal. Chim. Acta, 31 (1964) 397–399.

SPECTROPHOTOMETRIC DETERMINATION OF CATECHOLS WITH 4-AMINOANTIPYRINE

(Short Communication)

T. A. LARUE AND E. R. BLAKLEY,
Anal. Chim. Acta, 31 (1964) 400–403.

PRECAUTIONS A PRENDRE DANS L'EMPLOI DES THERMOBALANCES*

CLÉMENT DUVAL

Laboratoire de Recherches Micro-analytiques, 11 Rue Pierre Curie, Paris (France)

(Reçu le 3 janvier, 1964)

La thermogravimétrie a maintenant soixante années. C'est en effet en 1903 que NERNST associant avec RIESENFELD[1], un four électrique à la microbalance qui porte son nom, réalisa le moyen de déterminer la masse d'un corps pendant le chauffage. Puis, BRILL[2] construisit la première courbe de thermolyse sur les carbonates de calcium, de baryum et de magnésium, *chauffés d'une manière continue*, jusqu'à 1200°. Par la suite, en 1915, HONDA[3] devait proposer le mot thermobalance et un livre récent[4] indique, à la date du 1er janvier 1961, la publication de 2400 mémoires sur la question. Il faut en ajouter 560 autres depuis.

Il existe actuellement (fin 1963) 53 modèles de thermobalances dont 12 figurent sur le marché. Elles se classent en 3 grands groupes: celles de zéro, celles à déviation de fléau, celles de torsion; certaines sont manuelles, certaines sont automatiques, mécaniques ou électroniques; les unes tracent un enregistrement continu de la perte de poids en fonction de la température ou du temps, les autres en donnent la dérivée, d'autres encore associent la courbe de thermopesée avec la courbe d'analyse thermique différentielle. Pour quelques modèles, l'opération de thermolyse a lieu dans l'air sous la pression atmosphérique, pour d'autres, elle a lieu sous 20 atmosphères ou bien encore sous 10^{-5} mm de mercure ou encore dans un gaz autre que l'air; les vitesses de chauffe ou de refroidissement sont presque toujours linéaires et varient entre 0.5°/min à 600°/h; on peut aussi tracer des réseaux d'isothermes, opérer dans une boite à gants dans le cas de produits radioactifs, etc.

Il semblerait donc logique de comparer les avantages et les inconvénients des diverses thermobalances, leurs performances, leurs limites d'erreurs, etc. Nous n'en sommes pas encore là. Certains appareils sont sortis trop récemment et leurs quelques usagers se contentent, pour l'instant, d'accumuler les enregistrements. Il n'y a guère que les prix des catalogues à comparer.

Les thermobalances Chevenard ayant été les premières sur le marché, ayant retenu l'attention de plusieurs centaines d'acheteurs et, par suite, ayant produit le plus d'enregistrements bons et mauvais, sont nécessairement celles sur lesquelles on possède le plus de données critiques.

Cependant, je n'ai pas pu jusqu'ici obtenir les mêmes renseignements sur *quatre* thermobalances commerciales différentes relativement à une même préparation d'un

* Dédié à Monsieur le Professeur Denys Monnier pour son soixantième anniversaire.

même corps, chauffé à poids égal avec la même vitesse de chauffe, ce qui m'a fait dire *que la thermogravimétrie était encore en enfance et qu'elle perçait sa première dent.* Les températures auxquelles un corps se décompose ou semble se décomposer ne constituent pas un point fixe comme une température de fusion. Dix paramètres (et même sans doute douze) paraissent être indispensables à considérer. C'est ce que je vais essayer de faire en profitant des critiques qui m'ont été personnellement adressées.

Je rappelle tout d'abord que notre travail initial a été effectué dans un but de chimie analytique sur les précipités de la chimie minérale proposés en gravimétrie. La plupart des résultats ont été publiés depuis 1947 dans *Analytica Chimica Acta.* Les expériences ont toujours porté sur 200 à 300 mg de précipité humide, chauffé avec la plus grande vitesse possible de façon à se rapprocher du mode opératoire réel de la gravimétrie et en utilisant des creusets d'aluminite. On ne peut donc pas comparer, comme certains l'ont fait, la dissociation d'un cristal de calcite taillé en cube avec celle de carbonate de calcium obtenu transitoirement par dissociation de l'oxalate. De même, il est difficile de trouver une analogie entre la thermolyse de l'oxalate de lanthane chauffé dans le vide ou sous la pression atmosphérique; ce serait méconnaître la loi d'action de masse. On ne peut comparer que des choses comparables. Nous allons passer en revue les principaux facteurs en les discutant et en indiquant, au besoin, des remèdes aux insuccès. On verra que certaines critiques ne sont pas justifiées.

La marque de thermobalance

La plupart des thermobalances commerciales possèdent un fléau avec couteau (d'agate, d'acier, etc.); seules les thermobalances Chevenard font usage d'une suspension bifilaire. Ces dernières sont capables de fournir la même courbe, à 25 ans d'écart, même si l'on a cassé et remplacé les fils entre temps. Un couteau prismatique s'use et use son support; le tranchant s'émousse ou s'arrondit, ce qui peut devenir grave pour des enregistrements durant plusieurs jours, surtout dans une usine où règnent des trépidations. Il n'est pas sûr que pendant la pose, il conserve la même position. Il faut attendre le recul du temps pour pouvoir en juger. Je rappelle cependant que dès 1936 à Imphy, on faisait des enregistrements de contrôle s'effectuant sans interruption pendant 40 jours!

Interviennent aussi dans la construction: la position du four renversé ou non, au-dessus ou au-dessous de la balance, la position du couple thermo-électrique, la vitesse du courant d'air, le mode d'enregistrement, les amortisseurs s'il en existe, facteurs étudiés plus loin. Je crois qu'il est très difficile de comparer les balances des deux types, à déplacement de fléau et les appareils de zéro qui reposent sur des principes si différents et qui n'envisagent pas les frottements sous le même aspect ainsi que les balances à ressort qui obéissent à une loi différente et qui, généralement, ne fonctionnent pas dans l'air, sous la pression atmosphérique et les balances différentielles qui suppriment à peu près totalement l'influence des courants de convection.

Une thermobalance est généralement étalonnée à froid par adjonction sur un plateau ou une petite plateforme fixée à la tige porte-creuset d'un poids marqué; cela produit, soit une encoche plus ou moins profonde dans la courbe, soit le passage dans le champ du viseur d'un certain nombre de divisions, soit la création d'un courant induit; il semble difficile de comparer ces résultats. L'étalonnage n'est pas le

même pour toutes les températures et pour toutes les charges. La courbe de la Fig. 1 qui se rapporte à 300 mg d'oxalate de calcium, tracée par NEWKIRK[5] montre que le déplacement le long des 4 paliers reste bien constant, sur thermobalances de Chevenard inscrivant à la plume, à la vitesse de 300°/h, lorsqu'on ajoute une surcharge de 20 mg. Je crois que ceci reste vrai — toutes choses égales d'ailleurs — quand on ne dépasse pas la moitié de la charge permise, soit 5 g pour le creuset et son contenu.

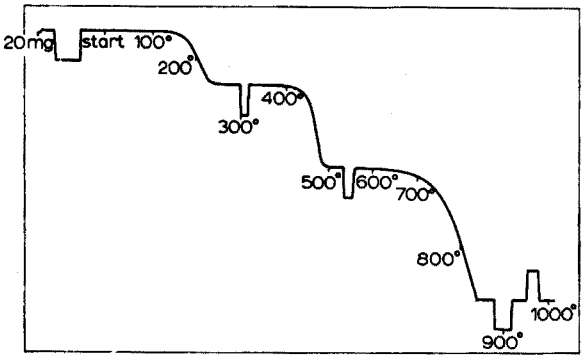


Fig. 1. Effet d'une surcharge de 20 mg le long des paliers de la courbe de pyrolyse de l'oxalate de calcium.

Lorsque la courbe température–temps n'est pas une droite (et cela dépend des dispositifs programme de l'appareil) dans le domaine 0–1200°, il est bon, comme l'ont fait divers auteurs (PAPAILHAU, ERDEY, etc.) d'inscrire la courbe d'échauffement sur le même papier que les courbes de thermolyse. Dans le système Chevenard–Joumier, un mouvement d'horlogerie règle ce programme. On peut donc en attendre une grande régularité. Dans la plupart des autres dispositifs, une pièce appropriée tourne sous l'action d'un moteur synchrone et l'on admet qu'il est alors toujours synchrone, ce qui n'était pas le cas en France pendant la deuxième guerre mondiale.

Sur le couple thermo-électrique

Nous distinguerons 4 choses: la nature, l'étalonnage, la position, les corrections. Jusqu'ici, les utilisateurs de thermobalances se sont servis du système platine–platine rhodié à 10 ou 20% et du couple chrome–nickel. Tout dépend du prix de vente de l'appareil et du domaine de chauffage. Dans le premier cas, la courbe d'étalonnage est une parabole très ouverte jusqu'à 200°, une droite ensuite. Dans certaines thermobalances, l'étalonnage est réalisé par le constructeur, mais le plus souvent, l'opérateur utilise les points d'ébullition de l'eau, de l'aniline, les points de fusion de l'étain, du plomb, le point d'ébullition du soufre, les points de fusion de l'antimoine, du chlorure de sodium, de l'argent et de l'or. La source froide n'est pas toujours disposée dans la glace fondante, mais dans un bloc métallique qui suit les fluctuations de température de la pièce. Pour économiser le platine, cette source "froide" est souvent placée assez près du four, aussi sa température propre doit elle être suivie et notée au cours du chauffage afin de corriger en plus ou en moins les nombres lus sur la courbe d'étalonnage. Une telle correction n'est valable que pour quelques

degrés. Elle est illusoire si l'étalonnage ayant été fait à 15°, la température de la pièce s'élève à 30°. L'étalonnage doit se faire avec la soudure chaude disposée dans sa gaine de silice protectrice et celle-ci immergée de moitié dans le produit d'étalonnage et sans en toucher les parois. Dans le cas de l'or, on peut économiser le métal en notant la température de coupure d'un fil entourant la gaine et parcouru par un courant électrique tout en étant disposé dans le four. Au cours du temps, les propriétés chimiques, mécaniques et électriques de la soudure et des fils s'altèrent. Il faut étalonner de temps en temps, par exemple, tous les ans, dans le cas d'un chauffage quotidien. En général, dans une thermobalance, on ne détermine pas la température de la substance, mais celle d'une couche d'air au voisinage du creuset. Cela n'a aucune importance dans le cas des dosages automatiques de l'auteur. Dans quelques modèles, la soudure chaude vient au contact du creuset, comme dans le "Derivatograph". Avec les balances à déflection, il est difficile de noyer la soudure chaude dans le produit du creuset. Cela est à la rigueur possible dans les balances de zéro, mais un danger se présente du fait de l'altération du platine par les produits dégagés (acide fluorhydrique, arsenic, etc.) ou fondus (nitrate de potassium, chlorates, etc.).

Notons aussi que si l'on veut disposer en thermostat un four à programme qui est préalablement le siège d'un échauffement continu, il est bon, à cause de l'inertie calorifique de ce four, d'arrêter la montée de température, une dizaine de degrés avant celle que l'on désire garder fixe.

La nature de la substance

Nous avons donné dans nos publications, depuis 1947, en règle générale, le mode opératoire ou tout au moins le principe de la méthode d'obtention des précipités chauffés. Même si un corps se trouvait tout préparé dans le commerce, comme le sulfate de baryum, nous l'avons toujours réalisé et lavé en suivant les indications des traités d'analyse. Les minéraux doivent être indiqués avec le lieu de leur provenance à cause des impuretés variables qu'ils sont susceptibles de renfermer. En général, on n'obtient pas la même courbe avec un produit gardé depuis des années dans les placards d'une collection minéralogique et le même minéral tel qu'il vient d'être extrait de la mine. En effet, au cours du temps, il durcit; il perd de l'humidité, du gaz sulfureux, de l'anhydride arsénieux, etc., à la température ordinaire; sa conductibilité calorifique change et sa vitesse d'échauffement se modifiant, la température commençante de décomposition s'élève, en général. A plus forte raison, on observe ce fait en traçant sur le même graphique, les courbes de thermolyse d'un minéral et du produit artificiel, par exemple, la brucite et la magnésie $Mg(OH)_2$. Les variétés allotropiques donnent aussi des courbes non superposables; on l'observe avec la calcite, l'aragonite et la vatérite. La courbe n'est pas la même non plus quand un précipité a muri, quand on l'a filtré au bout d'une heure, au bout de 24 heures ou d'une semaine. Le fait est le plus net avec les produits colloïdaux et s'explique aisément. De même, un précipité mouillé par les liquides de lavage tel qu'il se présente dans l'analyse courante ne donne pas la même courbe que le précipité séché plus ou moins partiellement à l'air. Dans le premier cas, on peut repérer la température de début du palier du corps sec, ce qui est un problème pratique, mais, là aussi, on trouve des différences avec les différents agents de précipitation (ce qui influe sur la grosseur des grains et leur pouvoir de rétentivité pour les solvants). Le

phénomène est marqué chez les différentes variétés d'alumine, de zircone et d'hydroxyde de béryllium.

Lorsque le précipité est volumineux, par suite de la quantité d'eau retenue, il est bon, comme le conseille NEWKIRK[5] d'utiliser de faibles vitesses de chauffe. Ainsi, en traitant le complexe de zinc et de salicylaldoxime avec une vitesse de 380°/h, il est impossible de trouver un palier pour le complexe. Sec, le même produit en fournit un allant de 25° à 285° d'après RYNASIEWICZ ET FLAGG[6]. Un produit partiellement hydraté (50%) montre un palier de 135° à 190° suivant la vitesse de 300°/h. Un échantillon initialement sec, puis mouillé à 63% fournit le sien entre 245° et 315°.

La quantité d'eau retenue par le précipité influe sur la conductibilité de la matière et la vapeur qui s'en dégage modifie considérablement l'atmosphère du four; de plus, toute vaporisation entraîne, comme on le sait, un refroidissement.

Beaucoup de chercheurs se sont préoccupés de la grosseur des grains de la substance mise en oeuvre lorsqu'ils n'étaient pas intéressés par les problèmes d'analyse mais, par le sort des substances sèches. Le premier, SAITO[7] a chauffé comparativement des minerais sulfurés et des arséniosulfures métalliques avec des grains passés par des trous de tamis déterminés. Ses recherches étaient naturellement guidées par le fonctionnement des fours à pyrites. Plus le grain est fin, plus la température commençante de décomposition est basse.

VALLET[8] s'est préoccupé aussi de l'influence de la grosseur des grains, notamment de sulfate de cuivre, puis, dans un mémoire écrit charitablement à mon intention, RICHER ET VALLET[9] comparent, dans l'azote, à raison de 150°/h, le seuil de décomposition du carbonate de calcium pur sec R.P., soit 783° avec celui de la calcite en poudre (802°) et de la calcite prise sous forme d'un cube pesant 352 mg (891°); ce cristal conserve sa forme mais devient un peu plus petit, l'opération étant faite dans l'air. Les auteurs ne donnent pas de résultat pour un carbonate de calcium précipité et encore humide, ce qui fournissait une raison d'être à leur critique, car il est assez rare pour un analyste de peser dans une atmosphère d'azote un précipité de carbonate de calcium disposé harmonieusement sous forme de cube!

Le poids de la substance

Le poids de la substance (et par suite, son volume) influe beaucoup sur les températures commençantes de décomposition. De plus, lorsqu'il s'agit de chélates formés d'un métal et d'un produit organique difficile à brûler, le goudron résiduel partira d'autant plus vite que son poids sera plus faible et le palier de l'oxyde (ou du carbonate final) commencera à des températures très variées; le fait est net surtout avec les dérivés de la quinoléine. C'est pourquoi nous avions donné une marge de sécurité très grande en chauffant très vite les précipités analytiques et en opérant sur des poids compris entre 100 et 300 mg. C'est ce que n'ont pas compris différents auteurs, en particulier WENDLANDT. Les chaleurs de réaction, quelles soient positives ou négatives, altèrent la différence entre la température de l'échantillon et celle du four.

NEWKIRK[5] rappelle que la méthode d'analyse thermique différentielle est possible parce que cette différence existe et il est bien significatif que, dans cette technique, on utilise des vitesses de chauffage élevées, par exemple, 600°/h pour accentuer la température différentielle.

Puisqu'une telle température différentielle peut très bien s'étaler au-dessus de 10°

avec cette vitesse de chauffe, les constantes cinétiques calculées à partir des thermogrammes doivent être de toute façon erronées quand la réaction est endothermique, mais si la réaction est exothermique, les effets vont tendre à se compenser. D'une manière frappante, l'effet de la chaleur de réaction peut être très grand comme le montre l'exemple de l'oxydation du carbure de tungstène dans l'air (NEWKIRK[10]). Les thermogrammes accusent que la même vitesse de réaction fut observée avec le four de la thermobalance maintenu constant à 527° ou chauffé uniformément d'une manière continue. L'effet apparait plus clairement sur la Fig. 2 quand la température

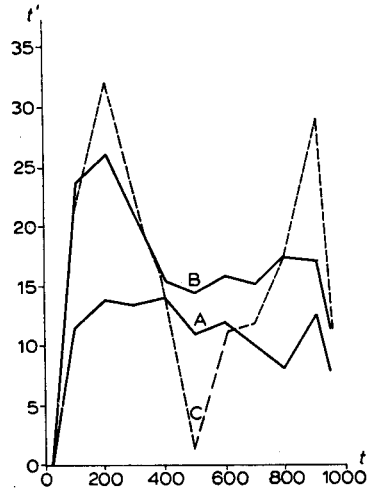


Fig. 2. Décalage de température dû à l'échantillon. Décomposition de l'oxalate de calcium monohydraté. Vitesse de chauffe: 600°/h. A, creuset vide; B, creuset + 0.2 g d'oxalate; C, creuset + 0.6 g d'oxalate.

différentielle entre le thermocouple t et celle du creuset t' a été mesurée directement pour la décomposition du monohydrate de l'oxalate de calcium pour la vitesse élevée de 600°/h pour 2 échantillons de taille différente. La courbe A pour le creuset seul montre un décalage de 10° à 14° dans l'intervalle 100° à 1000°. Quand on utilise un échantillon de 0.2 g, la perte endothermique de l'eau se manifeste par un décalage de 25 à 200°; la perte exothermique de l'oxyde de carbone ramène presqu'à la différence observée avec le creuset seul mais le décalage augmente encore pendant la perte endothermique du gaz carbonique. Avec un échantillon de 0.6 g, ces effets s'accentuent et en un point unique, la température de l'échantillon et celle du four sont presque les mêmes.

La vitesse de chauffe

La plupart des thermobalances sont maintenant équipées avec des dispositifs permettant de chauffer linéairement à des vitesses allant de 0.6° à 10°/min. Nous venons de voir qu'une si grande vitesse amenait des variations souvent considérables des températures finale et initiale des paliers et même certains d'entre eux peuvent disparaître ou n'être marqués que par un point de rebroussement ou un point d'in-

flexion. Le cas est très net chez les corps riches en eau de cristallisation comme les sulfates de la série magnésienne. Si l'on veut obtenir l'un de ces hydrates, on chauffera donc le plus lentement possible ou bien, l'on disposera le four en thermostat à l'approche du palier attendu.

Ainsi, FRUCHART ET MICHEL[11] chauffant l'heptahydrate de sulfate de nickel, à raison de 0.6°/min mettent en évidence l'hexahydrate, le tétrahydrate, le dihydrate

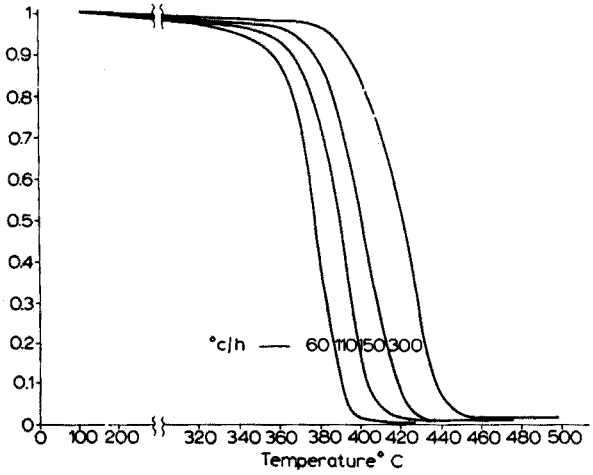


Fig. 3. Pyrolyse du polystyrolène dans l'azote pour différentes vitesses de chauffage.

et le monohydrate. A 2.5°/min, Mmes DEMASSIEUX ET MALARD ne signalent plus que le palier du monohydrate[12]. De son côté, NEWKIRK[5] montre l'altération de la courbe classique de l'oxalate de calcium monohydraté quand on passe de 300 à 150°/h (déplacement des paliers vers les basses températures). Sur la Fig. 3, on voit l'influence de la vitesse de chauffe sur la décomposition d'un échantillon de polystyrolène maintenu dans l'azote en portant en ordonnées la fraction restante. SABATIER[13] a observé un effet similaire au cours de la déshydratation du mica. Pour des échantillons chauffés entre 12° et 30°/h, les températures de seuil observées pour un poids donné vont de 735° à 815°, soit une différence de 80°.

L'effet de la vitesse de chauffe est très important si le thermogramme doit être ultérieurement utilisé pour des analyses cinétiques.

Nature de l'atmosphère environnante

Cette question a été étudiée par de nombreux auteurs depuis SAITO[7] qui prenait l'hydrogène, l'oxygène, l'azote, le gaz sulfureux, etc. La matière chauffée n'est cependant pas en équilibre avec l'atmosphère ambiante quand elle se décompose mais elle peut être assez voisine de cet équilibre pendant le tracé des paliers. Si l'atmosphère du four contient précisément un gaz dégagé par la substance, il faut s'attendre à une élévation de la température initiale et de la température finale. C'est ce qu'observent, d'une part, VALLET[8] en chauffant le pentahydrate du sulfate de cuivre, d'autre part, VALLET ET RICHER[9] en dissociant le carbonate de calcium dans le gaz carbonique. Il faut alors s'attendre à trouver des températures

variables car, quoique les gaz se mettent rapidement en équilibre de température, celui qui arrive froid à la base ou au sommet du four doit influer sur la diffusion de celui qui est dégagé par la substance du creuset. Comme la tension de vapeur règle la stabilité des hydrates, VALLET a pu, à volonté, faire apparaître ou disparaître le palier du trihydrate de sulfate de cuivre. HALADJIAN ET CARPÉNI[14] empêchent toute déshydratation de l'acide orthoborique, produisent ou empêchent à volonté la formation du palier de l'acide métaborique, etc.

Chauffant du carbonate de calcium dans le gaz carbonique puis dans l'azote, RICHER ET VALLET[9] observent que le seuil de décomposition est bien défini (900°) dans le premier cas, assez bien défini (environ 500°) dans l'azote. Il n'en est pas de même de la température à laquelle elle se termine, entre 914° et 1034° dans le gaz carbonique, entre 683° et 891° dans l'azote.

Fort heureusement, en analyse, où l'on chauffe une couche mince de substance (mouillée au départ) et sous la pression atmosphérique, les différences ne sont pas aussi grandes. Un gaz ou une vapeur dégagé par la substance peut influencer la forme de la courbe; en chauffant des sels ou des chélates organiques, l'oxyde de carbone notamment, peut partiellement réduire le résidu. Après son élimination, le courant d'air (ou l'oxygène) permet habituellement une remontée de la courbe. La réduction est très nette dans le cas de l'uranium du sel de Streng, du cuivre bivalent qui passe à l'état de métal ou d'oxyde cuivreux.

Nous avons utilisé le chauffage du gypse en atmosphère de vapeur d'eau, du deutérogypse en atmosphère d'eau lourde pour préparer les plâtres léger et lourd[15].

Utilisant la thermobalance de Chevenard, NEWKIRK[5] chauffe à raison de 300°/h, de l'oxalate de calcium précipité avec le même poids, mais en bouchant hermétiquement le sommet du four, puis en ventilant par un trou de 7 mm de diamètre. Les deux courbes coïncident sauf à haute température sur le palier de l'oxyde de calcium. Les différences s'exagèrent avec du carbonate de sodium chauffé dans l'air puis dans le gaz carbonique: A 300°/h, le seuil de décomposition est 850° dans l'air, mais n'est pas atteint à 1050° dans le gaz carbonique (250 ml/min). Le tungstate de sodium à 28 molécules d'eau fournit des courbes très différentes, tout au moins jusqu'à 175° suivant le degré d'humidification de l'air ambiant. Dans un travail plus récent, GARN ET KESSLER[16] opérant en atmosphère auto-engendrée, étudient la décomposition thermique du carbonate de cadmium, du carbonate d'ammonium, du carbonate de plomb, du carbonate de manganèse et de l'oxalate de cobalt dihydraté, en récipient ouvert ou fermé.

La vitesse du courant gazeux

L'importance de ce facteur est primordiale et s'explique aisément car nous venons de voir que la nature du gaz autour du creuset jouait un grand rôle. La plupart des fours de thermobalance sont ouverts, donc contiennent un mélange d'air, de gaz introduit, de gaz ou de la vapeur dégagé. On conçoit donc que d'après la vitesse du courant gazeux, la composition de l'atmosphère du four change, entraînant une modification dans la longueur et la position des paliers. Dans l'une de ses premières expériences, VALLET[8] chauffait 400 mg d'échantillons identiques de sulfate de cuivre pentahydraté à 42°/h, respectivement dans un courant d'air sec dont le débit était de 6.6 l/h pour le premier et 34.0 l/h pour le second (Fig. 4). L'apparition du trihydrate n'est pas influencée par l'augmentation du débit. Celle-ci entraîne une sorte de trans-

lation de la courbe masse–température parallèle à l'axe des températures, dans le sens des valeurs décroissantes. Cette translation est plus faible pour la portion de courbe relative à la déshydratation du pentahydrate que pour celle qui est relative au trihydrate et plus faible pour cette dernière que pour la portion finale relative à la déshydratation du monohydrate. SAITO[7] avait trouvé des résultats identiques, par exemple, avec le bioxyde de manganèse chauffé dans l'hydrogène.

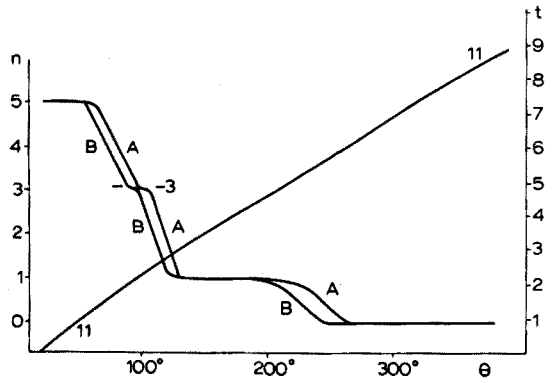


Fig. 4. Influence du débit d'air sec sur la déshydratation du sulfate de cuivre pentahydraté.

Dans le paragraphe précédent, nous avons fait allusion à la ventilation du four, facteur qui va trouver son importance ici. En effet, dans les thermobalances avec four ouvert en haut, c'est-à-dire, dans lequel "plonge" le creuset, la circulation des gaz s'effectue surtout par convection et le creuset est soumis à des poussées variables modifiant son poids réel et se traduisant par une montée d'ailleurs assez régulière de la courbe au fur et à mesure que la température s'élève. Dans les fours renversés, comme celui de la thermobalance Chevenard, on ménage dans le bouchon-couvercle, deux ou trois trous, pouvant être, à volonté, ouverts ou obturés, ce qui, naturellement, change la vitesse du courant d'air et le renouvellement plus ou moins rapide de l'atmosphère et l'évacuation plus ou moins rapide des gaz dégagés. Dans nos essais, de 1936 à 1946, nous avons toujours observé une droite parfaitement horizontale (autrement dit, venant recouvrir sa position initiale après un tour de cylindre) en chauffant jusqu'à 1050° un corps ne devant ni perdre, ni gagner de poids dans cet intervalle, comme un creuset d'or ou de platine. C'est en nous basant sur ce fait et sur ce réglage tout à fait certain que nous avons mis au point la gravimétrie automatique. De même, nous avons pu noter l'oxydation préalable à l'explosion des corps oxydants. Depuis cette époque, divers auteurs ont publié des courbes qui sont en désaccord avec les nôtres, toutes conditions de vitesse de chauffe étant respectées et ces auteurs trouvent notamment des gains de poids sur des échantillons qui nous accusent une valeur constante. Frappés de ces désaccords, les chercheurs de la General Electric Company (Schenectady, N.Y), SIMONS, NEWKIRK ET Mlle ALIFERIS[17] ont déterminé, en chauffant des objets de molybdène, de platine, de porcelaine, etc., une courbe de correction et ils estiment que la poussée de l'air chaud (Fig. 5) jusqu'à 1037° peut occasionner une montée de la courbe (une droite au-dessus de 200°) correspondant à 5 mg sur 200 mg. D'ailleurs, MIELENZ, SCHIELTZ ET KING[18] ont réalisé

une courbe de correction présentant plutôt une forme en S (Fig. 6) sur la même thermobalance avec concavité entre 700° et 800°, le gain de poids apparent étant encore de l'ordre de 5 mg sur des échantillons de 1.5 g jusqu'à 1000°. Il nous est apparu alors que les écarts relevés en chauffant un corps gardant un poids constant

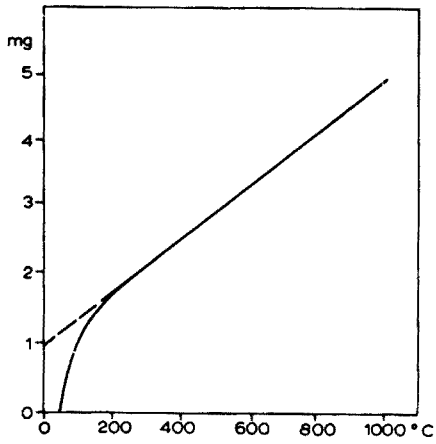


Fig. 5. Représentation générale du poids apparent en fonction de la température d'après SIMONS, NEWKIRK ET ALIFERIS.

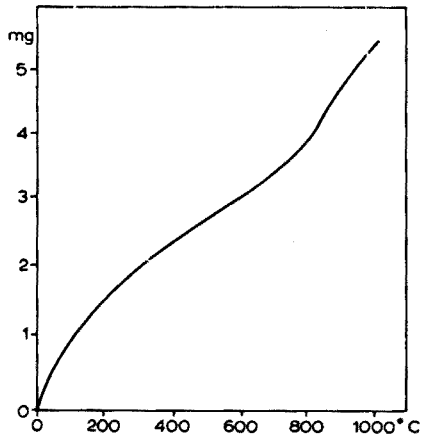


Fig. 6. Correction de poussée pour le cycle d'échauffement de la thermobalance.

étaient dus à la construction du bouchon métallique fermant le four à la partie supérieure. Ce bouchon porte normalement un trou pour loger la gaine de silice du couple et deux évents de 2 mm de diamètre, habituellement obstrués. Dans nos modèles de thermobalances initiaux, le bouchon portait des trous de différents diamètres (ayant servi pour faire des expériences préliminaires) que l'on pouvait escamoter à volonté. De plus, ces bouchons s'encastraient beaucoup moins jointivement dans le collier supérieur du four que dans les modèles récents (le diamètre de ce bouchon change d'ailleurs après chauffages répétés pendant une dizaine d'années).

Nous avons alors muni nos appareils de bouchons métalliques portant un trou central et 6 trous plus petits (Fig. 7) disposés symétriquement et pouvant être obstrués à volonté. D'après le nombre de trous débouchés, on peut réaliser, avec le même corps chauffé suivant la même loi, soit une droite qui s'élève (cas où tous les trous sont bouchés ou débouchés d'une façon dissymétrique), soit une droite horizontale (autrement dit, revenant passer exactement par le point de départ après un tour de cylindre et ceci, quel que soit le point de départ de ce cylindre), soit une droite décroissante. C'est donc tout à fait par hasard que nos propres appareils avaient été équipés d'un bouchon adéquat.

Nous avons ensuite songé à munir le trou central d'un système d'obturation progressif, analogue à celui des diaphragmes-iris des appareils photographiques mais cela complique beaucoup et ne fonctionne pas toujours surtout à chaud. Nous avons trouvé plus simple[19] de garnir le trou central de couronnes d'épaisseurs diverses pouvant s'adapter à la manière des ronds d'un poële ou d'un bain-marie (Fig. 8). On choisit alors des creusets ayant le même diamètre supérieur et inférieur et l'on

adapte, avant toute pyrolyse, celle des couronnes qui donne avec le creuset chauffé, une droite horizontale, c'est-à-dire, parallèle à l'axe des températures.

Nous ne possédons pas beaucoup de renseignements sur le sort des courbes réalisées avec un corps chauffé sous pression supérieure ou inférieure à la pression at-

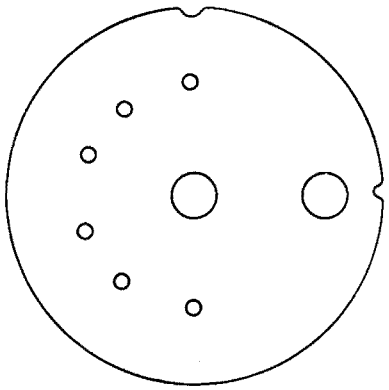


Fig. 7. Le bouchon du four avec 6 ouvertures symétriquement disposées.

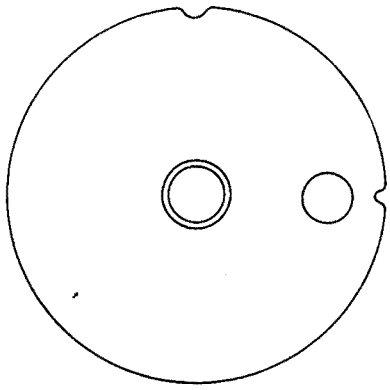


Fig. 8. Le bouchon avec ouverture centrale.

mosphérique. Le problème est très complexe et se modifie encore si l'on évacue ou non le gaz ou la vapeur émané. Il semble logique d'admettre qu'en évacuant les produits gazeux, il soit plus aisé de maintenir constante la pression et l'on doit abaisser le seuil de décomposition. On pouvait aussi s'attendre à voir s'amenuiser l'influence des courants de convection en diminuant la pression. C'est bien ce qu'ont constaté EYRAUD ET GOTON[20] mais, par contre, dès la pression de 1 mm de mercure une nouvelle cause d'erreur au moins aussi importante apparaît, due au choc des molécules sur l'échantillon. EYRAUD a pu y remédier dans la balance qu'il a construite.

Voici quelques valeurs de poussées radiométriques exprimées en mg, exercées sur des nacelles porte-échantillons, de diamètre 12 mm environ, suspendues dans un tube de quartz de 17 mm de diamètre intérieur et chauffées dans un four Chevenard vertical du type CT_2 ouvert aux deux extrémités. Les valeurs suivantes ont été enregistrées sous une pression de 0.1 mm de mercure et à une température de 500°:

Emplacement de la nacelle	*Poussée*
Au tiers supérieur du four	+ 2.2 mg
Au milieu du four	− 1.1 mg
Au tiers inférieur	− 5.0 mg

On constate en outre qu'au cours d'une élévation continue de la température, la nacelle étant dans une position invariable, la poussée radiométrique ne varie pas toujours dans le même sens; elle peut reprendre la valeur nulle puis, changer de signe.

A 800°, avec la nacelle en position invariable et la loi d'échauffement ayant été la même, la poussée atteint les valeurs suivantes, en fonction de la pression:

Pression	5	3	1	1/10	1/1000 mm
Poussée	0.0	0.5	1.0	2.2	3.3 mg

Pour des objets d'encombrement sensiblement identique et une même position dans l'intérieur du four, les résultats sont très variables. A 600° et sous la pression de 1/1000 mm, on note:

Objet utilisé	*Poussée*
Nacelle de quartz	+ 1.5 mg
Capsule de porcelaine	+ 2.9 mg
Barreau de quartz plein	+ 1.0 mg

Le phénomène résulte donc de la dissymétrie des pressions exercées sur les récipients par chocs de molécules pouvant provenir, soit des parties froides de l'enceinte, soit des parties chaudes. Une solution simple pour y remédier consiste à placer la nacelle dans une enceinte intermédiaire rendue sensiblement isotherme par sa forme réduite. Une petite ouverture est ménagée dans la partie supérieure afin de laisser passer les fils de suspension. L'efficacité d'un tel dispositif se montre très satisfaisante; il est nécessaire d'y recourir dès que la pression devient inférieure à 3 mm de mercure.

La nature et la forme du creuset

Jusqu'ici, on a fait usage pour contenir la substance chauffée de creusets tronconiques, forme haute et forme basse, de cônes, de capsules hémisphériques, de plaques carrées de 1 cm de côté, à bords relevés, de creusets dont le fond possède la forme d'un cul de bouteille; les matières de ces creusets ont varié depuis le platine, l'or, l'argent, le tungstène, le nickel jusqu'à la silice, la porcelaine, le verre, l'aluminite, la thorine, la zircone et la stéatite. Les essais systématiques n'ont pas toujours été faits avec une seule variable, mais, on peut dire qu'il existe une différence dans les seuils de décomposition entre l'emploi d'un creuset métallique, bon conducteur de la chaleur et un creuset formé d'oxydes ou de silicates. Les différences peuvent aller de 5 à 10°.

La forme joue un rôle prépondérant même sous la pression atmosphérique car les parois étant chauffées plus fortement que le centre, on se trouve en présence de la conductibilité propre de la substance et de la vitesse de diffusion des gaz évacués à travers les grains. La solution de la plaque et de la couche mince paraît être la meilleure et celle du creuset forme haute la plus mauvaise, mais, certaines substances font des projections ou se boursouflent beaucoup; on est bien obligé de les utiliser parfois. GARN ET KESSLER[16] ont étudié ces facteurs en détail par chauffage du carbonate de plomb et de l'oxalate de cobalt hydraté. NEWKIRK ET Mlle ALIFERIS[21] ont, de leur côté, étudié le comportement du carbonate de sodium sec dans des creusets d'or, de platine, d'alumine et de porcelaine. L'influence de la nature du métal semble négligeable. Par contre, la mise en place ou l'enlèvement d'un couvercle à ces creusets ne l'est pas. Nous retombons là sur un facteur déjà étudié: la vitesse du courant gazeux. Chacun sait que l'eau bout plus vite sur le gaz dans une casserole munie d'un couvercle. En pyrolyse, on gêne l'évacuation des gaz, par suite, les paliers des courbes s'allongent. On peut même susciter la formation de ces paliers. Dès 1936, nous avons constaté que dans le cas du phosphate ammoniacomagnésien, il n'apparaissait aucune discontinuité entre le départ d'eau et d'ammoniac en creuset ouvert; un court palier ou tout au moins une brisure se manifeste dès que l'ammoniac a cessé de se dégager et que le phosphate $MgPO_4H$ est pur, quand on munit le même creuset d'un couvercle.

Un dernier facteur qu'il ne faut pas négliger est l'attaque du creuset, c'est-à-dire une réaction à l'état solide entre la glaçure ou la matière même du creuset avec dégagement gazeux. Il est bien évident qu'il se produit une perte de poids parasite. Un exemple frappant est donné par NEWKIRK ET Mlle ALIFERIS[21] avec la pyrolyse du carbonate de sodium dans un creuset de quartz, l'attaque commençant alors dès 500°.

Faisons remarquer qu'en chauffant de l'anhydride molybdique dans un creuset de porcelaine, le fond de celui-ci se colore en bleu-vert mais sans changement de poids, plus exactement, sans montrer de variation supérieure à 0.1 mg sur 4 g.

La finesse du trait

Par enregistrement photographique sur papier glacé, on arrive à inscrire des traits, peu visibles il est vrai, ayant 0.1 mm d'épaisseur; avec la plume à bec triangulaire de platine, papier peu spongieux, encre au glycérol, il est difficile de descendre au-dessous de 0.5 mm. On conçoit l'importance de cette épaisseur pour effectuer les calculs quand les variations de poids sont faibles. Dans certains appareils, Chevenard trace la courbe à l'aide d'une série de points très rapprochés et produits par une aiguille venant toucher périodiquement le papier. Nous considérons cependant ce facteur comme peu important vis-à-vis de tous les autres. Il influe avant tout sur la sensibilité au même titre que les mouvements d'horlogerie, le jeu des engrenages, la paresse des cellules photo-électriques et les variations inopinées de la tension du courant électrique.

La nature et la pesée du résidu

C'est généralement avec le palier final que l'on effectue les calculs de poids moléculaires pour trouver, à l'aide des ordonnées des autres paliers, la nature du corps de départ et des produits intermédiaires. Cela suppose que le produit final possède une composition connue et qu'il ne capte pas d'eau au cours du refroidissement avant de le peser sur une balance analytique pour contrôle. Or, beaucoup d'oxydes métalliques sont carbonatés à la fin de l'opération et donnent cependant un palier bien horizontal. Nous avons fait allusion aussi à une combinaison possible avec la substance des parois internes du creuset. Des analyses à la touche sur le résidu s'imposent donc quand les calculs ne conduisent pas à une solution logique. Il serait bon de terminer ce mémoire par un calcul d'erreur relatif aux thermobalances. Nous n'avons pas encore, malheureusement, les matériaux nécessaires. Jusqu'ici nous ne possédons que des résultats partiels relatifs à la thermobalance de Chevenard et encore, les dix facteurs primordiaux que nous venons d'énumérer n'ont pas tous été pris en considération. Nous ne ferons que citer les tentatives fort louables de SIMONS, NEWKIRK ET Mlle ALIFERIS[17] avec l'appareil à plume et ceux de CLAISSE, EAST ET ABESQUE[22], de MIELENZ, SCHIELTZ ET KING[18] avec la thermobalance à enregistrement photographique.

RÉSUMÉ

Après avoir suivi pendant ces 25 dernières années la pyrolyse de plusieurs milliers de substances minérales ou organiques, notamment en vue de l'analyse gravimétrique, de l'étude des étalons volumétriques et de celle des réactions à l'état solide intervenant en analyse pour les mises en solution, l'auteur tire des remarques personnelles pour essayer d'expliquer les désaccords sur-

venant entre les usagers des divers modèles de thermobalance. Il rapporte actuellement ces désaccords à 10 causes principales: la marque de l'appareil, le couple thermo-électrique, la nature de la substance mise en oeuvre, le poids de cette substance, la vitesse de chauffe, la nature de l'atmosphère environnant le creuset, la vitesse du courant gazeux dans le four, la nature et la forme du creuset, la finesse du trait de l'enregistrement et, enfin, la nature et la pesée du résidu de chauffage.

SUMMARY

Based on 25 years of experience of the pyrolysis of several thousands of inorganic and organic substances with reference to gravimetric analysis, studies of volumetric standards and studies of solid-state reactions, the author attempts to explain the disagreements in results obtained by users of various models of thermobalances. These differences are traced to 10 main causes: the type of apparatus, the thermocouple, the nature of the substance involved, the weight of this substance, the rate of heating, the nature of the atmosphere around the crucible, the gas flow-rate in the furnace, the nature and shape of the crucible, the sensitivity of the recorder trace, and the nature and weight of the residue.

ZUSAMMENFASSUNG

Aufgrund 25-jähriger Erfahrung mit der Pyrolyse versucht der Autor unterschiedliche Ergebnisse, wie sie von Benutzern verschiedener Thermowaagen erhalten wurden, zu erklären. Er gibt als Grund 10 Hauptursachen an.

BIBLIOGRAPHIE

1 W. NERNST ET E. H. RIESENFELD, *Ber.*, 36 (1903) 2086.
2 O. BRILL, *Z. Anorg. Chem.*, 45 (1905) 275.
3 K. HONDA, *Sci. Rept. Tohoku Univ.*, 4 (1915) 97.
4 C. DUVAL, *Inorganic Thermogravimetric Analysis*, 2è Ed., Elsevier, Amsterdam, 1963.
5 A. E. NEWKIRK, *Anal. Chem.*, 32 (1960) 1558.
6 J. RYNASIEWICZ ET J. F. FLAGG, *Anal. Chem.*, 26 (1954) 1506.
7 H. SAITO, *Sci. Rept. Tohoku Univ.*, 16 (1927) 1.
8 P. VALLET, *Compt. Rend.*, 198 (1934) 1860; *Thèse*, Paris, 23 Mai 1936; *Ann. Chim.*, 7 (1937) 298.
9 A. RICHER ET P. VALLET, *Bull. Soc. Chim. France*, (1953) 148; *Compt. Rend.*, 249 (1959) 680.
10 A. E. NEWKIRK, *J. Am. Chem. Soc.*, 77 (1955) 452.
11 R. FRUCHART ET A. MICHEL, *Compt. Rend.*, 246 (1958) 1222.
12 N. DEMASSIEUX ET C. MALARD, *Compt. Rend.*, 245 (1957) 1514.
13 G. SABATIER, *J. Chim. Phys.*, 52 (1955) 60.
14 J. HALADJIAN ET J. CARPÉNI, *Bull. Soc. Chim. France*, (1956) 1679.
15 C. DUVAL, J. LECOMTE ET C. PAIN, *Compt. Rend.*, 237 (1953) 238.
16 P. D. GARN ET J. E. KESSLER, *Anal. Chem.*, 32 (1960) 1900.
17 E. L. SIMONS, A. E. NEWKIRK ET I. ALIFERIS, *General Electric Company Report Nr. 56-RL-1522.*
18 R. C. MIELENZ, N. C. SCHIELTZ ET M. E. KING, *Proc. 2nd Natl. Conf. Clays and Clay Minerals, Missouri, oct. 13–17, 1953*, Publication 327 of The National Academy of Science, 1954, p. 285.
19 C. DUVAL, *Mikrochim. Acta*, (1958) 705.
20 C. EYRAUD ET R. GOTON, *Bull. Soc. Chim. France*, (1953) 1009.
21 E. NEWKIRK ET I. ALIFERIS, *Phys. Chem. Research Nr. 405, General Electric Company; Anal. Chem.*, 30 (1958) 982.
22 F. CLAISSE, F. EAST ET F. ABESQUE, *the Use of the Thermobalance in Analytical Chemistry*, Dept. of Mines, Province of Quebec, Canada, 1954.

IMPROVED GRAVIMETRIC DETERMINATION OF COBALT AS $K_3Co(NO_2)_6$*

JAMES J. LINGANE

Department of Chemistry, Harvard University, Cambridge, Mass. 02138 (U.S.A.)

(Received April 2nd, 1964)

In a recent study[1] in this Laboratory the composition of "potassium cobaltinitrite" precipitated from a slightly acid solution of +2 cobalt containing a large excess of potassium nitrite, and finally dried at 110°, was investigated. Direct analyses of the precipitates for water and potassium, coupled with the weights of precipitate obtained over a wide range of cobalt concentrations, provided cogent evidence that when a relatively small amount of cobalt is precipitated the dried precipitate consists of a mixture of anhydrous and hydrated $K_3Co(NO_2)_6$. With increasing quantities of cobalt the precipitate also contains increasing amounts of a salt of +2 cobalt (probably $K_2Co(NO_2)_4$), due to incomplete oxidation of the +2 cobalt. Because the formula weight of $K_2Co(NO_2)_4$ is smaller than that of $K_3Co(NO_2)_6$, whereas that of hydrated $K_3Co(NO_2)_6$ is larger, these compounds compensate each other weight-wise and the weight of the precipitate is actually not very different than if it were pure $K_3Co(NO_2)_6$. The error resulting from assuming that the dried precipitate is $K_3Co(NO_2)_6$ varies from only +1% with 40 mg of cobalt (final volume 80–100 ml) to −1%, with 300 mg of cobalt, when the precipitation is performed at temperatures from 20° up to 70°.

In the previous study it was found that varying the initial temperature at which precipitation is begun from about 20° to 70° has very little effect on the composition of the precipitate finally obtained after the mixture is allowed to cool room temperature, and the effect of a higher temperature was not investigated. However, further investigation has now revealed that when the precipitation is begun with the cobalt solution and the potassium nitrite *both boiling hot*, and the mixture is allowed to cool to room temperature before filtration, the precipitate is very nearly pure, anhydrous $K_3Co(NO_2)_6$. This remarkable effect of a relatively small increase in temperature is due chiefly to the fact that in a boiling hot solution the precipitate does not begin to form immediately, and there is time for the +2 cobalt to become completely oxidized before precipitation commences. The higher temperature also eliminates the hydrated form of $K_3Co(NO_2)_6$.

With this simple change in precipitation conditions, quantities of cobalt from a few milligrams up to about 175 mg are determinable with an error of less than 0.1 mg.

* This paper is dedicated to Professor D. Monnier on the occasion of his sixtieth birthday.

ห้องสมุด กรมวิทยาศาสตร์

EXPERIMENTAL

Standard solution

The cobalt standard solution was prepared from spectroscopically pure metallic cobalt as previously described[1]. It was dispensed by weight, so that the uncertainty in the quantity of "cobalt taken" was smaller than ± 0.005 mg.

Procedure

The nearly neutral cobalt solution, in a 150-ml beaker, was diluted to *ca.* 50 ml and treated with 5 ml of glacial (17 *M*) acetic acid. It was then heated to the boiling point, the flame was removed, and 30 ml of *boiling hot*, 50% potassium nitrite solution (15 g KNO_2) was slowly added. To prevent spray loss due to the very lively gas evolution the beaker was kept covered with a watch-glass, and the potassium nitrite solution was added through the opening between the lip of the beaker and the watch-glass.

In the boiling hot solution the precipitate does not begin to form until nearly all the potassium nitrite solution has been added, and meanwhile the $+2$ cobalt is practically completely oxidized to reddish orange $Co(NO_2)_6^{3-}$. As the mixture is allowed to slowly cool the precipitation gradually becomes complete (apparent from the decrease in the color of the $Co(NO_2)_6^{3-}$), and finally the supernatant solution becomes colorless.

After the mixture had stood at room temperature for at least an hour (longer standing does no harm), the precipitate was filtered off on a medium-porosity sintered-glass crucible, washed, *seriatim* with 100 ml of 2% potassium nitrite solution, 5 10-ml portions of 80% (vol.) ethanol and once with acetone, and was then dried in an oven at 110° for 1 h before weighing. The rationale of this technique has been discussed in detail in the previous paper[1].

PERFORMANCE DATA

Results obtained in the determination of 17–258 mg of cobalt are summarized in Table I. With up to 174 mg of cobalt the average error is only ± 0.08 mg of cobalt, when the precipitation is begun in a boiling hot solution. The larger error of -0.54 mg with 258 mg of cobalt probably reflects some incomplete oxidation of the $+2$

TABLE I

GRAVIMETRIC DETERMINATION OF COBALT AS $K_3Co(NO_2)_6$

(In all cases the final solution volume was 85–100 ml and 5 ml of 17 *M* acetic acid and 30 ml of 50% potassium nitrite solution (15 g KNO_2) were used. The quantity "Co found" was calculated from the formula $K_3Co(NO_2)_6$ for the dried precipitate (13.031% Co))

Co taken (mg)	*Ppt. (g)*	*Co found (mg)*	*Error (mg)*
17.09	0.1318	17.18	0.09
44.52	0.3423	44.61	0.09
82.96	0.6371	83.02	0.06
120.19	0.9216	120.10	−0.09
174.15	1.3358	174.07	−0.08
258.65	1.9807	258.11	−0.54

cobalt when such a large quantity is precipitated. Obviously, it is a simple matter either to select a sample size so that the quantity of cobalt does not exceed 175 mg/50 ml, or to dilute the original cobalt solution to a correspondingly larger volume and use correspondingly larger amounts of acetic acid and potassium nitrite.

The small error when the precipitation is begun at the boiling point, coupled with the high degree of selectivity now makes this one of the very best methods for the accurate determination of cobalt in a wide variety of materials. As far as the writer is aware, no other method equals it in selectivity and freedom from interferences. Its accuracy is excelled only by the ferricyanide titration method[2].

One of the very few elements which interferes is sodium, which replaces part of the potassium to give the mixed potassium–sodium cobaltinitrite of notoriously variable composition[3]. When cobalt is to be determined in the presence of much sodium, the first precipitate should be dissolved and the precipitation as $K_3Co(NO_2)_6$ repeated. The precipitate can easily be dissolved in boiling 1:10 acetic acid, and then re-precipitated simply by adding boiling hot potassium nitrite solution.

In the course of systematic analyses of metallurgical materials, it is desirable on occasion to separate such elements as iron, aluminum, and chromium from cobalt (and nickel and manganese) by precipitating the former as the hydrous oxides. Of the various buffer systems that have been recommended for this separation a pyridine–pyridinium ion buffer provides the cleanest separation with no coprecipitation of cobalt (or nickel)[4]. When cobalt is precipitated from the pyridine-containing filtrate, it has been found that potassium ion is partly replaced by pyridinium ion, and the weight of the precipitate corresponds quite closely to $K_2(C_5H_5NH)Co(NO_2)_6$. Hence this first precipitate should be re-dissolved and the precipitation repeated to obtain pure $K_3Co(NO_2)_6$.

SUMMARY

The gravimetric determination of cobalt based on the precipitation of $K_3Co(NO_2)_6$ becomes highly accurate when the precipitation is begun in a *boiling hot* solution. Under this condition the precipitate is pure $K_3Co(NO_2)_6$, and, correspondingly, quantities of cobalt from a few milligrams up to 175 mg are easily determinable with an error of less than 0.1 mg.

RÉSUMÉ

Le dosage gravimétrique du cobalt, basé sur la précipitation de $K_3Co(NO_2)_6$, devient extrêmement précis lorsque la précipitation est commencée dans une solution à la témperature d'ébullition. Dans ces conditions, le précipité $K_3Co(NO_2)_6$ est pur; des teneurs en cobalt de quelques milligrammes à 175 g peuvent être facilement déterminées, avec une erreur inférieure à 0.1 mg.

ZUSAMMENFASSUNG

Die gravimetrische Bestimmung des Kobalts durch Fällung des $K_3Co(NO_2)_6$ wird sehr genau, wenn die Fällung in kochender Lösung durchgeführt wird. Unter dieser Bedingung besteht der Niederschlag aus reinem $K_3Co(NO_2)_6$ und ermöglicht eine einfache Bestimmung von wenigen Milligramm bis zu 175 mg Kobalt mit einem Fehler von weniger als 0.1 mg.

REFERENCES

1 J. J. LINGANE, P. J. LINGANE AND M. D. MORRIS, *Anal. Chim. Acta*, 29 (1963) 10.
2 J. J. LINGANE, *Anal. Chim. Acta*, 30 (1964) 319.
3 R. BELCHER AND J. W. ROBINSON, *Anal. Chim. Acta*, 27 (1962) 568.
4 J. J. LINGANE AND H. KERLINGER, *Ind. Eng. Chem., Anal. Ed.*, 15 (1943) 8; 16 (1944) 187.

SPECTROPHOTOMETRIC DETERMINATION OF MANGANESE WITH N,N,N′,N′-TETRAKIS(2-HYDROXYPROPYL)ETHYLENEDIAMINE*

LEROY PIKE** AND JOHN H. YOE

Pratt Trace Analysis Laboratory, Department of Chemistry, University of Virginia, Charlottesville, Va. (U.S.A.)

(Received March 24th, 1964)

In 1959, KEYWORTH[1] reported the stability constants and colors for the N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine (THPED) chelates of Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Hg^{2+} and Ag^{+}. His work suggested the possibility of using THPED as a spectrophotometric reagent in trace metal analysis. Preliminary investigation, however, failed to reveal absorptions of sufficient intensity for trace analysis of the chelates of the above-named metallic ions. However, a soluble yellow manganese THPED chelate was observed in strongly basic solutions. This paper describes a study of the manganese–THPED chelate and its use as a sensitive spectrophotometric reagent for the determination of manganese. The method has been applied successfully to a variety of National Bureau of Standards samples.

EXPERIMENTAL

Apparatus

A Beckman ratio recording spectrophotometer, Model DK-2, was used for obtaining absorbance curves as a function of wavelength. For absorbance measurements at a given wavelength a Beckman spectrophotometer, Model DU, was used. Matched 1-cm Corex cells were used for all absorbance measurements.

A Beckman pH meter, Model G, was used for all pH measurements. It was calibrated with certified standard buffers.

The ion-exchange columns were constructed from 7 mm (o.d.) Pyrex tubing sealed to a capillary stopcock at one end and to 10 cm of 20 mm (o.d.) Pyrex tubing at the other end. The columns were slurry packed with Dowex 1-X8, 50 to 100 mesh resin, and stoppered at each end with a glass wool plug. The resin beds were 20 cm high and about 5 mm in diameter.

Reagents

Standard manganese solution. Dissolve 1.000 g of manganese metal (Johnson-Matthey's "Specpure") in a minimum of reagent-grade hydrochloric acid and dilute to 1 l with distilled water. Alternatively, "Specpure" Mn_3O_4 may be used.

* This paper is dedicated to Professor D. Monnier on the occasion of his sixtieth birthday.
** Present address: Appalachian State Teachers College, Boone, N. C. (U.S.A.).

N,N,N',N'-Tetrakis(2-hydroxypropyl)ethylenediamine solution, 0.3 *M*. Dissolve 8.76 g of N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine (Wyandotte's "Quadrol") and 11.2 g of reagent-grade potassium hydroxide in distilled water and make up to 100 ml. The weights are not critical and small quantities of carbonate do not interfere.

Acetate buffer solution. Dissolve 62.5 g of sodium acetate in distilled water, add 60 ml of glacial acetic acid and dilute to about 500 ml. Adjust the volume so that 1 ml of buffer solution plus 1 ml of THPED solution diluted to 10 ml will yield a pH of 6.0 $\pm$ 0.2.

Other reagents. All other reagents were analytical grade and were used without further purification.

Properties of the reagent

N,N,N',N'-Tetrakis(2-hydroxypropyl)ethylenediamine (THPED) is marketed by Wyandotte under the trade name of "Quadrol". It is a clear viscous liquid at room temperature and completely miscible with water.

As indicated by its structure, this compound produces fairly basic water solutions that can be standardized acidimetrically. Figure 1 shows a titration curve obtained using 10 ml of a 0.2049 *M* solution of THPED with 0.4756 *N* hydrochloric acid.

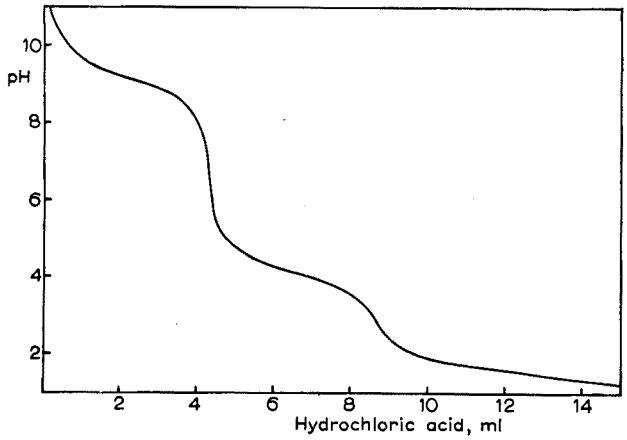


Fig. 1. Titration curve for N,N,N',N'-tetrakis(2-hydroxypropyl)ethylenediamine with hydrochloric acid.

In this study the compound was used either in aqueous solution (stable for several months) or in 2 *N* sodium or potassium hydroxide (stable for a week). Both solutions turn yellow on standing; only colorless solutions should be used for spectrophotometric work.

Properties of the chelate

Spectrum. The absorption spectrum of the pale yellow manganese–THPED chelate formed in strongly basic solutions is shown in Fig. 2, Curve II. Gradual lowering of the pH after color formation produces a family of curves between Curve

II and Curve III. As the pH is lowered the color changes from pale yellow to pink and the absorbance maximum shifts from about 440 mμ to 506 mμ, with about a two-fold increase in intensity. When the pH drops below 5 the color rapidly disappears. Curve I of Fig. 2 shows that the reagent alone has practically no absorbance between 300 and 700 mμ.

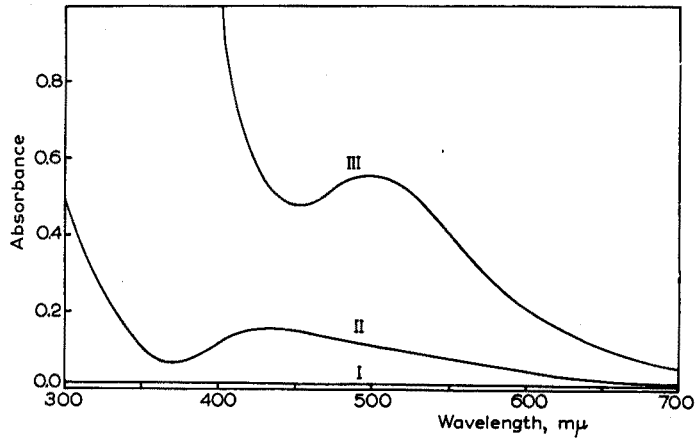


Fig. 2. Absorbance spectra of N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine (Curve I) and its manganese chelate in excess reagent at pH 13 (Curve II) and at pH 6 (Curve III).

Rate of complex formation and its stability. The rate of formation of the manganese–THPED chelate is dependent upon the pH of the solution and the availability of oxygen. Maximum absorbance develops within 3–5 min if the pH of the solution is 12.5 or higher and if it is shaken rapidly in air for about 15 sec. At either lower pH values or with less thorough mixing with air, the rate of formation decreases. After maximum absorbance develops, it remains constant for at least 90 min. Longer periods were not studied.

Effect of pH after chelate formation. The effect of the hydrogen-ion concentration on absorbance at 506 mμ after chelate formation is shown in Fig. 3. The rate of fading is also pH dependent, being about 5% per hour at pH 6.3 but increasing to about 10% per hour at pH 5.8. An optimum pH of 6.0±0.2 is indicated.

Order of addition of reagents. The reagent and manganese must be mixed before the solution is made basic in order to prevent the precipitation of hydrous oxides of manganese. However, the reagent and manganese mixture may be added to the base or *vice versa*. Alternatively, an alkaline solution of the reagent may be used, in which case the order of mixing has no effect on the absorbance of the chelate. The latter procedure was found most convenient in the majority of our work.

Nature of the chelate in solution. The chelate cannot be formed in a closed vessel from which air has been removed. Reducing agents, such as hydroxylamine, hydroquinone, ascorbic acid, tin(II), sulfite, etc. prevent formation of the chelate. They will also destroy the chelate if added to a solution containing it.

Addition of potassium iodide to the chelate solution followed by acidification releases iodine. Titration of the iodine with thiosulfate indicated that one equivalent of iodine per mole of manganese had been released. Hence, the manganese in the chelate is in the trivalent state.

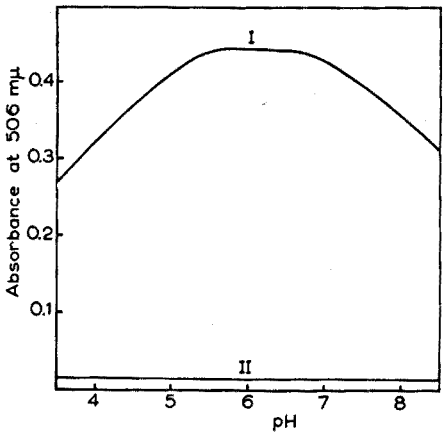


Fig. 3. Effect of pH on the absorbance of the manganese–THPED chelate in excess reagent (Curve I) and on THPED alone (Curve II).

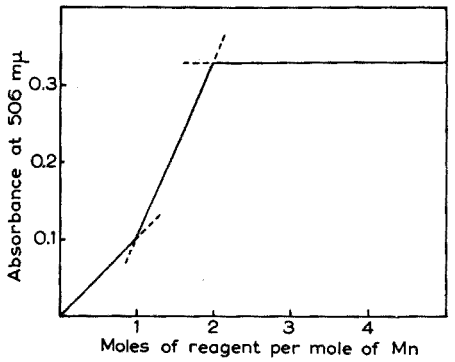
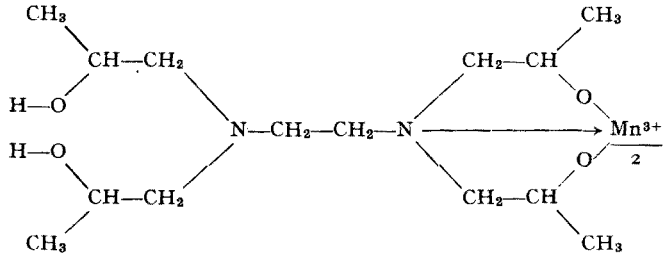


Fig. 4. Mole ratio method (YOE AND JONES) applied to the manganese–THPED chelate at pH 6.

Mole ratio studies. Three techniques were employed in these studies: the mole ratio method of YOE AND JONES[2], the continuous variations method of JOB[3] as modified by VOSBURGH AND COOPER[4], and the slope ratio method proposed by HARVEY AND MANNING[5]. All three methods indicated that the predominant species in excess reagent has a 1 : 2 ratio of manganese to reagent. Some evidence for a less stable 1 : 1 chelate was also observed (see Fig. 4). A plausible structural formula for the more stable chelate is:



Recommended procedure for preparing a calibration curve

Using a standard manganese solution (200 p.p.m.), transfer 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 ml, respectively, to 10-ml volumetric flasks and dilute to 5 ml with distilled water.

Add 1 ml of a 0.3 *M* reagent solution in either 2 *N* potassium or sodium hydroxide.

Mix well and shake the unstoppered flask rapidly for about 15 sec. Allow the solutions to stand 10 min, shake again, and add sufficient acetate buffer solution (about 1 ml) to produce a pH of 6.0 ± 0.2. Make to volume (10 ml) with distilled water, mix thoroughly, and measure the absorbance at 506 mμ immediately, using either a reagent blank or distilled water.

Plot the absorbance as a function of the manganese concentration. The suggested series contains 2, 5, 10, 20, 40, 60, 80 and 100 p.p.m. manganese when diluted to 10 ml.

Conformity to Beer's law

Beer's law is obeyed between 2 and 100 p.p.m. of manganese. Lower and higher concentrations were not investigated.

Optimum concentration range

If absorbance values between 0.2 and 0.7 are chosen as the optimum range[6], the values correspond to manganese concentrations between 25 and 85 p.p.m.

Sensitivity

The sensitivity is 0.12 μg of Mn per cm^2 for $\log I_0/I = 0.001$, *i.e.*, 1.2 parts of manganese in ten million parts of solution. A more practical sensitivity, however, based on an absorbance of 0.005 is about 0.5 p.p.m.

Reproducibility

Eleven 2-ml aliquots corresponding to 40 p.p.m. of manganese at the final dilution were analyzed by the recommended procedure. The standard deviation was ± 0.0024 absorbance unit, *i.e.*, 0.71%.

Effect of temperature

Absorbance measurements between 15° and 35° showed that normal variations in laboratory temperature have no significant effect upon the results.

Effect of diverse ions

The effect of 76 ions was investigated. The following ions had no effect on the absorbance at the 40 p.p.m. manganese level when present at a 10-fold excess: Li^+, Na^+, K^+, Ca^{2+}, Sr^{2+}, Ba^{2+}, BO_3^{3-}, CO_3^{2-}, SiO_3^{2-}, Pb^{2+}, NO_3^-, PO_4^{3-}, AsO_2^-, AsO_3^{3-}, Bi^{3+}, SO_4^{2-}, SeO_3^{2-}, F^-, Cl^-, Br^-, Zn^{2+}, Cu^{2+}, Cd^{2+}, MoO_4^{2-}, WO_4^{2-}, acetate, ammonium, bromate, cyanide, oxalate, tartrate, and thiocyanate. Reducing ions, such as Sn^{2+}, NO_2^-, S^{2-}, SO_3^{2-} and citrate must not be present. The following ions cause an interference greater than ±5% when present in a 10-fold excess but are tolerated with a change in absorbance no greater than ±5% at the concentration (in p.p.m.) given in parentheses: Mg^{2+}(20), Al^{3+}(190), Ti^{4+}(25), V^{5+}(60), Cr^{3+}(10), Fe^{3+}(20), Co^{2+}(50), Ni^{2+}(150), Sn^{4+}(200).

SEPARATION OF INTERFERING IONS

The interference of large quantities of iron(III), copper(II), cobalt(II), and nickel(II) was removed by an ion-exchange technique developed by KRAUS AND MOORE[7]. A separation is accomplished by adsorption on Dowex 1-X8 from 12 *M* hydrochloric acid and elution of the manganese with 6 *M* hydrochloric acid. Iron, copper and cobalt

are retained on the resin. A partial separation of non-adsorbed ions, such as nickel(II) and chromium(III), can also be obtained. The columns have a capacity of about 3 mequiv. of adsorbable ions. This sets the lower manganese concentration at about 0.2%, if all the ions in the sample are adsorbed on the resin. If a large percentage of the ions in the sample are not adsorbed, lower percentages of manganese can be determined. Of course larger ion-exchange columns could be used.

RECOMMENDED PROCEDURE FOR SAMPLES REQUIRING SEPARATION

Select the sample size and dilution so that a 1-ml aliquot will contain sufficient manganese for one determination (preferably 0.25 to 0.85 mg). Dissolve the sample in a minimum of dilute hydrochloric acid, or a mixture of hydrochloric and nitric acids if required, and evaporate almost to dryness. Dilute to volume with 12 *M* hydrochloric acid and mix thoroughly. Place a 1-ml aliquot on an ion-exchange column which has been conditioned with 12 *M* hydrochloric acid. When the aliquot has passed into the resin bed, add two 1-ml portions of 12 *M* hydrochloric acid, then elute with 6 *M* acid. Discard the first 4 ml of effluent and collect the next 10 ml which contains the manganese. Evaporate the effluent almost to dryness, transfer to a 10-ml volumetric flask, and analyze for manganese as described in the recommended procedure for preparing a calibration curve.

Analysis of NBS samples

In order to evaluate the accuracy of the method, 4 National Bureau of Standards samples were analyzed for manganese by the recommended procedure. The results are summarized in Table I. Twelve samples of NBS No. 62 (Mn-Bronze) were analyzed. The standard deviation was 1.25%.

TABLE I

RESULTS OF ANALYSES OF NBS SAMPLES

NBS sample		*NBS (% Mn)*		*New method (% Mn)*	
Type	*No.*	*Range*	*Average*	*Range*	*Average*
Mn Bronze	62	1.53–1.64	1.59	1.58–1.63	1.60
Mn–Al Bronze	164	4.65–4.70	4.68	4.63–4.75	4.68
Ni–Cu Alloy	162	2.31–2.36	2.34	2.29–2.43	2.36
Steel	13c	0.692–0.706	0.700	0.692–0.708	0.701

The authors wish to express their thanks to the Wyandotte Chemicals Corporation for their gift of the N,N,N′,N′-tetrakis(2-hydroxypropyl)ethylenediamine ("Quadrol") used in this work.

SUMMARY

A new spectrophotometric method for the determination of manganese with N,N,N′,N′-tetrakis-(2-hydroxypropyl)ethylenediamine has been developed. The sensitivity is 0.12 μg of Mn per cm^2 for log I_0/I = 0.001 and Beer's law is obeyed between 2 and 100 p.p.m. of manganese. An ion-exchange separation of interfering ions was satisfactory for the analysis of a variety of National Bureau of Standards samples with good accuracy. The standard deviation with a Mn-bronze was 1.25%.

RÉSUMÉ

Les auteurs proposent une nouvelle méthode pour le dosage spectrophotométrique du manganèse, au moyen de la N,N,N′,N′-tétrabis(2-hydroxypropyl)éthylènediamine. La sensibilité est de 0.12 μg de Mn par cm^2 pour log I_0/I = 0.001; la loi de Beer est applicable entre 2 et 100 p.p.m. de manganèse. Les ions gênants peuvent être séparés au moyen d'un échangeur d'ions.

ZUSAMMENFASSUNG

Es wurde eine neue spektralphotometrische Methode zur Bestimmung von Mangan mit N,N,N′,N′-Tetrakis(2-hydroxypropyl)-äthylendiamin entwickelt. Die Empfindlichkeit beträgt 0.12 μg Mn pro cm^2 für log I_0/I = 0.001; das Beersche Gesetz wird zwischen 2 und 100 p.p.m. Mangan befolgt. Die Abtrennung störender Ionen mit einem Ionenaustauscher gelang befriedigend. Die Standardabweichung betrug bei einer Mn-Bronze 1.25%.

REFERENCES

1 D. A. KEYWORTH, *Talanta*, 2 (1959) 383.
2 J. H. YOE AND A. L. JONES, *Ind. Eng. Chem., Anal. Ed.*, 16 (1944) 111.
3 P. JOB, *Ann. Chim. (Paris)*, [10] 9 (1928) 113.
4 W. C. VOSBURGH AND G. R. COOPER, *J. Am. Chem. Soc.*, 63 (1941) 437.
5 A. E. HARVEY AND D. L. MANNING, *J. Am. Chem. Soc.*, 72 (1950) 4488.
6 E. B. SANDELL, *Colorimetric Determination of Traces of Metals*, 3rd Ed., Interscience, New York, 1959.
7 K. A. KRAUS AND G. E. MOORE, *J. Am. Chem. Soc.*, 75 (1953) 1460.

SPECTROPHOTOMETRIC STUDY OF N,N'-BIS(*m*-SULPHOBENZYL)DITHIOOXAMIDE AS A REAGENT FOR NICKEL(II)*

A. A. JANSSENS, G. L. VAN DE CAPPELLE AND M. A. HERMAN

Department of General Chemistry, The University of Ghent, Ghent (Belgium)

(Received April 12th, 1964)

Dithiooxamide[1] and some of its derivatives have been used as analytical reagents for the spectrophotometric determination of nickel(II). Since none of these nickel chelates is water-soluble, several authors have proposed the addition of gelatine[2,3], gum acacia[4-6] or pyridine[7-9]. N,N'-Bis(β-1-piperazylethyl)dithiooxamide was claimed by BAKER AND PFLAUM[10] to give water-soluble complexes with copper(II), cobalt(II) and nickel(II). In an earlier paper[11], the preparation of N,N'-bis(*m*-sulphobenzyl)dithiooxamide ($(SB)_2DTO$) was mentioned. Unlike dithiooxamide and most of its derivatives, $(SB)_2DTO$ is very soluble in water and gives also water-soluble chelates with several metal ions. $(SB)_2DTO$ forms an intensely red-coloured chelate with nickel(II) and is a good colorimetric reagent for qualitative analysis. In this paper the spectrophotometric determination of nickel(II) with $(SB)_2DTO$ is examined.

EXPERIMENTAL

Apparatus

Spectral curves were recorded with a Beckman Model DK1 spectrophotometer using silica cells of 1- and 0.5-cm light path. Absorbances at a definite wavelength were measured with a Hilger Uvispek spectrophotometer and partly with a Zeiss P.M.Q II spectrophotometer. Calibrated volumetric ware was used.

Reagents

Standard nickel solution. A stock solution containing approximately 10 mg/ml of nickel(II) was prepared by dissolving 45 g of nickel(II) sulphate hexahydrate (Merck p.a.) in 1 l of distilled water. The nickel content was determined by the gravimetric dimethylglyoxime method. The standard solution contained 9.972 mg of nickel(II) per ml. Working solutions were obtained as needed by dilution of this stock solution.

Reagent solution. A 0.01 *M* solution of $(SB)_2DTO$ was prepared by dissolving 4.943 g of the di-ammonium salt in 1000 ml of distilled water. The working solutions were obtained by diluting this stock solution. Unlike others of the family of substituted dithiooxamides, solutions of this compound proved to be stable for several weeks.

* This paper is dedicated to Professor D. Monnier on the occasion of his sixtieth birthday.

Buffer solutions. Two different kinds of buffer solutions were used, *i.e.* SØRENSEN borate buffers as given by KORDATZKI[12], and an ammonia–ammonium chloride buffer which was made by mixing appropriate amounts of a 0.1 *N* ammonia solution with a 0.1 *M* ammonium chloride solution.

Solutions of foreign ions. Stock solutions containing 1 mg per ml of the ion were prepared from nitrates or chlorides of the metal. For the anions sodium salts were used.

Preliminary studies

Effect of pH on absorbance. Solutions containing the same amounts of nickel and reagent but with different pH values were measured against the appropriate reagent blank. The absorbance (Fig. 1) showed a flat maximum between pH 8 and 11 and remained practically constant in the pH range from 9.0 to 10.5. Therefore all measurements were made at pH 10 $\pm$ 0.3.

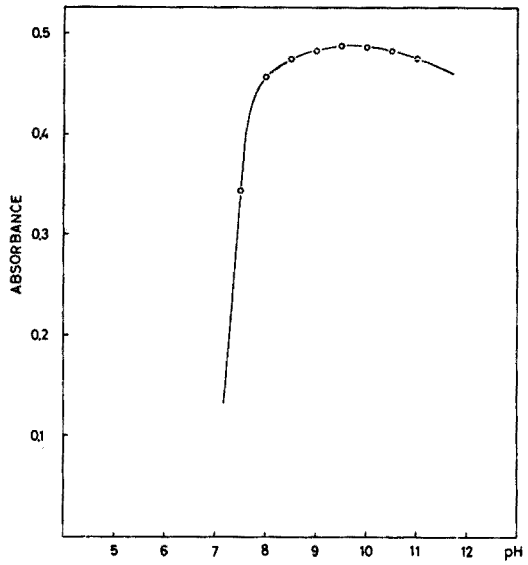


Fig. 1. Effect of pH on Ni(II)–$(SB)_2$DTO complex at 500 mμ.

Complex formation between nickel(II) and $(SB)_2$DTO. Absorbance curves obtained at pH 10 are shown in Fig. 2. The reagent showed an absorption maximum at 300 mμ and did not absorb above 400 mμ. The nickel(II) chelate showed maxima at 500 mμ and at 320 mμ. As the maximum at 320 mμ was partly due to the absorbance of the reagent, the maximum at 500 mμ was chosen for absorbance measurements. The composition of the complex formed was investigated by the continuous-variations method of JOB[13] as modified by VOSBURGH AND COOPER[14] (Fig. 3), the mole-ratio method of YOE AND JONES[15] (Fig. 4) and the slope-ratio method of HARVEY AND MANNING[16] (Fig. 5). It can be seen from these figures that only one complex corresponding to the ratio 1 Ni : 1 R was formed even in the presence of an excess of reagent.

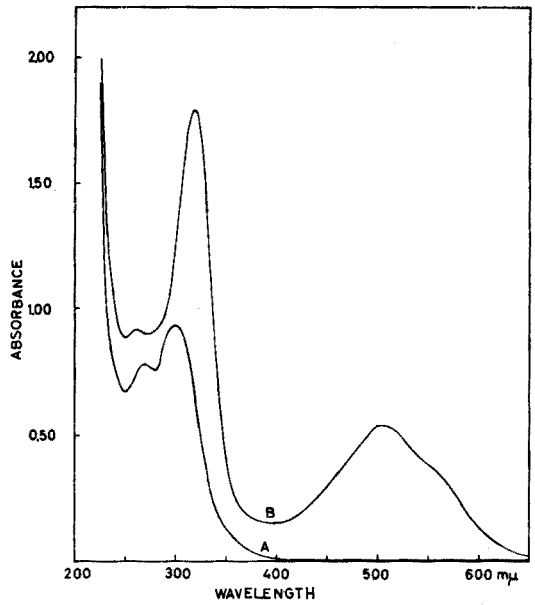


Fig. 2. Absorbance curves for Ni(II)–reagent mixtures at pH 10. A, reagent: $8 \cdot 10^{-5}$ *M*. B, reagent: $8 \cdot 10^{-5}$ *M*–Ni(II): $8 \cdot 10^{-5}$ *M*.

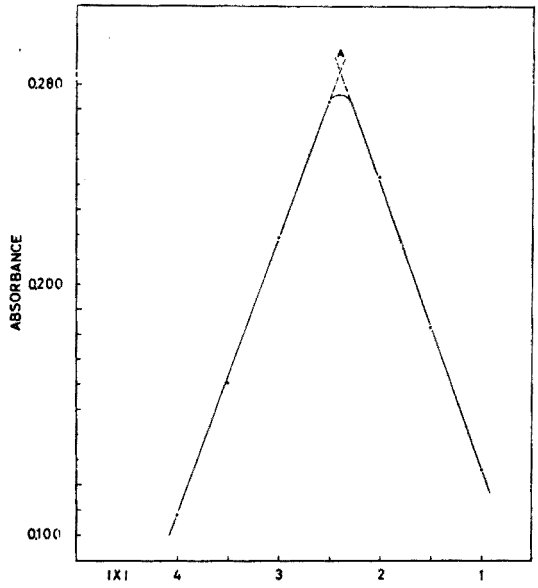


Fig. 3. Continuous-variations method at pH 10 and 500 mμ. Reagent: |5−x| ml 10^{-3} *M*; Ni(II): |x| ml 10^{-3} *M*.

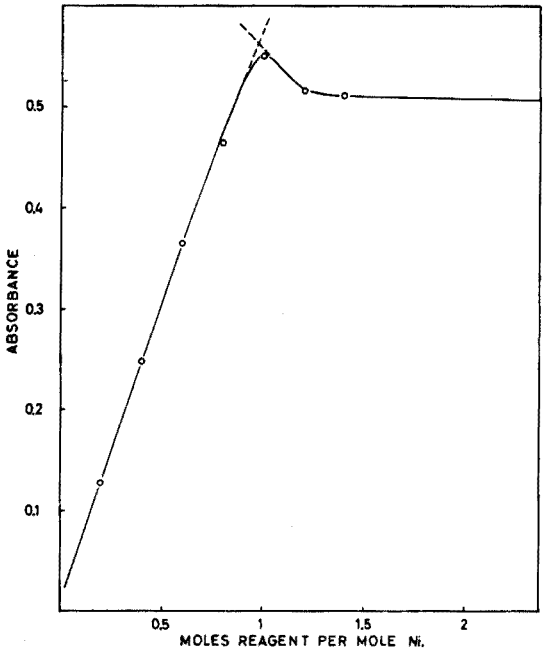


Fig. 4. Mole-ratio method at pH 10 and 500 mμ.

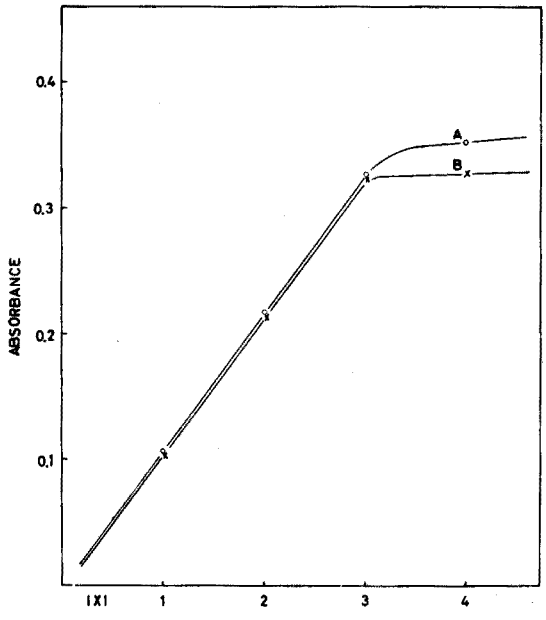


Fig. 5. Slope-ratio method at pH 10 and 500 mμ. A, reagent: |x| ml 10^{-3} M; Ni(II): 3 ml 10^{-3} M. B, reagent: 3 ml 10^{-3} M; Ni(II): |x| ml 10^{-3} M.

Effect of time on colour formation. Maximum absorbance was obtained after 90 min and the colour intensity then remained constant for at least 18 h.

Effect of the order of addition of reagents. The order of addition proved to be very important in developing the colour and in preventing the formation and precipitation of $Ni(OH)_2$. Experiments showed that the most suitable order of addition was: 1, nickel solution, 2, reagent solution, 3, buffer solution, 4, twice-distilled water. This sequence was always followed.

Adherence to Beer's law and sensitivity. Beer's law was obeyed over the range from 0.5 μg to 50 μg per ml. The average molar extinction coefficient, ε, was found to be 6962, with a mean deviation of 0.6 % and a mean square deviation of 0.79 %. The corresponding sensitivity as defined by SANDELL[17] was 0.0084 μg per ml.

Recommended procedure and standard deviation. To the sample containing an amount of nickel between 0.5 μg and 50 μg per ml, add 10 ml of 0.01 *M* reagent solution, followed by 10 ml of a pH 10 buffer solution. Dilute the solution to 50 ml with twice-distilled water. Measure the absorbance after at least 90 min, at 500 mμ against a reagent blank containing the same buffer solution.

Ten samples were measured in this way. The standard deviation was 0.002 absorption units, which corresponds to 0.017 μg of nickel per ml.

Effect of foreign ions

The influence of foreign ions on the colour intensity was studied. Samples were prepared containing 10 ml of a 0.01 *M* solution of reagent, 10 ml of a pH 10 buffer

TABLE I

EFFECT OF FOREIGN IONS

(Amount of Ni in the reference solution: 200 μg)

Foreign ion (X)	*Ratio X : Ni(II)*	*Nickel(II) found (μg)*	*% Error*
Cr^{3+}	0.05	194	−3
Mn^{2+}	0.06	204	+2
Co^{2+}	0.06	208	+4
Cu^{2+}	0.20	208	+4
Zn^{2+}	0.02	204	+2
Ru^{3+}	0.12	204	+2
Rh^{3+}	0.15	196	−2
Pd^{2+}	0.30	197	−1.5
Ag^{+}	1.25	196	−2
Cd^{2+}	0.12	204	+2
Ir^{3+}	2.00	204	+2
Pt^{4+}	2.50	194	−3
Au^{3+}	0.50	193	−3.5
Hg^{2+}	4.00	205	+2.5
Tl^{+}	6.00	205	+2.5
Pb^{2+}	0.20	205	+2.5
Nitrate	250.00	206	+3
Sulphate	150.00	204	+2
Tartrate	250.00	204	+2
Cyanide	0.01	194	−3

solution and 10 ml of a solution containing $2 \cdot 10^{-2}$ mg Ni(II)/ml. The order of addition of the solutions was the same as given above. Varying amounts of suitable solutions of foreign ions were added to the nickel(II) solution. The reagent solution was added, followed by the buffer solution. The sample was then diluted with twice-distilled water to 50 ml and measured against an appropriate reagent blank. The results are summarized in Table I.

SUMMARY

N,N'-Bis(*m*-sulphobenzyl)dithiooxamide forms a water-soluble intensely red chelate with nickel(II) in buffered solutions at pH 10. A 1 Ni : 1R complex is obtained. The absorption maximum of the complex lies at 500 mμ, where the absorbance of $(SB)_2DTO$ is negligible. The molar extinction coefficient is *ca.* 6962. Beer's law is obeyed over the range from 0.5 μg to 50 μg per ml.

RÉSUMÉ

La N,N'-bis(*m*-sulphobenzyl)dithioxamide réagit avec le nickel en solution tampon de pH 10, en formant un complexe rouge intense (1Ni : 1R), soluble dans l'eau. Le maximum d'absorption se situe à 500 mμ. Le coefficient d'absorption est d'environ 6962. La loi de Beer est valable entre 0.5 μg et 50 μg par ml.

ZUSAMMENFASSUNG

N,N'-Bis(*m*-sulfobenzyl)dithiooxamid bildet mit Nickel(II) in einer schwach alkalischen Pufferlösung einen wasserlöslichen, rot gefärbten Komplex. Der Komplex hat die Zusammensetzung 1Ni : 1R und besitzt ein Absorptionsmaximum bei 500 mμ. Der molare Extinktionskoeffizient beträgt etwa 6962. Das Beersche Gesetz gilt zwischen 0.5 und 50 μg/ml.

REFERENCES

1 P. RÂY AND R. M. RAY, *J. Indian Chem. Soc.*, 3 (1926) 118.
2 F. FARADY AND A. JANOSI, *Magy. Kem. Folyoirat*, 63 (1957) 19.
3 A. JANOSI AND F. FARADY, *Veszpremi Vegyip. Egyet. Tud. Ulesszakanak Eloadasai*, 2 (1957) 10.
4 W. D. JACOBS AND J. H. YOE, *Anal. Chim. Acta*, 20 (1959) 332.
5 W. D. JACOBS AND J. H. YOE, *Anal. Chim. Acta*, 20 (1959) 435.
6 W. D. JACOBS AND J. H. YOE, *Proc. Symp. Chem. Co-ord. Compounds, Agra, India, 1959*, Part III, Nat. Acad. Sci. India, 1960, p. 226.
7 J. XAVIER AND P. RÂY, *Sci. Cult. (Calcutta)*, 20 (1955) 455.
8 J. XAVIER AND P. RÂY, *Sci. Cult. (Calcutta)*, 21 (1956) 694.
9 J. XAVIER AND P. RÂY, *J. Indian Chem. Soc.*, 35 (1958) 432, 589, 633, 725.
10 H. W. BAKER AND R. T. PFLAUM, *Proc. Iowa Acad. Sci.*, 68 (1961) 174.
11 A. A. JANSSENS AND M. A. HERMAN, *Bull. Soc. Chim. Belges*, 70 (1961) 597.
12 W. KORDATZKI, *Taschenbuch der Praktischen pH-Messung*, Müller–Steinicke Verlag, München, 1941.
13 P. JOB, *Ann. Chim. Phys.*, 9 (1928) 113.
14 W. C. VOSBURGH AND G. R. COOPER, *J. Am. Chem. Soc.*, 63 (1941) 437.
15 J. H. YOE AND A. L. JONES, *Ind. Eng. Chem., Anal. Ed.*, 16 (1944) 111.
16 A. HARVEY AND D. L. MANNING, *J. Am. Chem. Soc.*, 72 (1950) 4488.
17 E. B. SANDELL, *Colorimetric Determination of Traces of Metals*, 3rd Ed., Interscience, New York, 1959, p. 83.

UNTERSUCHUNG DES WINKLER'SCHEN AMMONIAKMESSVERFAHRENS UNTER ANWENDUNG DER DAMPFRAUMANALYSE UND GLASELEKTRODE*

E. SCHULEK, J. TROMPLER, HOLLÓS E. ROKOSINYI, LÁNYI I. KONKOLY THEGE UND E. PUNGOR

Institut für Anorganische und Analytische Chemie, L. Eötvös Universität, Budapest

und

Institut für Analytische Chemie, Universität für Technische Chemie, Veszprém (Ungarn)

(Eingegangen den 27. November, 1963)

Wie allgemein bekannt ist, schlug WINKLER[1,2] im Jahre 1913 vor, beim Abdestillieren des Ammoniaks, z.B. im Rahmen des Stickstoffbestimmungsverfahrens von Kjeldahl, eine etwa 4 %ige Borsäurelösung vorzulegen. Auf diese Weise konnte er die zu befürchtenden Ammoniakverluste vermeiden und die direkt-titrimetrische Messung ermöglichen.

Können neben diesen Vorteilen überhaupt irgendwelche Nachteile auftreten? Wird WINKLER's Verfahren gegenwärtig in seiner ursprünglichen Form angewandt? Beide Fragen sollen nach der Beschreibung unserer Versuche und Auswertung der Messergebnisse beantwortet werden.

Die Frage aber, ob eine säurehaltige Lösung, sei es eine titerbeständige Mass- oder eine gesättigte Borsäurelösung, zur Bindung des übergehenden Ammoniaks überhaupt angewandt zu werden braucht, wurde in unserem Institut von SCHULEK, BURGER UND FEHÉR[3] in Anlehnung an die Arbeit von SCHULEK UND FÓTI[4] sowie an die bereits im Jahre 1958 begonnene vorliegende Arbeit eingehend untersucht.

An dieser Stelle wird das von WINKLER in Vorschlag gebrachte Verfahren eingehend und kritisch untersucht. Die Arbeit kann als Fortsetzung der bereits früher publizierten[3-6] angesehen werden.

BESTIMMUNG DES AMMONIAK-GLEICHGEWICHTSDRUCKES WÄSSRIGER AMMONIAK- UND AMMONIAK–BORSÄURE-LÖSUNGEN

Literaturwerte wässriger Ammoniak-Lösungen dienten uns als Ausgangspunkt beim Schätzen der zu bestimmenden Druckhöhe und Ammoniakmenge. Es galt demgemäss, Ammoniakmengen von 200 μg bis zu 10 μg herab in maximal 150-ml grossen Gasproben quantitativ zu erfassen. Die Gasproben entnahmen wir mit Hilfe des von uns entwickelten Dampfraumanalysenapparates[7-9].

Das sich zuerst anbietende photometrische Nessler-Verfahren, welches von BOLTZ,

* Dieser Artikel ist Professor D. Monnier gewidmet zu seinem sechzigsten Geburtstag.

bzw. TARAS[10] sehr eingehend behandelt wird, lieferte keine genauen, zuverlässigen Messergebnisse. Oft erhielten wir ganz falsche Messwerte, ohne irgendeinen Grund hierfür feststellen zu können. Wir schlugen deshalb einen anderen Weg ein und wählten die direkt-titrimetrische Methode von SCHULEK UND FÓTI[4], gebrauchten aber als Masslösung nicht Schwefelsäure, sondern Perchlorsäure[5]. Zur Absorption des Ammoniaks wandten wir im Falle des photometrischen Messvorganges das sogenannte Überführungs- oder Gaswaschflaschen-, beim titrimetrischen hingegen das Saugflaschenprinzip an. Letzteres wurde in unserem Institut schon früher bei der Bestimmung geringer Mengen Sauerstoffs[11] und Kohlendioxids[12] erfolgreich angewandt.

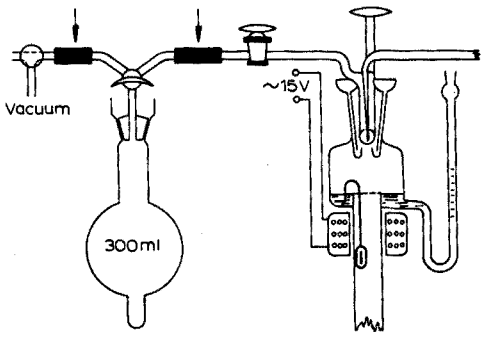


Fig. 1

Als Sauggefässe kamen 300-ml Rundkolben gemäss Fig. 1, als Absorptionsflüssigkeit etwa 3 ml Wasser und als Spülgas mit KOH-Lösung und konz. H_2SO_4 gereinigte Luft zur Anwendung. Titriert wurde mit 0.002 *N*, in einigen Fällen mit 0.001 *N* Perchlorsäure. Zur Dichtung der Glashähne gebrauchten wir Paraffinöl, zur Äquivalenzpunktanzeige den von HAWES UND SKAVINSKI[13] sowie MA UND ZUAZAGA[14] in Vorschlag gebrachten Mischindikator Methylrot–Bromkresolgrün. Die Endpunktanzeige von grün über grau nach rot hin erfolgt äusserst scharf, so dass keine Vergleichslösung angewendet zu werden brauchte. Wegen der Alkaliabgabe des Glases musste der Kolben mit schwach saurem und destilliertem Wasser gründlich gespült werden.

Vorversuche bewiesen, dass das direkt-titrimetrische Saugflaschenverfahren zuverlässige Messergebnisse liefert.

Das zur Absorption verwendete Wasser wurde mit 1 Tropfen 0.02 % Methylrot- sowie 2 Tropfen 0.04% Bromkresolgrün-Indikatorlösung versetzt und mit Hilfe von 0.002 *N* $HClO_4$- und NaOH-Lösungen ein eben rötlicher Farbton eingestellt. Die Probenahme erfolgte auf die in Ref. 7 und 12 beschriebene Art und Weise, aber ohne Pentanverschluss. Nach Schliessen des Kolbens wurden die Rohrverbindungen (siehe Pfeile in Fig. 1) gelöst und der Kolbeninhalt etwa 10 Min. lang geschüttelt. Titriert wurde mit einer 3-ml Mikrobürette. Die 0.002 *N* $HClO_4$-Masslösung wurde aus einer genau eingestellten 0.1 *N* Lösung hergestellt und höchstens 4 Tage lang gelagert. (Erst nach dieser Zeit war eine nicht unbedeutende Titeränderung festzustellen.)

TABELLE I

SYSTEM: AMMONIAK–WASSER

Temperatur (°)	NH_3-Gehalt der Flüssigkeit		Grösse der Gasprobe (ml) (N.Z.)	Verbraucht 0.002 N $HClO_4$-Lösung (ml)	NH_3-Gehalt der Gasprobe (µg)	p_{NH_3} (mm Hg)		Abweichung vom Mittelwert (Δ%)
	Molenbruch	g/g%				Einzelwerte	Mittelwert	
	$1.980 \cdot 10^{-4}$	0.01872	92.62	0.246	8.38	0.0905		−0.3
			92.01	0.244	8.31	0.0903	0.0907	−0.4
	$2.035 \cdot 10^{-4}$	0.01924	138.63	0.372	12.67	0.0914		+0.8
			92.25	0.246	8.38	0.0908		+0.01
			45.85	1.395	47.52	1.031		−3.9
			45.83	1.450	49.39	1.072		−0.1
20.21	$1.972 \cdot 10^{-3}$	0.1865	46.10	1.450	49.39	1.097	1.073	+2.2
			46.09	1.482	50.48	1.096		+2.1
			46.50	1.451	49.43	1.064		−0.8
			9.72	3.029	103.2	10.62		0
			9.69	3.015	102.7	10.60		−0.2
	$1.906 \cdot 10^{-2}$	1.804	8.72	2.755	91.71	10.52	10.62	−0.9
			8.10	2.528	86.11	10.64		+0.2
			8.40	2.650	90.27	10.74		+1.1
			90.25	0.377	12.84	0.1423		−2.7
	$2.031 \cdot 10^{-4}$	0.01920	89.35	0.391	13.32	0.1491	0.1463	+1.9
			89.53	0.390	13.28	0.1485		+1.4
			45.00	0.192	6.540	0.1455		−0.6
			46.26	2.314	78.82	1.705		+1.2
30.00	$1.966 \cdot 10^{-3}$	0.1859	19.04	0.935	31.84	1.674	1.685	−0.6
			21.59	1.053	35.87	1.662		−1.4
			10.98	0.548	18.67	1.701		+0.9
			10.30	5.119	174.4	16.94		−0.7
	$1.906 \cdot 10^{-2}$	1.776	10.73	5.335	181.7	16.94	17.06	−0.7
			6.05	3.033	103.3	17.07		+0.06
			4.72	2.399	81.72	17.30		+1.4

TABELLE II

SYSTEM: AMMONIAK–WASSER–BORSÄURE ($t = 30.00°$)

NH_3-Gehalt der Flüssigkeit		H_3BO_3-Gehalt der Flüssigkeit		Grösse der Gasprobe (ml) (N.Z.)	Verbraucht 0.002 N $HClO_4$-Lösung (ml)	NH_3-Gehalt der Gasprobe (μg)	p_{NH_3} (mm Hg)		Abweichung vom Mittelwert (Δ%)
Molenbruch	g/g%	Molenbruch	g/g%				Einzelwerte	Mittelwert	
				10.48	0.407	13.86	1.324		−1.3
$1.982 \cdot 10^{-3}$	0.1871	$5.822 \cdot 10^{-4}$	0.199	45.35	1.799	61.28	1.352	1.341	+0.8
				45.37	1.798	61.25	1.351		+0.7
				10.31	0.405	13.80	1.339		−0.1
				9.75	0.251	8.55	0.876		−1.2
$2.007 \cdot 10^{-3}$	0.1890	$1.747 \cdot 10^{-3}$	0.597	45.05	1.180	40.19	0.892	0.887	+0.5
				44.94	1.158	39.45	0.878		−1.1
				17.74	0.470	16.01	0.902		+1.7
				44.55	0.773	26.33	0.591		−3.2
				44.37	0.805	27.42	0.618		+1.2
$2.023 \cdot 10^{-3}$	0.1899	$2.917 \cdot 10^{-3}$	0.994	44.96	0.800	27.25	0.606	0.6106	−0.7
				44.66	0.802	27.32	0.612		+0.2
				44.91	0.825	28.10	0.626		+2.5
				88.68	0.723	24.63	0.276		−0.1
$2.046 \cdot 10^{-3}$	0.1908	$5.811 \cdot 10^{-3}$	1.967	44.73	0.356	12.13	0.271	0.276	−1.8
				44.70	0.361	12.30	0.281		+1.9
$2.032 \cdot 10^{-3}$	0.1867	$1.188 \cdot 10^{-2}$	3.963	89.41	0.077	2.69	0.0293	0.0296	−0.8
				134.69	0.233[a]	3.97	0.0298		+0.8

[a] Mit 0.001 N $HClO_4$-Lösung titriert.

Das Ammoniak der Flüssigkeitsprobe wurde gemäss der in Ref. 3 angegebenen Vorschriften direkt-titrimetrisch gemessen. Zur Äquivalenzpunktanzeige wurde auch in diesem Falle statt Methylrot der oben erwähnte Mischindikator angewandt.

Aus den analytischen Messergebnissen wurden mittels der in Ref. 7 angeführten Gleichung die Ammoniak-Gleichgewichtsdrucke berechnet. Die Ergebnisse für Ammoniak–Wasser-Gemische sind in Tabelle I, für Ammoniak–Wasser–Borsäure in Tabelle II zusammengestellt. Zwischen unseren, in Tabelle I und den in der Literatur[15,16] angeführten Messwerten besteht kein wesentlicher Unterschied. Letztere ergeben für den Ausdruck p_{NH_3}/x_{NH_3}, der bei Ammoniak–Wasser-Gemischen nicht konstant ist, den Wert von 918 (bezogen auf etwa molare Ammoniak-Lösungen bei 30°). Gemäss Tabelle I würde dies bei 30° und dem Ammoniak-Molenbruch von $1.906 \cdot 10^{-2}$ einem Ammoniak-Druck von 17.49 mm Hg entsprechen. Tabelle I führt als Mittelwert 17.06 mm Hg an. Die Differenz beträgt bloss —2.5%, ist also verhältnismässig gering, und muss damit erklärt werden, dass ein grundsätzlicher Unterschied zwischen den in der Literatur angeführten und unserem Verfahren besteht. PERMAN[15,17] wandte z.B. das Überführungsverfahren an und erhielt unserer Meinung nach deshalb etwas höhere Werte. Auf die praktische Bedeutung der Werte in Tabelle I bei der direkt-titrimetrischen Ammoniakbestimmung im Rahmen des Kjeldahlverfahrens wiesen bereits SCHULEK, BURGER UND FEHÉR[3] hin.

Die Lösungen der Versuchsreihe Ammoniak–Wasser–Borsäure wurden in 50-ml Messkolben hergestellt, u.zw. unter Anwendung kristalliner Borsäure und etwa 0.1 *N*

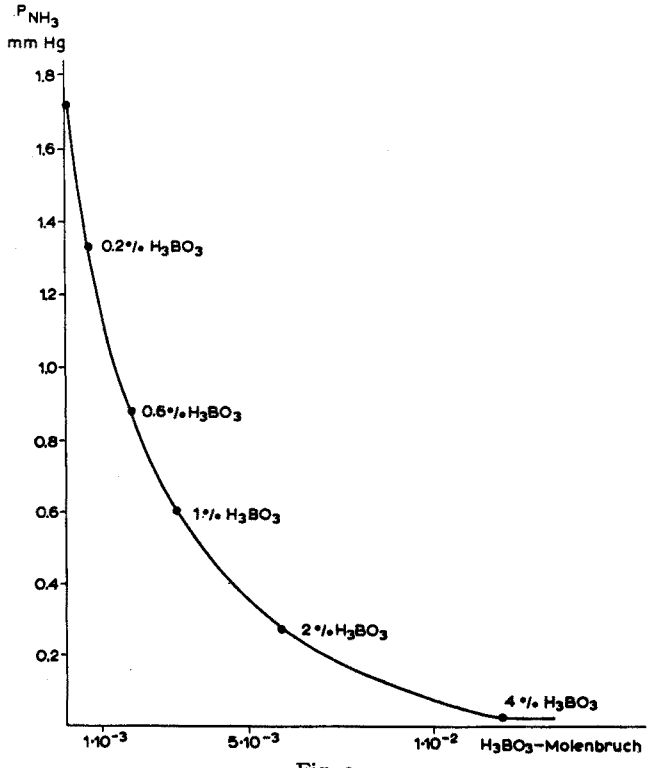


Fig. 2

Ammoniak-Lösung. Der Ammoniakgehalt der Versuchslösungen betrug 0.187–0.191 g/g%, und der dem Mittelwert entsprechende, nach Angaben der Tabelle I berechnete partielle Ammoniakdruck 1.71 mm Hg. Bei der graphischen Darstellung der Messergebnisse auf Fig. 2 wurde dieser Wert der borsäurefreien Ammoniak-Lösung zugeordnet und nicht der aus Tabelle I. Sowohl die Werte in Tabelle II als auch der Verlauf der Kurve in Fig. 2 zeigen, wie gross die partielle Druckerniedrigung ist. Ein Zusatz von 2% Borsäure zu einer 0.1 *N* Ammoniak-Lösung (Molverhältnis von $NH_3:H_3BO_3=1:3$) erniedrigt den Ammoniakdruck um nicht ganz eine Grössenordnung, von 4% (Molverhältnis 1:6) um eine weitere. Die Flüchtigkeit des Ammoniaks wird also nur unter solchen Bedingungen wesentlich erniedrigt. Von diesem Standpunkt aus betrachtet, ist daher ein 2%iger Borsäuregehalt, geschweige denn ein 1%iger unzureichend.

VERSUCHE AUF DEM GEBIETE DER DIREKT-TITRIMETRISCHEN AMMONIAKBESTIMMUNG

Wir waren bei der Untersuchung der Frage, unter welchen Bedingungen die direkt-titrimetrische Ammoniakbestimmung durchgeführt werden muss, bestrebt, exakte Messergebnisse unter Anwendung der elektrometrischen Endpunktanzeige zu erhalten und diese mit denen der Farbindikatormethode zu vergleichen. Um Ammoniakverluste, die beim Rühren einer im offenen Becherglas befindlichen Ammoniak-Lösung unbedingt auftreten würden, von vornherein auszuschliessen, titrierten wir mit der Ammoniak-Lösung. Die 0.1 *N* Ammoniak-Lösung dosierten wir unter Anwendung der auf Fig. 3 abgebildeten Bürette. (Ein ähnliches Gerät wird in unserem Institut bei Versuchen mit Halogenen angewandt[18].) Angewandt wurde 5.00 ml 0.1 *N* Perchlorsäure und 0.5–2.0 g kristalline Borsäure. Das Ausgangs-Lösungsvolumen betrug 55 ml.

Die Äquivalenzpunkte wurden sowohl graphisch als auch rechnerisch ermittelt und sind in der Unterschrift der Fig. 4 angeführt. Ein Borsäurezusatz von etwa 1, 2 und 4% ändert bloss den Kurvenast nach dem Inflexionspunkt, wie dies übrigens theoretischen Erwägungen zufolge und experimentell auf Grund der Kurve *e* auch zu erwarten war. Der Verlauf dieser Kurve erklärt also das Verhalten einer borsäurehaltigen, speziell einer etwa 4% Borsäure enthaltenden Perchlorsäure-Lösung. Die maximale pH-Differenz zwischen zwei aufeinander folgenden Messpunkten—bei 4.80 und 4.90 ml—nimmt von 2.7 über 1.5 und 0.8 bis 0.4 stark ab (siehe die Kurven *a*, *b*, *c* und *d*). Auch der dem Äquivalenzpunkt entsprechende pH-Wert sinkt rasch von 5.7 über 5.0 und 4.4 nach 3.8. Man sieht deutlich, dass sich einerseits das Umschlagsintervall von Methylorange nicht mit dem pH-Sprung der Kurve *a* und anderseits das von Methylrot nicht mit dem der Kurve *d* deckt. In der einschlägigen Literatur findet man diesem Befund zuwidersprechende Angaben. So schlägt VOGEL[19] beim Verwenden einer gesättigten Borsäurelösung Bromkresolgrün, Bromphenolblau sowie den Mischindikator Methylrot–Bromkresolgrün vor. Bei FRESENIUS UND JANDER[20] wird neben anderen WINKLER's Verfahren kurz erwähnt und Methylrot, gegebenenfalls in Kombination mit Methylenblau, Bromkresolgrün oder Bromphenolblau empfohlen.

Es soll ausdrücklich darauf hingewiesen werden, dass das pH der im Äquivalenzpunkt vorliegenden 4% Borsäure enthaltenden Ammoniumsalzlösung zwischen 3.60–3.80 und nicht zwischen 5–6 liegt, wie dies VOGEL in seinem Buche anführt. Die pH-Werte wurden übrigens elektrometrisch unter Anwendung bis zu 0.1 *N*

Ammoniumsalzlösungen (bezogen auf NH_3) bestimmt und entsprechen dem Inflexionspunkt der Kurve *d* in Fig. 4.

Unsere Untersuchungen zeigten also eindeutig, dass bei der direkt-titrimetrischen Ammoniakbestimmung in erster Linie die Wahl des Indikators bedeutend ist, was

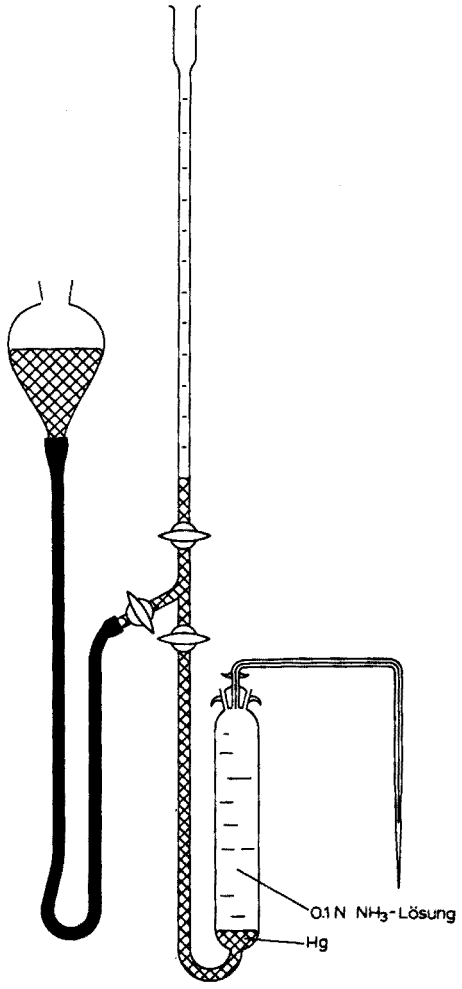


Fig. 3

Versuche unter Anwendung der Farbindikationsmethode noch bestätigten. Beim Titrieren der doppelten Perchlorsäuremenge (10.00 ml 0.1 *N*) erhielten wir als Mittelwert mehrerer Parallelversuche einen Verbrauch von 9.68 ml 0.1 *N* Ammoniak-Lösung, wobei sowohl die Glaselektrode als auch der bereits oben erwähnte Mischindikator gebraucht wurde. Die Einzelwerte wichen voneinander maximal 0.2% ab. Bei unseren weiteren Versuchen verfuhren wir nun so, dass 9.68 ml 0.1 *N* Ammoniak-Lösung (gemessen mit der Bürette in Fig. 3) zur Einwaage gelangten. Die Versuche

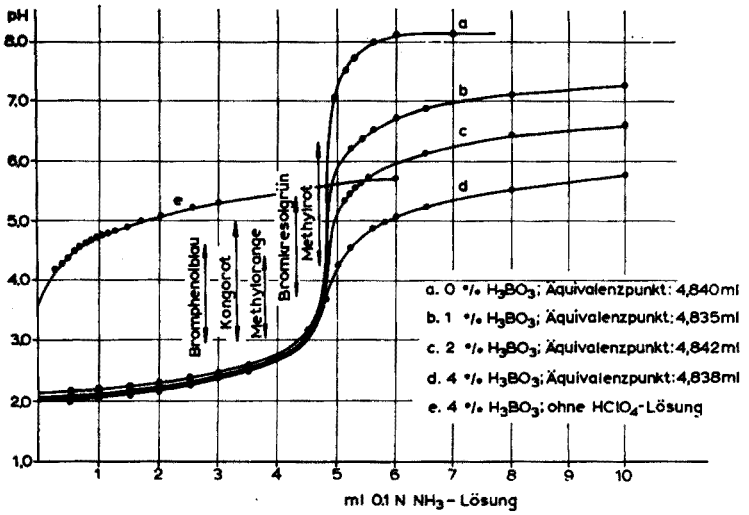


Fig. 4

TABELLE III

Indikator	*Borsäure-gehalt (g/v%)*	*Verbraucht 0.1 N $HClO_4$-Lösung (ml)*	*Abweichung vom Sollwert (Δ%)*
Mischindikator Methylrot–Bromkresolgrün (titriert bis rötlich grau)	0	9.99	−0.1
	1	9.97	−0.3
	2	9.90	−1.0
	4	8.99	−10.1
Methylorange (titriert bis orange)	0	10.05	+0.5
	1	10.06	+0.6
	2	10.04	+0.4
	4	9.95	−0.5
		9.99[a]	−0.1
	Sollwert:	10.00	

[a] In diesem Fall wurde nach WINKLER's Vorschrift eine Vergleichslösung angewandt.

TABELLE IV

MISCHINDIKATOR METHYLROT-BROMKRESOLGRÜN

Einwaage 0.1 N NH_3-Lösung (ml)	*0.1 N $HClO_4$-Lösung*		*Abweichung vom Sollwert (Δ%)*
	Verbraucht (ml)	*Sollwert (ml)*	
1 × 9.68	8.99	10.00	−10.1
2 × 9.68	19.38	20.00	−3.1
3 × 9.68	29.55	30.00	−1.5

wurden in 150-ml Stehkolben gemäss[21] durchgeführt und stets ein Ausgangsvolumen von 50 ml eingehalten.

Methylrot kann in borsäurefreier Lösung, wie Tabelle III zu entnehmen ist, sehr gut bei der direkt-titrimetrischen Ammoniakbestimmung eingesetzt werden, noch besser aber der Mischindikator. Unter gleichen Bedingungen erhielten wir bei Methylorange positive Fehler, was auf Grund der Fig. 4 auch verständlich ist.

Anders steht es beim Titrieren borsäurehaltiger, besonders 4% Borsäure enthaltender Ammoniaklösungen. Methylrot darf in diesem Falle nicht angewandt werden. WINKLER wies in seiner Arbeit[2] ausdrücklich darauf hin, dass die Indikatoren Methylorange und Kongorot zu verwenden sind. Der Ablauf der Kurve *d* auf Fig. 4 zeigt übrigens sehr deutlich, dass WINKLER richtige Indikatoren wählte. Im Falle von Methylorange ist eine Vergleichslösung (siehe Ref. 2 und Tabelle III) zu verwenden.

Die Messergebnisse der Tabelle IV stehen im Einklang mit dem Ablauf der Kurve *d* in Fig. 4. Die Messgenauigkeit hängt demnach sehr stark von der zu bestimmenden Ammoniakmenge ab.

Die Frage also, ob WINKLER's Verfahren gegenwärtig in seiner ursprünglichen Form gebraucht, bzw. vorgeschlagen wird, muss, wie aus den zahlreichen Literaturangaben hervorgeht, verneint werden.

Bei der zweiten Frage, ob neben den Vorteilen der WINKLER'schen Methode überhaupt irgendwelche Nachteile auftreten, darf folgendes festgestellt werden: Ein grosser Nachteil ist, dass die pH-Differenz im Äquivalenzpunkt, wie Fig. 4 zeigt, und worauf ausdrücklich hingewiesen wurde, stark abnimmt. Experimentell zeigt sich dies im Hinziehen des Farbumschlages und der Ungenauigkeit der Äquivalenzpunktbestimmung (siehe den grossen Unterschied im Ablauf der Kurven *a* und *d* in Fig. 4). Dies hat zur Folge, dass die Bestimmung nur mit geringer Sicherheit und sehr langsam durchgeführt werden kann. Wenn keine Vergleichslösung angewandt wird, treten diese nachteiligen Faktoren noch mehr in den Vordergrund. Wegen der erwähnten Umstände kann das Verfahren im Mikro- und Ultramikromassstab nicht eingesetzt, bzw. vorgeschlagen werden.

ZUSAMMENFASSUNG

Die Verfasser untersuchten das WINKLER'sche Messverfahren und stellten folgendes fest. Der partielle Ammoniakdruck einer zur Analyse vorliegenden 0.1, bzw. 0.01 *N* Lösung wird nur durch einen Borsäuregehalt von 4% bedeutend erniedrigt. Ammoniakverluste sind unter solchen Bedingungen nicht zu befürchten. Die Messergebnisse bewiesen anderseits, dass das Ammoniak ohne Borsäurezusatz direkt-titrimetrisch sicherer, rascher und genauer bestimmt werden kann. Beim Titrieren borsäurefreier Ammoniaklösungen liegt der Äquivalenzpunkt im Umschlagsintervall von Methylrot, bei borsäurehaltigen (4%) in dem von Methylorange. Die in der Literatur angeführten Indikatoren werden als völlig falsch befunden.

SUMMARY

A detailed study of WINKLER's method shows that the partial pressure of ammonia in a 0.1 or 0.01 *N* solution for analysis is significantly lowered only by a boric acid content of 4%; under such conditions there is no danger of loss of ammonia. However, the ammonia can be determined with greater certainty, speed and accuracy by direct titration without addition of boric acid. In the titration of boric acid-free ammonia solutions, the equivalence point lies within the pH range of methyl red; if boric acid (4%) is present, methyl orange should be used. Literature data on the choice of indicator are shown to be false.

RÉSUMÉ

Une étude détaillée de la méthode de WINKLER a montré que la pression partielle de l'ammoniac peut être abaissée considérablement par addition d'acide borique. Dans ces conditions, il n'y a plus de risque de perte d'ammoniac. Cependant son dosage est possible sans addition d'acide borique, avec rouge de méthyle comme indicateur; alors qu'en présence d'acide borique on utilisera le méthylorange.

LITERATUR

1 L. W. WINKLER, *Angew. Chem.*, 26 (1913) 231.
2 L. W. WINKLER, *Angew. Chem.*, 27 (1914) 630.
3 E. SCHULEK, K. BURGER UND M. FEHÉR, *Z. Anal. Chem.*, 167 (1959) 28.
4 E. SCHULEK UND GY. FÓTI, *Anal. Chim. Acta*, 3 (1949) 665.
5 E. SCHULEK UND L. Z. SZABÓ, *Z. Anal. Chem.*, 157 (1957) 405.
6 E. SCHULEK UND G. VASTAGH, *Z. Anal. Chem.*, 92 (1933) 352.
7 E. SCHULEK, E. PUNGOR UND J. TROMPLER, *Mikrochim. Acta*, (1956) 1005.
8 E. SCHULEK, E. PUNGOR UND J. TROMPLER, *Mikrochim. Acta*, (1958) 52.
9 E. SCHULEK, E. PUNGOR UND J. TROMPLER, *Acta Chim. Acad. Sci. Hung.*, 26 (1961) 157.
10 D. F. BOLTZ, *Colorimetric Determination of Nonmetals*, Interscience, New York–London, 1958, S. 75.
11 E. SCHULEK UND E. PUNGOR, *Magy. Kém. Folyóirat*, 56 (1950) 250.
12 E. SCHULEK, J. TROMPLER, Á. ENDRÖI HAVAS UND I. REMPORT, *Anal. Chim. Acta*, 24 (1961) 11.
13 R. C. HAWES UND E. R. SKAVINSKI, *Ind. Eng. Chem., Anal. Ed.*, 14 (1942) 917.
14 T. S. MA UND G. ZUAZAGA, *Ind. Eng. Chem., Anal. Ed.*, 14 (1942) 280.
15 LANDOLT-BÖRNSTEIN, *Physikalisch-chemische Tabellen*, Hptw., Springer, Berlin, 1923, S. 1397.
16 LANDOLT-BÖRNSTEIN, *Physikalisch-chemische Tabellen*, Ergbd. I, Springer, Berlin, 1927, S. 764.
17 E. P. PERMAN, *J. Chem. Soc.*, 83 (1903) 1168.
18 E. SCHULEK UND E. PUNGOR, *Anal. Chim. Acta*, 5 (1951) 137.
19 A. J. VOGEL, *Text-Book of Quantitative Inorganic Analysis*, 3. Ausgabe, Longmans-Verlag, London, 1961, S. 257.
20 W. FRESENIUS UND G. JANDER, *Handbuch der analytischen Chemie*, 3. Teil, Band Va, bearbeitet von W. LEITHE, Springer, Berlin–Göttingen–Heidelberg, 1957, S. 35.
21 E. SCHULEK, K. BURGER UND M. FEHÉR, *Z. Anal. Chem.*, 167 (1959) 423.

2,3,5,6-TETRAKIS-(2'-PYRIDYL)-PYRAZINE AS A COLORIMETRIC REAGENT FOR IRON

R. T. PFLAUM, C. J. SMITH, JR.*, E. B. BUCHANAN, JR. AND R. E. JENSEN

Department of Chemistry, State University of Iowa, Iowa City, Iowa (U.S.A.)

(Received February 24th, 1964)

Organic compounds containing the —N=C—C=N— and the —N=C—C=N—C—C=N— groupings have been recognized as chelate ligands since the latter part of the 19th century. Intensely colored complexes are formed in the reactions of 1,10-phenanthroline, 2,2'-bipyridine, 2,2',2''-terpyridine and their derivatives with transitional metal ions. The terroin grouping, —N=C—C=N—C=C—N=, is also found in pyridine-substituted pyrazines and triazines. The pyrazine compound, 2,3,5,6-tetrakis-(2'-pyridyl)-pyrazine, has been prepared and some of its metal chelate salts isolated[1].

The intensely colored reaction product formed between the ligand and the iron(II) ion was thoroughly investigated in this work. The optimum conditions for complex formation, the stoichiometry of the chelate, and its extractability into nonaqueous solvents were determined. The analytical possibilities of the reagent were recognized and evaluated. A spectrophotometric method for the determination of iron is proposed.

EXPERIMENTAL

Apparatus and reagents

Absorptimetric measurements were made with a Cary Model 14 recording spectrophotometer. One-cm silica cells were used for all measurements. A Beckman Model G pH meter equipped with glass-calomel electrodes was used for all pH measurements.

The organic reagent, 2,3,5,6-tetrakis-(2'-pyridyl)-pyrazine was prepared by the method described by GOODWIN AND LIONS[1]. α-Pyridoin (K and K Laboratories, Inc.), 40 g (0.2 mole), was heated with 180 g (0.42 mole) of ammonium acetate at 180° for 2 h. The solid reaction product obtained upon cooling was recrystallized from pyridine. The reaction yielded 8.0 g of the ligand (20% yield), m.p. 282–284° (lit. 284°).

Stock solutions of the reagent were prepared by dissolution of the solid compound in 25 ml of 1:1 hydrochloric acid and dilution to volume with distilled water.

A standard iron solution was prepared by dissolving reagent-grade iron wire (Merck and Co., Inc.) in 15 ml of concentrated hydrochloric acid and diluting to 1 l with deionized water. Aliquots of this solution were titrated with sulfatocerate solution. The concentration was found to be $1.83 \cdot 10^{-3}$ moles/l.

* Present address: Tuskegee Institute, Tuskegee, Alabama.

The buffer solution used was 2 *M* in acetic acid and 2 *M* in sodium acetate. Hydroxylamine hydrochloride (10%) was used as the reducing agent.

Nitrobenzene, used as an extractant, was purified by vacuum distillation. Reagent-grade chloroform was used without further purification. All other reagents were analytical grade and were used as received.

Investigation of the reagent

The solubility of the reagent was determined in a variety of common organic solvents. Since the reagent is insoluble in water, mixed solvent systems of water and miscible organic solvents were also investigated.

Ultraviolet characteristics of tetra-(2'-pyridyl)pyrazine, TPP, were ascertained from absorption spectra obtained on aqueous solutions of the reagent over the pH range of 1.8–10.4. The pH in these solutions was adjusted with varying amounts of standard buffer solutions.

The reactivity of the reagent toward selected transition metal ions was determined spectrophotometrically. Stock solutions approximately 0.1 *M* in nickel(II), copper(II), and ruthenium(III) were prepared by the dissolution of weighed amounts of the chloride salts in water. The standard iron solution was used for the iron system. An excess of hydroxylamine hydrochloride was added to the ruthenium and iron solutions. Solutions approximately $1.0 \cdot 10^{-3}$ *M* in metal ion and $4.0 \cdot 10^{-3}$ *M* in reagent were prepared from the above stock solutions and were measured spectrophotometrically.

The iron(II)–2,3,5,6-tetrakis-(2'-pyridyl)-pyrazine system

The absorption spectrum of the iron(II) complex was examined in solutions of varying acidity. Solutions of pH 1.0–9.2 were prepared containing 4 mmoles of iron and an excess of reagent and hydroxylamine hydrochloride.

The stoichiometry of the complex was determined by the mole ratio method[2] and the method of continuous variations[3]. Solutions were prepared containing $5 \cdot 10^{-5}$ *M* iron, 2 ml of 10% hydroxylamine hydrochloride, 5 ml of 2 *M* acetate buffer, and varying amounts of $1 \cdot 10^{-3}$ *M* TPP solution. The final concentration of TPP varied from $2.25 \cdot 10^{-4}$ to $2.5 \cdot 10^{-5}$ *M*. The absorption spectra of these solutions were recorded.

The combining ratio of tetra-2'-pyridylpyrazine and iron(II) by the method of continuous variations was carried out on a series of solutions using $2.5 \cdot 10^{-4}$ *M* TPP and $2.5 \cdot 10^{-4}$ *M* iron stock solutions. The solutions were combined in amounts to vary the iron to reagent ratio from 0.1 to 0.9. Two ml of hydroxylamine hydrochloride solution and 5 ml of acetate buffer were added to each solution. Data were recorded from the measurement of the absorption spectrum of each solution.

The conformity of the complex to Beer's law was investigated. A series of solutions containing an excess of TPP and iron(II) ion in a concentration range of $5.0 \cdot 10^{-6}$ to $1.0 \cdot 10^{-4}$ *M* were prepared. These solutions also contained 2 ml of hydroxylamine hydrochloride and 5 ml of 2 *M* acetate buffer. The absorbance values for these solutions were obtained at 575 mμ.

In the presence of perchlorate ion, the tetra-(2'-pyridyl)pyrazine iron(II) complex was extracted from aqueous solution into chloroform and nitrobenzene. Aliquots (25 ml) of the complex solution containing 2 mmoles of perchlorate ion were

extracted with 2–3 10-ml volumes of the extractant. The combined extracts were filtered through cotton into a 50-ml volumetric flask and diluted to volume with additional solvent. Absorbance values were recorded against corresponding solvent reference solutions.

The effects of diverse ions on the iron(II) system were investigated by the addition of varying amounts of diverse ion to the complex system containing 0.56 p.p.m. of iron. Into a 25-ml volumetric flask containing 1 ml of the ion to be tested, one ml of $2.5 \cdot 10^{-4}$ *M* standard iron solution, 5 ml of $2.5 \cdot 10^{-4}$ *M* TPP, 5 ml of 20% sodium acetate and 2 ml of 10% hydroxylamine hydrochloride were mixed together. Stock solutions of interfering ions were prepared from reagent-grade salts at concentrations such that a 25-fold dilution would equal the concentrations shown in Table IV.

Recommended procedure

Dissolve the sample to be analyzed by appropriate means. Adjust the pH of the solution to approximately 4. To a 5-ml aliquot containing from 0.3 to 5.6 p.p.m. of iron in a 50-ml volumetric flask, add 2 ml of 10% hydroxylamine hydrochloride, 5 ml of 2 *M* acetate buffer, and 10 ml of $1.0 \cdot 10^{-3}$ *M* TPP solution. Dilute to volume with distilled water and mix thoroughly. Measure the absorption of the solution in 1-cm cells with an appropriate spectrophotometer at a wavelength of 575 mμ. From a previously prepared calibration curve, determine the amount of iron in the unknown sample.

DISCUSSION

Tetra-(2′-pyridyl)pyrazine, TPP, was found to be soluble in chloroform, nitrobenzene, and dilute hydrochloric acid. The reagent is insoluble in water and only slightly soluble in water-miscible solvents. The reagent is insoluble in methyl cellosolve and most other common water-immiscible solvents.

The reagent in very acidic solutions exhibited two absorption maxima. In 50% sulfuric acid, absorption peaks were located at 280 mμ ($\varepsilon = 25{,}000$) and 342 mμ ($\varepsilon = 28{,}400$). At pH 1.0, the two peaks had shifted to 290 mμ ($\varepsilon = 21{,}500$) and 333 mμ ($\varepsilon = 24{,}500$). The compound in solutions of pH greater than 4.2 exhibited wavelengths of maximum absorption at 320 mμ ($\varepsilon = 19{,}200$) and 297 mμ ($\varepsilon = 20{,}000$).

The tridentate ligand, 2,3,5,6-tetrakis-(2′-pyridyl)-pyrazine reacts with transition metal ions to form soluble colored complexes. Absorptimetric data for a series of metal ions are presented in Table I. The molar absorptivity of the iron complex was

TABLE I

ABSORPTIMETRIC DATA ON METAL ION COMPLEXES

Metal ion	λ_{max}	ε
Co(II)	525	320
Cu(II)	700	80
Fe(II)	575	19,800
Ni(II)	775	28
Ru(II)	530	1638

found to be tenfold greater than that of any other transitional metal ion examined. Absorption spectra for the iron complex are shown in Fig. 1.

The iron complex is stable over the pH range from 3.55 to 7.00. The pH maintained in other solutions used in this study was 4.6, well within the region covered satisfactorily by the acetate buffer system. Solutions of pH 4.6 retained maximum color

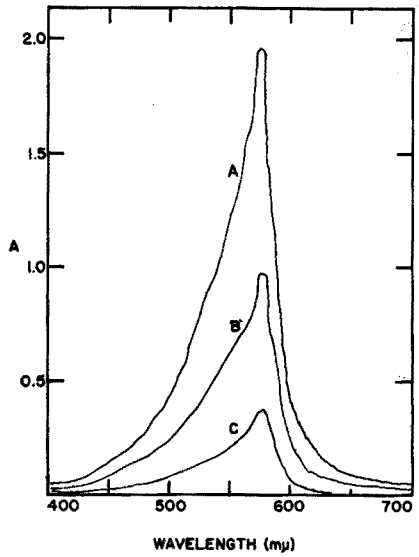


Fig. 1. Absorbance of iron(II)–tetra-2′-pyridylpyrazine complex. A, $100.48 \cdot 10^{-6}$ *M* Fe(II); B, $50.24 \cdot 10^{-6}$ *M* Fe(II); C, $20.10 \cdot 10^{-6}$ *M* Fe(II).

for a period of one week. The addition of a large amount of acid to a solution containing the iron(II) complex caused the wavelength of maximum absorption to shift from 575 mμ to 580 mμ. A new equilibrium is established at a lower pH with a decrease in absorbance.

TABLE II

MOLE RATIO STUDY OF THE IRON(II)–TETRA-(2′-PYRIDYL)PYRAZINE SYSTEM

Ratio of ligand to iron(II)	*A 575 mμ*
0.5	0.200
1.0	0.445
1.5	0.670
1.8	0.835
1.9	0.881
2.0	0.835
2.1	0.970
2.2	1.000
2.5	1.005
3.0	1.005
9.0	0.985

The results of the mole ratio study are presented in Table II. The data indicate that two moles of the ligand react with one mole of iron(II). The formula of the iron(II) complex was also determined by the method of continuous variations. The data obtained are shown graphically in Fig. 2. The results show the existence in solution of one complex with a composition of one mole of iron(II) and two moles of the ligand. This is all in accord with the results of GOODWIN AND LIONS[1], who reported the isolation of solid $Fe(TPP)_2(ClO_4)_2$.

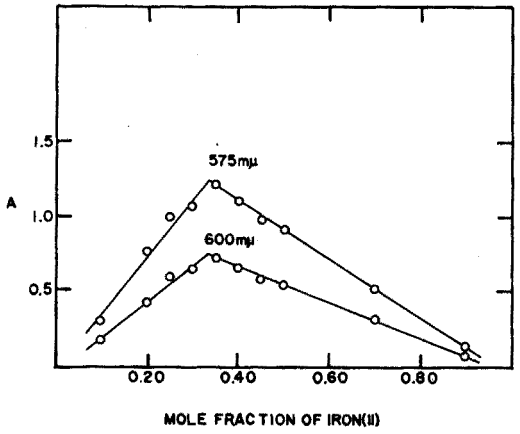


Fig. 2. Continuous variations study.

The iron(II)–TPP complex was found to obey Beer's law over a concentration range of $2.5 \cdot 10^{-6}$ to $100 \cdot 10^{-6}$ M, as shown in Table III. A molar absorptivity of 19,800 for the complex was determined from a least squares treatment of the data.

In the presence of perchlorate ion, the iron(II) complex can be extracted from

TABLE III

DATA FROM BEER'S LAW STUDY

Concn. of iron present ($M \cdot 10^6$)	*A* 575 mμ
2.512	0.060
5.024	0.100
20.096	0.397
50.24	0.995
100.48	1.983

aqueous solution into nitrobenzene or chloroform. The wavelength of maximum absorption was 570 mμ for chloroform and the molar absorptivity determined as 19,200. Nitrobenzene extracts of the complex exhibited an absorption maximum of 573 mμ with a molar absorptivity of 17,400. The chloroform system required at least 3 extractions whereas one extraction with nitrobenzene appeared to be sufficient.

The results from a study of the effect of diverse ions on the iron system are reported

TABLE IV

EFFECT OF DIVERSE IONS ON IRON(II) COMPLEX

Ion	*Amount permissible (p.p.m.)*	*Ion*	*Amount permissible (p.p.m.)*
Ba^{2+}	100	Br^-	500
Bi^{3+}	50[a]		
Cd^{2+}	100	Cl^-	500
Ca^{2+}	100		
Cr^{3+}	25	ClO_4^-	100
Co^{2+}	5	CN^-	5
Cu^{2+}	2	F^-	15
Pb^{2+}	100	I^-	100
Li^+	1000	NO_2^-	500
Mg^{2+}	100	NO_3^-	500
Mn^{2+}	100	PO_4^{3-}	500
Hg^{2+}	100	SO_4^{2-}	500
Ni^{2+}	5	$S_2O_3^{2-}$	500
K^+	500		
Sn^{2+}	50[a]	SCN^-	500
Zn^{2+}	100		

[a] Precipitate formed.

TABLE V

SUMMARY OF IRON DETERMINATIONS

Sample	*Iron present (mg)*	*Iron found (mg)*
S_1	1.85	1.83
		1.83
S_2	2.13	2.08
		2.05
S_3	1.65	1.65
		1.66
NBS-104	48.97	48.20
		49.19
		49.36
	50.01	50.21
		50.32
		49.82
	52.73	52.27
		52.10
		52.04

in Table IV. The transition metals, cobalt, copper, nickel, and ruthenium interfere because of the formation of colored compounds. Ions which complex strongly with the iron(II) ion also cause large interferences. Phosphate and thiocyanate ions, which cause considerable error in some determinations, can be tolerated with this reagent as with 1,3,5-tripyridyl-2,4,6-triazine[4].

The usefulness of tetra-2'-pyridylpyrazine as a colorimetric reagent for iron was demonstrated. The results presented in Table V show that the method is both precise and accurate.

SUMMARY

The reactivity of the tridentate organic ligand, 2,3,5,6-tetrakis-(2'-pyridyl)-pyrazine, toward hydrogen ion and transition metal ions was investigated. The purple color of the bis-2,3,5,6-tetra-(2'-pyridyl)-pyrazine–iron(II) complex can be used in a colorimetric method for iron(II). The complex in aqueous solution exhibits a wavelength of maximum absorption at 575 mμ and a molar absorptivity of 19,800 in the pH range of 3.0–7.0. Beer's law is obeyed over the concentration range 5.0–100 · 10^{-6} *M*. The perchlorate salt of the complex is extractable into chloroform and nitrobenzene with absorption maxima of 570 mμ and 573 mμ and molar absorptivities of 19,200 and 17,400, respectively. The effects of diverse ions and the results of determinations on selected samples are discussed.

RÉSUMÉ

La 2,3,5,6-tétrakis-(2'-pyridyl)-pyrazine réagit avec les métaux de transition pour donner des complexes colorés. Avec le fer(II) en particulier, elle donne un complexe pourpre, permettant un dosage colorimétrique: absorption maximum à 575 mμ et absorption molaire 19,800. Le perchlorate de ce complexe peut être extrait dans le chloroforme et le nitrobenzène (absorption maximum à 570 mμ et 573 mμ, respectivement; absorption molaire 19,200 et 17,400). Les auteurs ont examiné également l'influence de divers ions.

ZUSAMMENFASSUNG

Die Reaktivität des dreizähnigen organischen Liganden 2,3,5,6-Tetrakis-(2'-pyridyl-)-pyrazin auf Wasserstoffionen und Übergangsmetallionen wurde untersucht. Die Purpurfarbe des Bis-2, 3,5,6-Tetrakis-(2'-pyridyl-)-pyrazin–Eisen(II)-Komplexes kann für die kolorimetrische Bestimmung des Eisen(II) benutzt werden. Der Komplex zeigt in wässriger Lösung bei 575 mμ ein Absorptionsmaximum und einen molaren Extinktionskoeffizienten von 19,800 im pH-Bereich von 3.0–7.0. Das Beersche Gesetz wird befolgt für den Konzentrationsbereich von 5.0–100 · 10^{-6} *M*. Das Perchloratsalz des Komplexes ist mit Chloroform und Nitrobenzol extrahierbar mit Absorptionsmaxima bei 570 mμ und 573 mμ und molaren Extinktionskoeffizienten von 19,200 bzw. 17,400. Der Einfluss verschiedener Ionen und Ergebnisse von ausgewählten Proben werden diskutiert.

REFERENCES

1 H. GOODWIN AND F. LIONS, *J. Am. Chem. Soc.*, 81 (1959) 6415.
2 J. YOE AND A. JONES, *Ind. Eng. Chem., Anal. Ed.*, 16 (1944) 111.
3 P. JOB, *Ann. Chim.*, 9 (1928) 113.
4 P. COLLINS, H. DIEHL AND G. SMITH, *Anal. Chem.*, 31 (1959) 1862.

THE COMPOSITION OF THE ZIRCONIUM–ARSENAZO COMPLEX

HIROSHI ONISHI AND HITOSHI NAGAI

Japan Atomic Energy Research Institute, Tokai-mura, Ibaraki-ken (Japan)

(Received February 17th, 1964)

KUZNETSOV *et al.*[1] first reported a spectrophotometric arsenazo method for zirconium. KUTEĬNIKOV[2] reported that zirconium formed 1:1 and 1:2 complexes with arsenazo in the pH ranges 0.5–1.5 and 4.0–8.0, respectively. He gave a molar absorptivity of 9400 at the low pH range and at a wavelength of 600 mμ. Later, SHIBATA *et al.*[3] described another spectrophotometric arsenazo method for zirconium. They suggested that a 2:1 complex of zirconium with arsenazo was formed at pH 1.7. However, in a Japanese paper on the same subject[4], they reported that the mole ratio of zirconium:arsenazo was 1:2. Their molar absorptivity value was only 2400.

The purpose of the present work was to establish the empirical formula of the zirconium–arsenazo complex at low pH. The results of spectrophotometric methods indicate that at pH 1.3, 1.7 and 3.5, zirconium forms a 1:1 complex with arsenazo.

EXPERIMENTAL

Reagents

Zirconium solution (1 mg per ml) was prepared by dissolving $ZrOCl_2 \cdot 8H_2O$ in 1 *N* hydrochloric acid. This solution was standardized by titration with ethylenediaminetetraacetic acid, disodium salt, using xylenol orange as indicator.

Arsenazo was purified by the method of FRITZ AND JOHNSON-RICHARD[5]. The purified reagent was assumed to be pure 3-(2-arsonophenylazo)-4,5-dihydroxy-2,7-naphthalenedisulfonic acid monohydrate (molecular weight 566) and was dissolved in water.

A pH 1.7 buffer solution was prepared by mixing 0.2 *M* potassium chloride solution with 0.2 *M* hydrochloric acid[3].

A pH 3.5 buffer solution was prepared by mixing 1 *M* acetic acid with 1 *M* sodium acetate solution.

Gelatin solution was prepared by dissolving 1.0 g of gelatin in 50 ml of water on a water bath. After cooling, the solution was diluted to 100 ml with water. This solution was prepared fresh daily.

Apparatus

Absorbance measurements were made with a Hitachi EPU-2A spectrophotometer, using 1-cm cells. A Horiba Model M-3 pH meter was used for pH measurements.

Methods

The composition of the zirconium–arsenazo complex was studied under the conditions developed for the determination of zirconium by KUZNETSOV *et al.*[1] and SHIBATA *et al.*[3]. Their methods are described below. For comparison a method at pH 3.5 is given.

Method of KUZNETSOV *et al.* (slightly modified). Take 0–200 μg of zirconium in a 25-ml volumetric flask. This range of zirconium is the same for the other 2 methods. Adjust the amount of hydrochloric acid to 2.5 mequiv, add 2.0 ml of 0.1% (w/v) arsenazo solution and 1.0 ml of 1% (w/v) gelatin solution, and dilute to the mark with water. (The pH of the solution was 1.3.) Measure the absorbance of the solution in a 1-cm cell at 575 mμ, using water as the reference.

Method of SHIBATA *et al.* Add 5.0 ml of pH 1.7 buffer solution and 2.0 ml of 0.1% arsenazo solution to the zirconium solution. Dilute to 25 ml with water. Measure the absorbance at 575 mμ. Contrary to the observation by SHIBATA *et al.*, a full color was developed within a few minutes at room temperature.

Method at pH 3.5. Add 2.0 ml of 0.1% arsenazo solution and 2.5 ml of pH 3.5 buffer solution to the zirconium solution. Dilute to 25 ml with water. Measure the absorbance at 575 mμ.

The composition of the complex in solution was studied by the mole ratio method[6], the continuous variation(s) method[7] and the slope ratio method[8]. The final volume of each solution was 25 ml. Absorbances were measured against water. All experiments were carried out at room temperatures with a range of 16° to 26°.

RESULTS AND DISCUSSION

Absorption curves

Absorption curves for the zirconium–arsenazo complex were recorded by previous workers[1,3]. Our results showed that the absorbance curves of zirconium plus reagent against reagent blank are very similar at pH 1.3, 1.7 and 3.5 (Fig. 1). All the curves show maximum absorbance at 570–590 mμ.

Composition of the complex

The results of the mole ratio method, continuous variation(s) method and slope

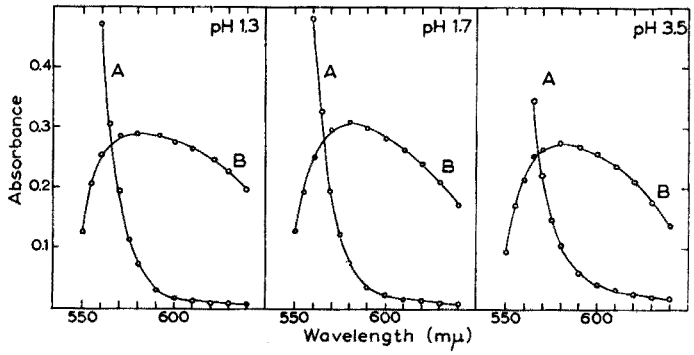


Fig. 1. Absorption curves. (A) Reagent blank against water; (B) 2.53 p.p.m. Zr against reagent blank.

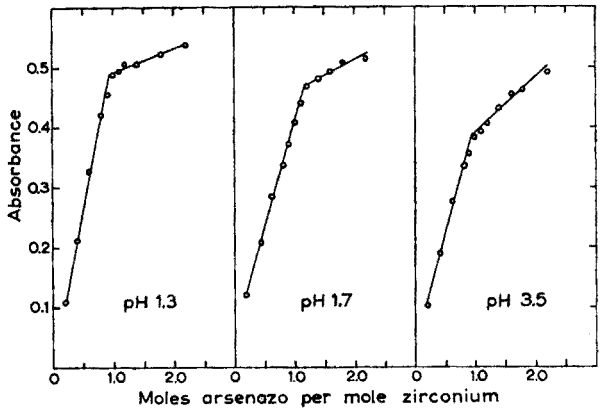


Fig. 2. Mole ratio method. 1.0 μmole Zr in 25 ml.

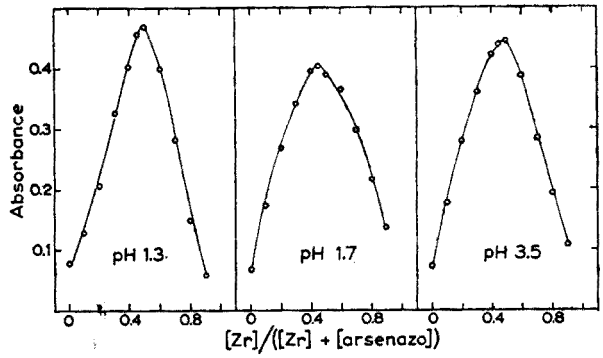


Fig. 3. Continuous variation(s) method. [Zr] + [arsenazo] = 2.0 μmole in 25 ml.

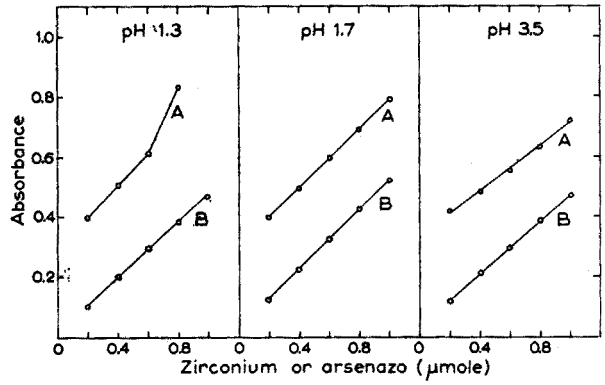


Fig. 4. Slope ratio method. (A) Arsenazo concentration constant, 10 μmole in 25 ml; (B) Zr concentration constant, 10 μmole in 25 ml.

ratio method at pH 1.3 (0.1 *M* in hydrochloric acid), 1.7 (0.04 *M* in chloride) and 3.5 (0.1 *M* in acetate) are shown in Figs. 2, 3 and 4. Absorbances were measured at 575 mμ. At pH 1.3 the slope ratio method did not yield a definite result (Fig. 4). This is probably due to the appreciable turbidity observed when zirconium was greater than 0.8 μmole (Fig. 4, pH 1.3, curve A).

The results shown in Figs. 2, 3 and 4 indicate that at pH 1.3, 1.7 and 3.5 (or probably between pH 1.3 and 3.5), the complex is formed by one mole of zirconium reacting with one mole of arsenazo (R), giving the empirical formula ZrR. The same conclusion was reached by measuring the absorbances at 585 mμ in the 3 methods.

Zirconium and thorium show similar reactions with arsenazo. Thorium also is known[9] to form a 1 : 1 complex with arsenazo at pH 1.5 ± 0.5.

Molar absorptivity

The molar absorptivities of the zirconium complex at 575 mμ were calculated from calibration curves. They are $1.4 \cdot 10^4$, $1.1 \cdot 10^4$ and $1.4 \cdot 10^4$ at pH 1.3, 1.7 and 3.5, respectively. The value of $1.1 \cdot 10^4$ is much greater than the value reported by SHIBATA *et al.* (2400 at 578 mμ) and is in agreement with the value given by KUTEĬNIKOV (9400 at 600 mμ). In this connection, it may be mentioned that a dilute working standard solution of zirconium, *e.g.* 100 p.p.m. Zr and 0.1 *N* hydrochloric acid, should be prepared from the stock solution just before use. The absorbance obtained by using such a working standard solution was about twice as much as that obtained by using the standard solution which had been neutralized with ammonium hydroxide. Therefore, the neutralization of the sample solution in the analysis of magnesium–base alloys[3] may not be suitable.

SUMMARY

There is discordant information in the literature on the composition of the zirconium–arsenazo complex in solution. A detailed spectrophotometric study indicates that at pH 1.3, 1.7 and 3.5 the complex is formed by one mole of zirconium reacting with one mole of arsenazo (R), giving the empirical formula ZrR.

RÉSUMÉ

Les auteurs ont effectué une étude spectrophotométrique sur la composition du complexe zirconium–arsénazo en solution. Aux pH 1.3, 1.7 et 3.5, le complexe est formé d'une mole de zirconium et d'une mole d'arsénazo (R), donnant la formule empirique ZrR.

ZUSAMMENFASSUNG

In der Literatur werden widersprechende Angaben über die Zusammensetzung des Zirkonium–Arsenazo-Komplexes in Lösung gemacht. Eine eingehende spektralphotometrische Untersuchung zeigt, dass beim pH-Wert 1.3, 1.7 und 3.5 der Komplex aus einem Mol Zirkonium und einem Mol Arsenazo gebildet wird.

REFERENCES

1 V. I. KUZNETSOV, L. M. BUDANOVA AND T. V. MATROSOVA, *Zavodsk. Lab.*, 22 (1956) 406.
2 A. F. KUTEĬNIKOV, *Zavodsk. Lab.*, 24 (1958) 1050.
3 S. SHIBATA, Y. ISHIGURO AND T. MATSUMAE, *Anal. Chim. Acta*, 23 (1960) 384.
4 S. SHIBATA, Y. ISHIGURO AND T. MATSUMAE, *Nagoya Kogyo Gijutsu Shikensho Hokoku*, 9 (1960) 561.
5 J. S. FRITZ AND M. JOHNSON-RICHARD, *Anal. Chim. Acta*, 20 (1959) 164.
6 J. H. YOE AND A. L. JONES, *Ind. Eng. Chem., Anal. Ed.*, 16 (1944) 111.
7 P. JOB, *Ann. Chim. (Paris)*, (10) 9 (1928) 113.
8 A. E. HARVEY, JR. AND D. L. MANNING, *J. Am. Chem. Soc.*, 72 (1950) 4488.
9 H. P. HOLCOMB AND J. H. YOE, *Microchem. J.*, 4 (1960) 463.

CHELATE DES NITROSYLRUTHENIUM(III)-IONS MIT NITROSONAPHTHOLEN

CTIRAD KONEČNÝ

Institut für Kernforschung,
Tschechoslowakische Akademie der Wissenschaften,
Řež bei Prag
(Tschechoslowakei)

(Eingegangen den 3. Februar 1964)

Den Komplexbildungsreaktionen der Nitrosonaphthole mit Nitrosylruthenium(III)-Ionen ($Ru(NO)^{3+}$) wurde bisher nur wenig Aufmerksamkeit gewidmet. Es wurde nur festgestellt, dass sich mehrere farbige Komplexe bilden, die in Wasser meistens schlecht löslich, dagegen in einer Reihe organischer Lösungsmittel gut löslich sind[1]. Daher wurden die Gleichgewichte in wässrig–äthanolischen Lösungen von Chelaten der Nitrosonaphthole mit Nitrosylruthenium(III)-Ionen eingehend untersucht.

EXPERIMENTELLER TEIL

Reagenzien und Apparatur

Dikalium-pentachloronitrosylruthenat(III) wurde aus der Pentachloronitrosylruthenium(III)-säure und gesättigter Lösung des Kaliumchlorids hergestellt[2] und aus 0.1 *M* HCl umkristallisiert. Die Rutheniumkonzentration der Stammlösung betrug 0.01 mg-at Ru/ml.

Die Stammlösung der Nitrosylruthenium(III)-nitratokomplexe in 3 *M* HNO_3 wurde nach FLETCHER *et al.*[3,4] aus dem Nitrosylruthenium(III)-hydroxid frisch hergestellt. Die Rutheniumkonzentration war $2.3 \cdot 10^{-2}$ mg-at Ru/ml.

Der Rutheniumgehalt in allen Präparaten wurde gravimetrisch als Metall bestimmt.

0.012 *M* Lösungen der Nitrosonaphthole in 96%igem Äthanol wurden durch genaues Einwägen der Präparate, die aus Äthanol umkristallisiert wurden, zubereitet.

Die Ionenstärke der einzelnen Lösungen wurden durch Natriumchlorid, bzw. durch Natriumnitrat eingestellt.

Die spektralphotometrischen Messungen wurden mit einem Quarzspektralphotometer SF-4 sowjetischer Herkunft in Quarzküvetten durchgeführt. Die pH-Werte der Lösungen wurden mit Glaselektrode und Potentiometer E 187 (Metrohm A.G., Herisau, Schweiz) gemessen. Für die Berechnungen der Komplexgleichgewichte im Medium von 30%igem Äthanol wurden die pH-Werte ähnlich wie in der vorangehenden Arbeit korrigiert[5].

Arbeitsvorgang

Die Proben in 30%igem Äthanol (Gesamtmenge 25 ml) wurden durch Mischen einzelner Reaktionskomponenten in nachstehender Reihenfolge hergestellt: Säure, Reagens, Äthanol, Lösung des Nitrosylrutheniumsalzes. Zur Erzielung maximaler Verfärbung wurden die Proben 4 Stunden lang bei 80° im Wasserbad erwärmt.

ERGEBNISSE UND DISKUSSION

Die spektralphotometrische Untersuchung der Nitrosonaphtholatkomplexe

Die Komplexe sind meistens im Wasser schlecht löslich, aber gut löslich in einer Reihe organischer Lösungsmittel. In schwach salpeter- und salzsaurem Milieu (0.05–0.1 *M* Säure) haben die Absorptionskurven ein deutliches Maximum bei 570 mμ für 1-Nitroso-2-naphthol, bei 580–585 mμ für 2-Nitroso-1-naphthol (Fign. 1 und 2), das sich mit zunehmender Konzentration der organischen Reagenzien zu längeren Wellenlängen verschiebt. Bei Molverhältnissen Ru: R kleiner als 1:2 (R = Nitrosonaphthol) ist in den Spektren ein deutliches Maximum bei 525 mμ für 1-Nitroso-2-naphthol und bei λ_{max} 545 mμ in verdünnter Salpetersäure, bei 540 mμ in Salzsäure für 2-Nitroso-1-naphthol zu beobachten. Mit zunehmender Salpetersäurekonzentration wird die Bildung der Komplexe einerseits durch den Einfluss

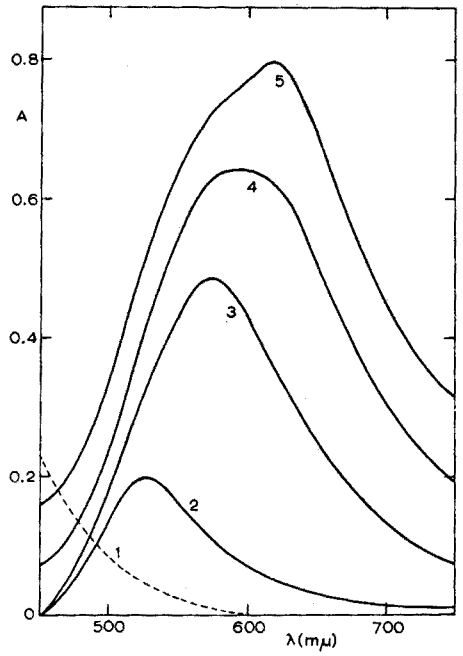


Fig. 1. Die Absorptionskurven der Komplexe mit 1-Nitroso-2-naphthol (Lösung in 30%igem Äthanol). 0.05 *M* HNO_3. Rutheniumkonzentration: Kurven *1* und *2*, $4.95 \cdot 10^{-3} M$; *3–5*, $3.2 \cdot 10^{-4} M$. $c_{Ru}:c_R$: Kurve *2*, 1:0.1; *3*, 1:2; *4*, 1:7.5; *5*, 1:20. Kurve *1*: Absorptionskurve der Nitrosylruthenium(III)-nitratokomplexe in 0.05 *M* HNO_3.

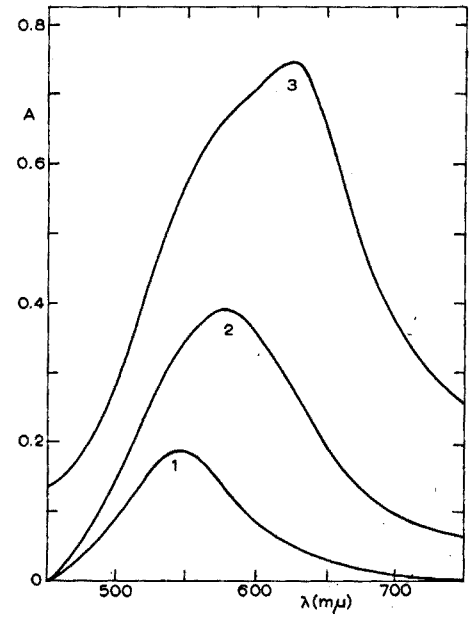


Fig. 2. Die Absorptionskurven der Komplexe mit 2-Nitroso-1-naphthol (Lösung in 30%igem Äthanol). 0.05 *M* HNO_3. Rutheniumkonzentration: Kurve *1*, $4.95 \cdot 10^{-3} M$; *2* und *3*, $3.2 \cdot 10^{-4} M$. $c_{Ru}:c_R$: Kurve *1*, 1:0.1; *2*, 1:1.5; *3*, 1:10.

der Wasserstoffionen, anderseits durch störende Reaktionen des organischen Reagens mit der Salpetersäure wesentlich unterdrückt (Fig. 3). Durch einen Überschuss an Nitrosonaphthol und Verminderung der Wasserstoffionenkonzentration wird das Auftreten einer Komplexverbindung mit $\lambda_{max} = 620$ mμ für 1-Nitroso-2-naphthol bzw. $\lambda_{max} = 625$–628 mμ für 2-Nitroso-1-naphthol bedingt.

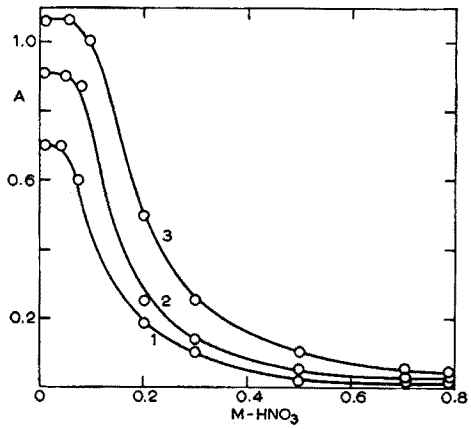


Fig. 3. Abhängigkeit der Extinktion von der Salpetersäurekonzentration (1-Nitroso-2-naphthol). $c_{Ru} = 1.23 \cdot 10^{-4} M$; $c_R = 1.23 \cdot 10^{-3}$M; 30%igem Äthanol. Kurve *1*, 535 mμ; *2*, 580 mμ; *3*, 620 mμ.

Studium der komplexen Gleichgewichte mittels spektralphotometrischen Methoden

Die Anzahl der Komplexe in gegebenen System, ihre Zusammensetzung und ihre Assoziationskonstanten wurden mittels vorliegender graphisch-numerischer Methoden bestimmt:

(*a*) aus den Gleichungen für die Extinktion $\bar{A}$ der Gemische *zweier* absorbierender Komplexe MR_n und MR_{n+p}

$$\bar{A} \equiv A - (\varepsilon_M[M] + \varepsilon_R c_R) = [M] \cdot c_R{}^n(\beta_n \cdot \varepsilon_n + \beta_{n+p} \cdot \varepsilon_{n+p} \cdot c_R{}^p) \quad (1)$$

und für die Gesamtkonzentration des Metalls

$$c_M = [M]c_R{}^n(\beta_n + \beta_{n+p} \cdot c_R{}^p) + [M] \quad (2)$$

geht nach der Eliminierung von [M] folgender Ausdruck hervor:

$$\beta_n + \beta_{n+p} \cdot B_{n+p} \cdot B_n{}^{-1} = -\bar{A} \cdot B_n{}^{-1} \quad (3)$$

wo

$$B_i = c_R{}^i(\bar{A} - \varepsilon_i \cdot c_M), \quad i = n, n+p \quad (4)$$

Es wurden dieselben Symbole wie in der vorangehenden Arbeit[5] benutzt. Für geeignete Werte von n und p entspricht die Beziehung (3) geradliniger Form $-\bar{A}/B_n = f(\beta_{n+p} \cdot B_n{}^{-1})$; aus dem Richtungskoeffizienten und dem Abszissenabschnitt kann man die Werte der Assoziationskonstanten errechnen, falls molare Extinktionskoeffizienten jeder Komponente bekannt sind. Bei verhältnismässig grossem Überschuss an organischem Reagens kann die Gleichgewichtskonzentration des Kations vernachlässigt werden ([M] $\doteq$ 0) und folglich

$$-\beta_n \cdot B_n \cdot B_{n+p}{}^{-1} = \beta_{n+p}, \text{oder } \beta_{n,n+p} = B_n \cdot B_{n+p}{}^{-1} = \text{konst.} \quad (5)$$

($\beta_{n,\,n+p} = \beta_{n+p} \cdot \beta_n^{-1}$ ist die teilweise Stabilitätskonstante). Wenn bei gegebener Wellenlänge nur ein Komplex absorbiert, z. B. MR_{n+p} (d.h. $\varepsilon_n = 0$), geht die Gleichung (3) über in

$$\beta_n + \beta_{n+p} \cdot c_R^n(1 - \varepsilon_{n+p} \cdot c_M \cdot A^{-1}) = -c_R^{-n} \tag{6}$$

und die Gleichung (5) in

$$\beta_{n,\,n+p} = (\varepsilon_{n+p} \cdot c_M \cdot A^{-1} - 1)^{-1} \cdot c_R^{-p} = \text{konst.} \tag{7}$$

(*b*) Für den Fall, dass im System *drei absorbierende Komplexe* vorkommen und gleichzeitig [M] ≠ 0, ergibt sich:

$$\beta_{n,\,n+p} + \beta_{n,\,n+p+r} \cdot B_{n+p+r} = -B_n \cdot B_{n+p}^{-1} \tag{8}$$

wo

$$B_{n+p+r} = c_R^{n+p+r}(A - \varepsilon_{n+p+r} \cdot c_M) \tag{9}$$

Wenn nur ein Komplex absorbiert, z.B. MR_{n+p+r}, wird die rechte Seite der Gleichung (8) zu $-c_R^{-p}$ vereinfacht.

(*c*) Wenn im System nur *ein Komplex* vorkommt, folgt aus den vorangegangenen Gleichungen:

$$\beta_n = -A \cdot B_n^{-1} = \text{konst.} \tag{10}$$

Die Anzahl der Nitrosonaphtholatkomplexe, ihre Zusammensetzung und Assoziationskonstanten wurden in Serien von Lösungen mit konstanter Metallkonzentration ($c_{Ru} = 1.23 \cdot 10^{-4}$–$3.2 \cdot 10^{-4}$ mg-at Ru/ml) und veränderlichen Konzentration des organischen Reagens in 0.05 *M* HNO_3 oder HCl nach oben angegebenen Bestimmungsverfahren festgestellt (die Abhängigkeit $A = f(c_R/c_{Ru})$ ist in Fig. 4 dargestellt).

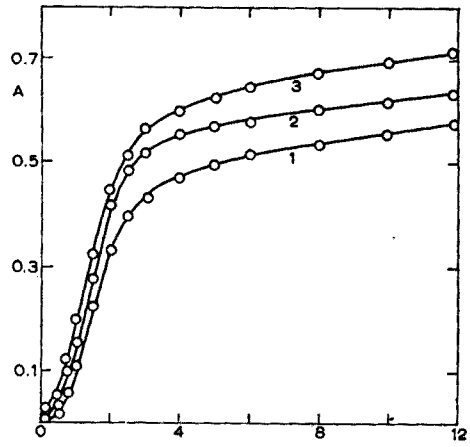


Fig. 4. Photometrische Titration von Nitrosylruthenium(III)-Ionen mit 1-Nitroso-2-naphthol (30%igem Äthanol). $c_{Ru} = 3.2 \cdot 10^{-4} M$; $c_{HNO_3} = 0.05\ M$. Wellenlängen: Kurve *1*, 525 mμ; *2*, 575 mμ; *3*, 620 mμ.

Die Werte der molaren Extinktionskoeffizienten einzelner Komplexe wurden nach den Methoden von JACIMIRSKIJ[6] und von YAFFE UND VOIGT[7] bestimmt. Es wurde gefunden, dass in gegebenen Systemen der Komplex mit einem Verhältnis der Komponenten Ru : R = 1 : 2 überwiegt, mit zunehmender Nitrosonaphtholkonzen-

tration bildet sich auch der Komplex 1 : 3. Bei Molverhältnissen $c_{Ru} : c_R$ grösser als 1:2 entstehen die Gemische der Verbindungen mit Ru: R = 1:1 und 1:2. Zur genaueren Berechnung der Konstanten wurde die Beziehung

$$\bar{\varepsilon} + (\bar{\varepsilon} - \varepsilon_M) \cdot \beta_i^{-1} \cdot c_R^{-i} = \varepsilon_i + (\varepsilon_j - \bar{\varepsilon}) c_R^{j-i} \cdot \beta_{i,j} \qquad (11)$$

verwendet, in der $\bar{\varepsilon} = A \cdot c_M^{-1}, \varepsilon_R \doteq 0$; dem Index i ist immer der Index j in den Paaren $i, j = n, n+p; n+p, n$ zugeordnet. Wenn wir jetzt die linke Seite der letzten Gleichung als Funktion des Ausdrucks $c_R^{j-i} \cdot (\varepsilon_j - \bar{\varepsilon})$ ausdrücken, erhalten wir bei geeigneter Wahl von n und p eine geradlinige Abhängigkeit, bei der der Richtungskoeffizient den Wert von $\beta_{i,j}$, der Abszissenabschnitt den Wert von ε_i angibt. Die Ergebnisse über die Zusammensetzung und Assoziationskonstanten sind mit den mittels graphisch-numerischen Methoden nach YAFFE UND VOIGT bzw. nach NEWMAN UND HUME[8] errechneten Ergebnissen in guter Übereinstimmung.

DIE ASSOZIATIONS- UND BILDUNGSKONSTANTEN DER NITROSONAPHTHOLATKOMPLEXE

Die Durchschnittwerte der Assoziationskonstanten β_i der Chelate mit Verhältnissen Ru : R = 1:1 bis 1:3 im Milieu von 0.05 M HNO_3 und 30%igem Äthanol bei der Ionenstärke $\mu = 0.2$ M sind in der Tabelle I zusammengefasst. Dabei ist

$$\beta_i = [Ru(NO)R^{(3-i)+}]/[Ru(NO)]_0 \cdot [R]_0^i \quad (i = 1, 2)$$

$$\beta_3 = [RuR_3]/[Ru(NO)]_0 \cdot [R]_0^3$$

$$[R]_0 = c_R - (3[RuR_3] + \sum_{i=1}^{2}{}' [Ru(NO)R_i])$$

$$[Ru(NO)]_0 = c_{Ru} - ([RuR_3] + \sum_{i=1}^{2}{}' [Ru(NO)R_i]).$$

TABELLE I

ASSOZIATIONSKONSTANTEN DER NITROSONAPHTHOLATKOMPLEXE

Komplex	*log* β_i	
	1-Nitroso-2-naphthol	*2-Nitroso-1-naphthol*
$Ru(NO)R^{2+}$	3.80 ± 0.05	4.23 ± 0.05
$Ru(NO)R_2^+$	6.22 ± 0.05	6.40 ± 0.07
RuR_3	10.12 ± 0.10	10.34 ± 0.12

TABELLE II

GLEICHGEWICHTS- UND BILDUNGSKONSTANTEN DER NITROSONAPHTHOLATKOMPLEXE

β_i ($\varkappa_i$)	*log* β_i (*log* $\varkappa_i$)	
	1-Nitroso-2-naphthol	*2-Nitroso-1-naphthol*
$[Ru(NO)R^{2+}]/[Ru(NO)^{3+}][R^-]$	11.8	11.8
$[Ru(NO)R_2^+]/Ru(NO)^{3+}][R^-]^2$	21.2	20.5
$[Ru(NO)R_2^+]/Ru(NO)R^{2+}][R^-]$	9.4	8.7
$[Ru(NO)R^{2+}][H^+]/[Ru(NO)^{3+}][HR]$	3.7	4.1
$[Ru(NO)R_2^+][H^+]/[Ru(NO)R^{2+}][HR]$	1.3	1.0

Zur Berechnung der Bildungskonstanten der Komplexe wurden folgende Basizitätskonstanten K_b der Nitrosonaphthole verwendet: $\log K_{b,1} = 8.1$ für 1-Nitroso-2-naphthol, $\log K_{b,2} = 7.7$ für 2-Nitroso-1-naphthol, die aus den Angaben von DYRSSEN *et al.*[9] abgeleitet wurden (siehe auch[5]). Die teilweisen und Gesamtgleichgewichtskonstanten $\varkappa_{i,j}$, bzw. $\varkappa_i$ wurden nach den Beziehungen (17) in Ref. 5 berechnet. Die Werte der Komplexitäts- und Gleichgewichtskonstanten der Nitrosonaphtholatchelate sind in der Tabelle II, die Werte der molaren Extinktionskoeffizienten der einzelnen Chelate in der Tabelle III zusammengefasst.

TABELLE III

DURCHSCHNITTWERTE DER MOLAREN EXTINKTIONSKOEFFIZIENTEN ($\varepsilon \cdot 10^3$)DER NITROSONAPHTHOLATKOMPLEXE

	Wellenlänge (mμ)		
1-Nitroso-2-naphthol	*525*	*570*	*620*
$Ru(NO)R^{2+}$	5.65[a,b]	4.28[a,b]	1.60[a,b]
$Ru(NO)R_2^+$	5.11[b,c]	7.75[b,c]	4.30[b,c]
RuR_3	5.25[c]	7.40[c]	8.65[c,d]
2-Nitroso-1-naphthol	*545*	*585*	*625*
$Ru(NO)R^{2+}$	5.02[a,b]	3.47[a,b]	2.00[a,b]
$Ru(NO)R_2^+$	4.72[b,c]	8.10[b,c]	4.23[b,c]
RuR_3	5.39[c]	8.82[c]	9.38[c,d]

Bestimmungsmethoden: [a] Numerisch.
[b] Nach YAFFE UND VOIGT.
[c] Nach JACIMIRSKIJ, graphisch.
[d] Nach NEWMAN UND HUME.

Die Reaktionen des Nitrosylruthenium(III)-Ions mit Nitrosonaphtholen sind denen des Ruthenium(III)-Ions im Zitratpuffermilieu[5] analog und es bilden sich stufenweise drei gefärbte Chelate mit den Komponentenverhältnissen Ru : R = 1 : 1, 1 : 2, 1 : 3, deren Maxima sich mit wachsender Koordination der Liganden nach den längeren Wellenlängen verschieben. Die für die Ausarbeitung einer exakten spektralphotometrischen Methode wichtigen genauen Abgrenzungen von Einzelexistenzen der Chelate wurden aber nicht gefunden. In beiden Fällen is die Existenz der Komplexe 1 : 1 vor allem durch zunehmende Wasserstoffionenkonzentration und kleine Molverhältnisse Ruthenium: Nitrosonaphthol bedingt. In schwach salpeter- und salzsaurem Milieu bildet das $[Ru(NO)]^{3+}$-Ion mit Nitrosonaphtholen überwiegend die 1 : 2 Verbindungen, denen wahrscheinlich die Zusammensetzung $Ru(NO)R_2$ X ($X^- = Cl^-, NO_3^-$) zugeschrieben werden kann. Im Gegensatz zu dem in der vorangegangenen Arbeit[5] verfolgten System ist in Anwesenheit vom Nitrosylruthenium (III)-Ion die Bildung des Chelates 1 : 3 wesentlich unterdrückt. Die Ursache ist wahrscheinlich darin zu suchen, dass die an das Rutheniumatom äusserst fest gebundene NO-Gruppe des Nitrosylruthenium(III)-Ions verdrängt werden muss, damit der dritte Nitrosonaphtholat-Chelatring entstehen kann.

ZUSAMMENFASSUNG

Bei der spektralphotometrischen Untersuchung der Komplexe des Nitrosylruthenium(III)-Ions mit Nitrosonaphtholen wurde gezeigt, dass in schwach salpeter- und salzsaurem Milieu Gemische von Komplexen mit Verhältnissen Ru : R = 1 : 1 bis 1 : 3 entstehen, deren relatives Auftreten

einerseits von der Wasserstoffionenkonzentration, anderseits von den Molverhältnissen der komplexbildenden Komponenten abhängen.

SUMMARY

A spectrophotometric study of the reactions of nitrosylruthenium(III)-ion with nitrosonaphthols in dilute nitric and hydrochloric acid media is described. The complexes formed contain Ru:R ratios of 1:1 to 1:3, depending on the hydrogen ion concentration and on the molar ratios of the complex-forming species. Mixtures are usually obtained.

RÉSUMÉ

L'auteur a effectué une étude spectrophotométrique des réactions de l'ion nitrosylruthénium ($Ru(NO)^{3+}$) avec les nitrosonaphtols (R). Les rapports Ru:R dans les complexes obtenus sont de 1:1 à 1:3, suivant le pH et les proportions des constituants.

LITERATUR

1 C. KONEČNÝ, *Collection Czech. Chem. Commun.*, 28 (1962) 2596.
2 O. E. ZVJAGINCEV UND A. KURBANOV, *Zh. Neorgan. Khim.*, 3 (1958)2305.
3 J. M. FLETCHER, I. L. JENKINS, F. M. LEVER, F. S. MARTIN, A. R. POWELL UND R. TODD, *J. Inorg. & Nucl. Chem.*, 1 (1955) 378.
4 J. M. FLETCHER, P. G. M. BROWN, E. R. GARDNER, C. J. HARDY, A. G. WAIN UND J. L. WOODHEAD, *J. Inorg. & Nucl. Chem.*, 12 (1959) 154.
5 C. KONEČNÝ, *Anal. Chim. Acta*, 29 (1963) 423.
6 K. B. JACIMIRSKIJ, *Zh. Neorgan. Khim.*, 1 (1956) 2306.
7 R. P. YAFFE UND A. F. VOIGT, *J. Am. Chem. Soc.*, 74 (1952) 2503, 3163.
8 L. NEWMAN UND D. N. HUME, *J. Am. Chem. Soc.*, 79 (1957) 4576.
9 D. DYRSSEN, M. DYRSSEN UND E. JOHANSSON, *Acta Chem. Scand.*, 10 (1956) 106.

THIN-LAYER CHROMATOGRAPHIC SEPARATION AND ANALYSIS OF POLYNUCLEAR AZA HETEROCYCLIC COMPOUNDS

E. SAWICKI, T. W. STANLEY, J. D. PFAFF AND W. C. ELBERT

Laboratory of Engineering and Physical Sciences, Division of Air Pollution, Robert A. Taft Sanitary Engineering Center, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati 26, Ohio (U.S.A.)

(Received February 1st, 1964)

The carcinogenicity to animals of a moderate number of polynuclear aza heterocyclic hydrocarbons has been well established[1]. In addition many methylated derivatives of benz(a)acridine, benz(c)acridine, dibenz(a,h)acridine, and dibenz(a,j)acridine are carcinogenic. Dibenz(a,j)acridine and dibenz(a,h)acridine, both carcinogens, have been found in cigarette-smoke condensate in amounts up to 0.12 and 0.004 μg per gram of cigarette-smoke condensate, respectively[2]. Because of their toxic properties the possible presence of aza compounds in polluted air or in effluents from pollution sources merits investigation.

Several systems of separation of the aza heterocyclic compounds from each other and from polynuclear hydrocarbons and other types of aromatic compounds are presented in this paper. In addition, spectroscopic procedures have been organized for eventual application to the characterization and determination of these basic compounds in polluted air and in effluents from pollution sources.

EXPERIMENTAL

Reagents

All solvents were distilled and the middle cuts were used in the analytical work. The aza heterocyclic compounds and the polycyclic aromatic hydrocarbons were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, and K and K Laboratories, Inc., Jamaica 33, N.Y.

The alumina, cellulose, carboxymethylcellulose and silica gel G adsorbents were obtained from Brinkmann Instruments, Inc.; adsorbil from Applied Science Laboratories, Inc., State College, Pa.; cellulose, 21% acetylated from Carl Schleicher and Schuell Co., Keene, N. H.; and florisil from Floridin Co., Tallahassee, Fla.

Apparatus

A Cary Model 11 Quartz Recording Spectrophotometer with 1-cm cells was used for all absorption spectral work. An Aminco-Bowman spectrophotofluorimeter was used in the fluorescence studies; an Aminco-Keirs spectrophosphorimeter was used in

the phosphorescence studies. All developed plates were examined in a Chromato-Vue cabinet, which is equipped with 3660 Å, 2537 Å, and white-light lamps (Kensington Scientific Corp., Berkeley 10, Calif.).

Solvents and standards

Pentane and pentane containing 2% trifluoroacetic acid (v/v) were used as solvents in the absorption spectral and spectrophotofluorimetric work. Quinine was used as a fluorimetric standard in the comparison of the fluorescence intensities of the different compounds. A mixture of ethyl ether, isopentane, and ethyl alcohol (EPA) in the volume ratio of 5 : 5 : 2 was used as the solvent in the phosphorimetric analysis; it was obtained from American Instrument Co., Inc., Silver Spring, Maryland. Amounts of chloroform up to 5% were used with the EPA. Indole was used as a phosphorimetric standard. The following wavelength maxima and *MM·T* values were obtained for indole (10^{-4} *M*): with instrument set at an emission wavelength maximum of 426 mμ, the excitation spectral values were 220 (0.14) and 281 (0.80); with instrument set at an excitation wavelength of 281 mμ, the emission spectral values were 400 (0.52), 425 (0.94), 445 (0.75), and 458 (0.75). The *MM·T* value is the product of the meter multiplier and the percent transmission readings obtained on the photomultiplier microphotometer. This value is a constant independent of concentration for low concentrations of compounds, when readings are taken at the same excitation and emission wavelengths. The *MM·T* values are useful mainly for characterization purposes; they indicate the relative intensity of the various bands in the fluorescence excitation and emission spectra of a compound. The relative fluorescence intensity of the various compounds compared to quinine can be ascertained with the K_Q value[3]. The K_Q value is calculated at the most intense excitation and emission wavelengths of a compound. For quantitative analysis a standard should be run with the unknown.

Preparation of plates

All plates except florisil were made by the supplier's recommended procedure. Florisil was plated by the silica-gel procedure. The adsorbosil, alumina, and silica-gel plates were coated to a thickness of 250 μ; the remainder of the plates were coated to a thickness of 500 μ.

Separation procedure

A solution (1–5 μl) containing about 5–15 μg of aromatic compound was spotted with a pipet 1.5 cm from the base of a coated glass plate (20 × 20 cm). The spotted plates were placed in a glass jar containing 100 ml of the appropriate solvent system and were developed in an ascending manner. The fastest development time for a 15-cm run was obtained with alumina — about 1 h; the slowest development time was obtained with cellulose — 2.5–5 h. After the plates were developed, they were placed under the ultraviolet light in the cabinet; all fluorescent spots were marked, and any necessary tests were made quickly.

Trifluoroacetic acid test

The spots on the thin-layer plate were treated with a small burst of trifluoroacetic acid fumes from a throwaway pipet fitted with a squeezebulb. Any changes in fluorescence color were noted.

DISCUSSION

Separation

The basic fraction of a complicated mixture can be separated usually from the water-insoluble acidic and neutral fractions by extraction with a dilute aqueous acid solution. In this fashion the aza heterocyclic hydrocarbons can be concentrated into a fraction that could contain aromatic amines and other basic compounds as well as a miscellaneous assortment of water-soluble compounds.

Where only small amounts of a mixture are at hand, the aza heterocyclic hydrocarbons can be readily separated from the aromatic hydrocarbons by any of the systems presented in Table I. With an adsorbent such as cellulose, and aqueous 50% (v/v)

TABLE I

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF AZA HETEROCYCLIC HYDROCARBONS FROM POLYNUCLEAR AROMATIC HYDROCARBONS

Adsorbent	*Solvent*	R_F				
		P	*BaP*	*Cor*	*ACR*	*Pyrenoline*
Cellulose	Formic acid–water (3 : 7)	0.02	0.00	0.00	0.8	0.3
Cellulose	Formic acid–water (1 : 1)	0.1	0.00	0.00	0.9	0.6
Cellulose	Acetic acid–water (2 : 3)	0.2	0.04	0.00	0.9	0.6
Carboxymethyl-cellulose	Acetic acid–water (3 : 7)	0.2	0.04	0.00	0.9	0.4
Cellulose acetate	Ethanol–toluene–water (17 : 4 : 4)	0.5	0.2		0.8	0.6
Alumina	Hexane–benzene (1 : 1)	0.9	0.9		0.4	0.7
Adsorbil	Hexane–benzene (1 : 1)	0.97	0.97		0.04	0.3
Silica gel	Hexane–benzene (1 : 1)	0.99	0.96		0.09	0.3
Florisil	Hexane–benzene (1 : 1)	0.9	0.9		0.00	0.02
Florisil	Pentane–benzene (19 : 1)	1.0	1.0	0.96	0.00	0.00
Florisil	Pentane–chloroform (19 : 1)	0.8	0.5	0.00	0.00	0.00
Florisil	Pentane–furan (19 : 1)	0.7	0.4	0.3	0.00	0.00
Florisil	Hexane	0.7	0.4		0.00	0.03
Florisil	Carbon tetrachloride	0.9	0.8	0.3	0.00	0.05
Florisil	Benzene–methanol (99 : 1)	1.0	1.0	1.0	0.2	0.7

formic acid as the developing solvent, the polynuclear aromatic hydrocarbons remain at the origin and the aza heterocyclic hydrocarbons are found in the R_F range of 0.6 to 0.9 With an adsorbent such as florisil, and the developing solvent pentane–benzene (19:1), the aromatic hydrocarbons are found at the solvent front, the aza compounds at the origin, and the aromatic amines and carbazoles in between. Figure 1 shows a separation of some of the large polycyclic aromatic hydrocarbons from the aza hydrocarbons.

Three thin-layer chromatographic systems for the separation of 23 aza heterocyclic compounds are compared in Table II. In the cellulose–aqueous dimethylformamide system the adsorption of a class of compounds increases with increasing conjugation and ring number. The R_F value of anthracene was 0.35; the R_F values of the larger hydrocarbons were lower. The R_F values of carbazole and the 4 benzocarbazoles ranged from 0.7 to 0.2; it is obvious, therefore, that the carbazoles would be found with the

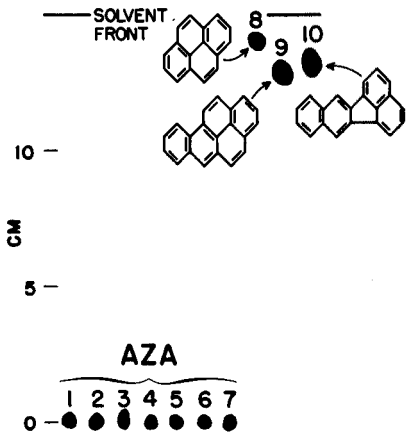


Fig. 1. TLC separation of the aza heterocyclic compounds from some polycyclic aromatic hydrocarbons (florisil; hexane–benzene, 1:1). The numbers 1–7 represent acridine, benz(a)acridine, pyrenoline, benzo(lmn)phenanthridine, acenaphtho(1,2-b)pyridine, phenanthridine and 12-methylbenz(a)acridine, respectively.

TABLE II

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF AZA HETEROCYCLIC COMPOUNDS

Compound	*R_F values*			*Color change on Al_2O_3 (on addition of TFA)*
	Cellulose		*Alumina P–E(19:1)*	
	DMF–H_2O (35:65)	*AcOH–H_2O (3:7)*		
7-Phenyldibenz(c, h)acridine	0.00	0.00	0.70	B → G
8,12-Dimethylbenz(a)acridine	0.20	0.62	0.60	B → B
8,10-Dimethylbenz(c)acridine	0.13	0.40	0.54	B → B
7-Methylbenz(c)acridine	0.20	0.61	0.53	B → BG
Benz(c)acridine	0.26	0.55	0.53	B → BG
Benzo(h)quinoline	0.71	0.77	0.51	lB → B
7,10-Dimethylbenz(c)acridine	0.10	0.53	0.46	B → BG
7,9-Dimethylbenz(c)acridine	0.10	0.46	0.45	B → BG
Dibenz(a, h)acridine	0.06		0.26	Y → B
Pyrenoline	0.20	0.26	0.22	B → O
Indeno(1,2,3-ij)isoquinoline	0.64	0.71	0.19	Y → O
Acridine	0.74	0.83	0.18	B → G
3-Methylbenzo(f)quinoline	0.75	0.79	0.18	B → B
Phenanthridine	0.72	0.78	0.16	B → B
Benzo(f)quinoline	0.76	0.79	0.14	B → B
Acenaphtho(1,2-b)pyridine	0.60	0.75	0.13	B → B
Benzo(lmn)phenanthridine	0.57	0.71	0.12	BG → B
Benz(a)acridine	0.46	0.53	0.12	B → B
12-Methylbenz(a)acridine	0.41	0.61	0.11	B → B
9,12-Dimethylbenz(a)acridine	0.28	0.49	0.11	B → B
14-Phenyldibenz(a, j)acridine	0.00	0.12	0.08	BG → BG
Dibenz(a, j)acridine	0.22		0.04	Y → B

aza derivatives. With this system benz(c)acridine is readily separated from benz(a)-acridine. Figure 2 shows separation of a mixture of aza heterocyclic hydrocarbons.

In the cellulose–aqueous 30% acetic acid method the large aza heterocyclic compounds, such as the dibenzacridines and pyrenoline are readily separated from the

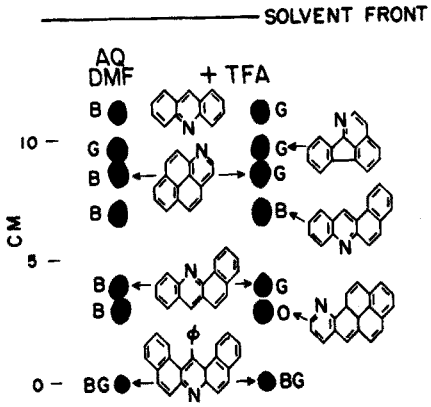


Fig. 2. TLC separation of some aza heterocyclic compounds (cellulose; DMF–H_2O, 35 : 65).

other aza compounds. Carbazole and the 4 benzocarbazoles (R_F 0.24 to 0.05) would present an interference only for the larger aza compounds. Many types of carbonyl compounds would be separated with the aza compounds, but these latter derivatives could be characterized through absorption, fluorescence, and phosphorescence spectra in neutral and weakly acidic solutions. The aza compounds are readily separated from the polynuclear aromatic hydrocarbons since the latter compounds remain at, or very close to, the origin (the R_F values of anthracene and benzo(a)pyrene are 0.10 and 0.00, respectively).

The system in which alumina is used as the adsorbent and pentane–ether (19:1) as the developing solvent gave the most interesting results in the separation of the aza compounds, in that compounds with a non-sterically hindered aza nitrogen are strongly attracted to the adsorbent. If the aza nitrogen is sterically hindered, then the attraction is weakened and the compound is less strongly adsorbed. Of all the aza compounds the 6-ring 7-phenyldibenz(c,h)acridine has the highest R_F value, 0.70. This weaker adsorption is due to the much greater steric hindrance around the aza nitrogen in this molecule, which causes the compound to be adsorbed as weakly as a polynuclear hydrocarbon. For example, the R_F value of the 6-ring hydrocarbon, anthanthrene, is 0.70 also. The substitution of a methyl group or one end of a benzo group in the 4-position of acridine has enough of a steric effect to decrease the strong attraction of the aza nitrogen to the alumina. This effect is readily used to separate isomers such as benzo(f)quinoline and benz(h)quinoline, benz(a)acridine and benz(c)-acridine, dibenz(a,h)acridine and dibenz(a,j)acridine, and 7-phenyldibenz(c,h)-acridine and 14-phenyldibenz(a,j)acridine (Table II). Interestingly, the R_F values of the last two isomers are 0.70 and 0.08, a very wide separation. Many other interesting separations of isomers are given in Table II. Among the sterically hindered compounds the lowest R_F values are for dibenz(a,h)acridine, pyrenoline, and indeno(1,2,3-ij)-

isoquinoline. In the last 2 compounds there is probably less steric hindrance around the nitrogen atom than is found in benzo(h)quinoline and more hindrance than is found in acridine, but because of their larger size their R_F values are roughly equivalent to that of acridine. The R_F value of dibenz(a,h)acridine is approximately halfway between the values of benz(c)acridine and benz(a)acridine.

Polycyclic aromatic hydrocarbons containing 2 to 5 rings will not interfere, for their R_F values are greater than 0.7. The R_F values of many hexacyclic hydrocarbons range between 0.6 and 0.8. The greatest amount of interference would come from heptacyclic or larger-ring hydrocarbons and from compounds more polar than the hydrocarbons.

Preliminary indications show that these various systems are of value in separating acridine and the benzacridines from airborne particulates.

Absorption spectra

Ultraviolet-visible absorption spectral methods are best for characterizing or determining polycyclic compounds in mixtures especially after some separation. The advantages are the large amount of fine structure and the reproducibility of band position and intensity. The main disadvantage is that the sensitivity is in the microgram to milligram range, whereas sensitivities in the nanogram to microgram range are needed.

The detection limits are usually much higher than those obtained in fluorimetric or phosphorimetric work. This limit is defined as the nanograms of compound that will give an absorbance of 0.1 for the least intense band in a 1-ml cell of 1 cm path length. Thus at the detection limit, every band would be present in the absorption spectrum of the compound. These would be the ideal conditions for characterization. In preliminary investigative work with minute amounts of material, however, the search would be for the most intense long-wavelength band of a compound. Once this is found and if the detection limit is known, the proper amount of unknown can be collected to obtain a complete absorption spectrum for characterization.

The aza heterocyclic hydrocarbons can be distinguished by their spectra in neutral and acid solution (Table III). On the addition of acid, the spectrum shows a loss in fine structure and the long-wavelength band shifts toward longer wavelength, usually with an increase in intensity.

By means of the spectra in neutral and acid solution the following isomers can be readily differentiated: acridine and phenanthridine, indeno(1,2,3-ij)isoquinoline and acenaphtho(1,2-b)pyridine, benz(a)acridine and benz(c)acridine, dibenz(a,h)-acridine and dibenz(a,j)acridine, and 14-phenyldibenz(a,j)acridine and 7-phenyldibenz(c,h)acridine. In addition, methylated derivatives of benz(a)acridine and benz(c)acridine can be differentiated.

The lowest detection limit was found for 9,12-dimethylbenz(c)acridine in pentane–trifluoroacetic acid (50:1). The value was 1500. If the strong band of a compound, like dibenz(a,h)acridine, in pentane were used in calculating the detection limit, a value of 200 would be obtained. The highest value, 40,000 is obtained for 3-methylbenzo(f)quinoline in pentane solution. If the limit were found at the long-wavelength band of 345 mμ, the value would be 4000. These various values are given because in preliminary work the whole spectrum is preferable for characterization, whereas in routine work, derived from a strong background of characterization studies, one band can be used.

TABLE III

ABSORPTION SPECTRA OF AZA HETEROCYCLIC HYDROCARBONS IN PENTANE AND PENTANE-TRIFLUOROACETIC ACID (50 : 1)

Pentane		*Pentane–trifluoroacetic acid*		*Pentane*		*Pentane–trifluoroacetic acid*	
λ max(mμ)	$\varepsilon \cdot 10^{-3}$	λ max(mμ)	$\varepsilon \cdot 10^{-3}$	λ max(mμ)	$\varepsilon \cdot 10^{-3}$	λ max(mμ)	$\varepsilon \cdot 10^{-3}$
Benzo(f)quinoline				*Benzo(h)quinoline*			
242	29.0	270s	23.5	~240s	~37.0	257s	5.0
247	28.0	282	32.0	~246s	~29.0	~266s	~ 8.8
265	25.5	310s	3.9	265	24.0	277s	15.0
270s	25.0	338s	3.9	290s	4.7	282	17.0
300s	1.8	356	7.4	295	5.5	312	4.0
308	1.3	368	8.4	309	0.8	328s	3.0
314	2.3			316	1.9	356	3.3
322	1.60			323	1.3	372	3.3
329	4.3			325s	1.0		
337	1.7			330	3.9		
339	1.2			338	1.4		
345	5.5			340	1.0		
				346	5.3		
3-Methylbenzo(f)quinoline				*Phenanthridine*			
234	30.0	*280s*	21.6	243s	~54.0	245	~45.0
240	33.0	283	23.3	247	57.0	276	5.6
246s	18.0	*310s*	3.9	252s	~52.0	318	7.1
269s	21.0	*338s*	4.4	269	10.7	355	3.7
272	21.5	252	8.0	284	5.5	368	3.6
301s	1.0	368	9.7	296	4.5		
308	0.45			314	1.3		
315	1.6			321	0.8		
322	1.0			328	2.0		
329	3.9			336	0.6		
337	1.2			342	2.0		
345	5.2						
Acridine				*Indeno(1,2,3-ij)isoquinoline*			
242s	55.0	256	92.0	246	14.5	244	28.6
249	135.0	288	9.0	256	13.8	283	14.4
310s	60.0	300s	9.0	268	16.8	293	14.5
325	1.9	311s	1.3	279	19.2	308	9.5
~332s	2.5	325	3.1	285	17.2	*354s*	3.6
340	3.9	~333	4.8	288	17.2	371	8.4
350	3.6	341	9.5	333	4.4	390	11.1
356	5.4	350	10.4	347	8.2		
372	1.9	357	20.5	357	6.8		
		286	3.0	363	10.0		
		405	3.0				
		432s	1.7	*Benzo(lmn)phenanthridine*			
				264	18.2	244	24.5
Acenaphtheno(1,2-b)pyridine				275	28.0	288	15.7
240	13.0	302	25.0	287	5.6	300	18.0
~245s	~10.0	307s	24.0	300	7.4	370s	~ 7.0
270	6.0	~346s	~ 5.5	318	12.0	386	9.8
275	10.0	360	6.1	330	16.6	~407	~ 8.2
281	18.0			340	13.6		
287	24.0			346	16.0		
292	40.0			~352s	~ 5.4		
307	4.0			363	0.8		
317s	4.4			370	2.2		

TABLE III (continued)

Pentane		*Pentane–trifluoroacetic acid*	
λ max(mμ)	ε · 10⁻³	λ max(mμ)	ε · 10⁻³
322	5.3		
329	5.0		
338	4.8		
345	5.2		
362s	0.51		
Benz(a)acridine			
240	34.0	~248s	87.0
265s	~55.0	258	72.0
273	81.0	276s	~ 53.0
282	65.0	288	88.0
316	5.4	294	123.0
324s	5.9	386	31.0
330	7.0	405	35.0
339	6.6		
345	8.4		
354	5.5		
362	11.6		
371	3.2		
380	14.4		
8,10-Dimethylbenz(c)acridine			
244	13.0	234	104.0
260s	25.5	260	35.0
269	48.0	286s	72.0
278	73.5	295	98.0
289	65.5	332	4.1
310	4.0	372s	10.6
324	6.8	385	14.5
344s	7.6	403	10.3
349	9.7	427	7.0
361	7.0		
366	12.5		
378	3.1		
384	12.6		
9,12-Dimethylbenz(a)acridine			
250	23.0	262	27.0
268s	~44.0	280s	~32.0
277	64.0	389	44.0
285s	56.0	370s	~ 7.7
320s	3.5	386	14.1
334	4.6	406	17.8
348	5.8		
365	6.8		
384	6.2		
7-Methylbenz(c)acridine			
258s	29.0	257s	31.0
268	47.0	284	53.0
277	71.0	294	81.0
288	65.0	332	3.6
319	4.0	~350	~ 3.9
327s	4.7	362	6.8

Pentane		*Pentane–trifluoroacetic acid*	
λ max(mμ)	ε · 10⁻³	λ max(mμ)	ε · 10⁻³
12-Methylbenz(a)acridine			
245	42.0	260	40.0
266s	~58.0	284s	~57.0
275	91.0	291	72.0
284	85.0	385s	11.0
320s	5.3	402	12.6
334	6.7		
348	8.0		
365	9.4		
384	9.1		
8,12-Dimethylbenz(a)acridine			
269s	~44.0	264	28.0
278	71.0	285s	~49.0
287	62.0	293	65.0
322s	~ 3.4	385s	9.3
336	4.5	405	9.7
350	5.8		
360s	5.0		
366	6.9		
385	5.9		
Benz(c)acridine			
~258s	29.0	250s	17.0
266	50.0	256	25.0
274	72.0	282	50.0
286	60.0	294	78.0
317	4.6	335	4.4
324	5.0	365s	8.3
330	5.8	380	10.8
338	5.6	410	6.0
346	7.2	432	5.0
355	4.8		
363	11.4		
373	3.0		
~378s	~ 3.2		
382	14.8		
7,9-Dimethylbenz(c)acridine			
244	21.0	241	~ 9.0
279	46.0	254s	~15.0
288	66.0	260	20.0
291	58.0	285s	~53.0
320s	3.6	295	77.0
334	5.0	332	2.8
343s	~ 5.5	370s	~ 7.4
348	6.8	386	10.8
360	5.0	428	5.3
366	8.7		
378	2.5		
384	9.1		
392s	0.5		

TABLE III (continued)

Pentane		*Pentane–trifluoroacetic acid*		*Pentane*		*Pentane–trifluoroacetic acid*	
λ max(mμ)	*ε · 10⁻³*	*λ max(mμ)*	*ε · 10⁻³*	*λ max(mμ)*	*ε · 10⁻³*	*λ max(mμ)*	*ε · 10⁻³*
333	5.6	379	9.5		*Dibenz(a,h)acridine*		
342	5.8	406	6.3	248	31.0	245s	40.0
348	7.2	429	5.5	260	32.5	262	14.5
359	5.1			268	47.0	290s	36.0
366	9.6			288	125.0	300	80.0
378s	2.8			295	147.0	310	91.0
384	11.0			318	16.4	393	6.3
				331	13.2	415	8.8
	7,10-Dimethylbenz(c)acridine			346	7.2	438	9.8
260s	~36.0	285	52.0	354	7.2		
268	57.0	296	74.0	363	4.5		
278	87.0	336	4.0	371	17.3		
289	86.0	361	5.6	381	6.1		
320s	5.2	377	7.5	391	21.6		
327s	~ 5.4	409	5.1				
334	6.6	430	4.6		*14-Phenyldibenz(a,j)acridine*		
341s	6.1			241	21.8	258	13.4
350	7.7			265s	15.2	302	43.6
368	10.0			290	46.4	386s	6.12
386	11.6			300	46.0	408	11.8
				324	9.2	432	18.0
	Dibenz(a,j)acridine			338	8.4		
248s	26.0	258	19.0	354	5.0		
257	35.0	*290s*	40.0	375	5.0		
270	35.0	301	90.0	394	6.4		
285	60.0	387s	6.2				
293	83.5	408	9.7				
300s	60.0	429	12.2		*Pyrenoline*		
320	11.5			240	32.0	*259s*	15.0
334	10.2			261	35.5	270	17.5
344	4.6			278	35.5	*310s*	48.0
348	4.8			287	50.5	319	71.0
352	6.4			298	46.5	354	3.5
361	3.8			*318s*	6.3	373	4.3
370	13.7			336	6.9	391	3.0
380	4.8			340	6.7	*432s*	3.3
389	19.1			353	12.2	456	4.5
				357	12.9	*475s*	4.3
	7-Phenyldibenz(c,h)acridine			368	17.6		
243	20.4	253	13.6	*372s*	16.5		
248	20.6	258s	12.7	376	16.5		
256	21.8	269	16.0	385	17.6		
270s	19.0	280	11.4	*390s*	8.5		
290	55.0	314	54.0	396	4.0		
304	48.0	344	5.6	403	5.5		
327	7.6	385s	5.2	409	19.2		
341	8.7	403	10.6	*428s*	3.1		
356	7.4	430	13.6	*451s*	2.4		
375	6.6			457	2.8		
394	8.8			457	2.8		

Fluorescence spectra

The disadvantages of this spectral method are the relatively poorer reproducibility

as compared to absorption spectral methods and the quenching effects, which can drastically alter the relative and absolute intensities of the bands. Compared to absorption spectral methods, the selectivity and sensitivity of spectrophotofluorimetric methods are usually much greater.

The detection limit used in our fluorimetric work is defined as the smallest quantity in nanograms of a compound (dissolved in a 0.1-ml cell) which will give unchanged fluorescence excitation and emission spectra. The detection limits range from 1.2 for 7-phenyldibenz(c,h)acridine to 80 for 12-methylbenz(a)acridine and average about 17. Thus, at least 1.2 nanograms of 7-phenyldibenz(c,h)acridine is necessary to obtain its fluorescence excitation and emission spectra.

The K_Q values range from about 40 for 7-phenyldibenz(c,h)acridine to 0.3 for indeno(1,2,3-ij)isoquinoline. The extremely high fluorescence intensity of the former compares favorably with the K_Q value of 45 of anthanthrene. Anthanthrene was shown to have the highest K_Q value in pentane compared to a large number of polycyclic hydrocarbons[3].

The fluorescence spectra of the aza compounds usually have less fine structure than do the absorption spectra. The spectral changes that occur in the change from neutral to acid solution are similar to those described under absorption spectra (Table IV).

The following isomers can be differentiated from each other: benzo(f)quinoline and benzo(h)quinoline, phenanthridine and acridine, indeno(1,2,3-ij)isoquinoline and acenaphtho(1,2-b)pyridine, benz(a)acridines and benz(c)acridines, dibenz(a,h)acridine and dibenz(a,j)acridine, and 14-phenyldibenz(a,j)acridine and 7-phenyldibenz(c,h)acridine. Acridine can be differentiated from 3-methylacridine; benzo(lmn)phenanthridine and pyrenoline are readily distinguishable from each other and from other aza heterocyclic compounds.

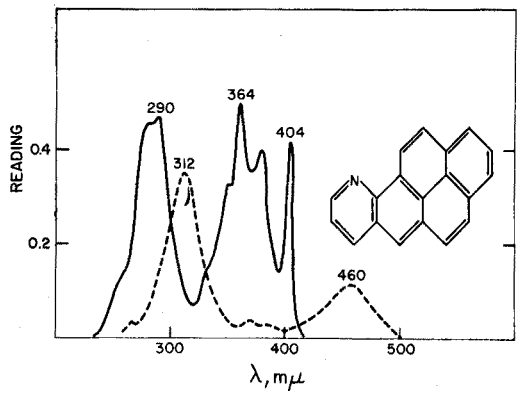


Fig. 3. Fluorescence excitation spectra of pyrenoline in pentane (10^{-7} M) with emission wavelength 410 mμ (———) and in pentane–trifluoroacetic acid ($10^{-6}M$) with emission wavelength 545 mμ (– –).

Examples of the excitation and emission spectra of pyrenoline in pentane and in pentane–trifluoroacetic acid are given in Figs. 3 and 4. These spectra were taken with an Aminco-Keirs spectrophosphorimeter with a fluorescence attachment a year before those presented in Table IV, and the spectra were measured by different observers. Slight differences in the positions of the wavelength maxima and somewhat

TABLE IV

FLUORESCENCE SPECTRA OF AZA HETEROCYCLIC HYDROCARBONS IN PENTANE AND PENTANE – TRIFLUOROACETIC ACID (50 : 1; V/V)

Emission spectra				*Excitation spectra*			
P		*P-TFA*		*P*		*P-TFA*	
λ	*MM · T*	*λ*	*MM · T*	*λ*	*MM · T*	*λ*	*MM · T*
DL[a] = 12		*Benzo(f)quinoline (10⁻⁶M)*				K_Q^{340} = 0.7[b]	
Exc. λ 275		*Exc. λ 282*		*Emiss. λ 360*		*Emiss. λ 420*	
340	0.19	420	0.76	250	0.09	282	0.80
360	0.18			275	0.17	370	0.77
380	0.08			318	0.02		
400	0.02			329	0.06		
				341	0.08		
DL = 20		*3-Methylbenzo(f)quinoline (10⁻⁶M)*				K_Q^{340} = 1	
Exc. λ 275		*Exc. λ 364*		*Emiss. λ 360*		*Emiss. λ 407*	
340	0.03	407	1.29	250	0.14	282	1.08
360	0.29			275	0.24	318	0.42
380	0.13			314	0.05	364	1.28
400	0.02			329	0.08		
				335	0.05		
				341	0.10		
DL = 6		*Benzo(h)quinoline (10⁻⁶M)*				K_Q^{345} = 0.7	
Exc. λ 262		*Exc. λ 279*		*Emiss. λ 345*		*Emiss. λ 425*	
345	0.18	425	0.20	262	0.15	279	0.20
362	0.15			330	0.05	315s[c]	0.07
382	0.06			345	0.07	368	0.12
~400	0.01						
DL = 6		*Phenanthridine (10⁻⁶M)*				K_Q^{400} = 0.8	
Exc. λ		*Exc. λ 320*		*Emiss. λ*		*Emiss. λ 400*	
—[d]		400	0.20	—		254	0.10
						271s	0.04
						320	0.19
						362	0.15
DL = 6		*6-Methylphenanthridine (10⁻⁶M)*				K_Q^{400} = 1	
Exc. λ		*Exc. λ 320*		*Emiss. λ*		*Emiss. λ 398*	
—		400	0.23	—		258	0.13
						319	0.20
						348	0.19
						361	0.20
DL = 3		*Acridine (10⁻⁶M)*				K_Q^{475} = 3	
Exc. λ		*Exc. λ 350*		*Emiss. λ*		*Emiss. λ 475*	
—		450	0.55	—		260	0.89
		475	0.71			343	0.39
						350	0.78
						400	0.18
DL = 18		*3-Methylacridine (10⁻⁶M)*				K_Q^{484} = 2	
Exc. λ 290		*Exc. λ 262*		*Emiss. λ*		*Emiss. λ 484*	
—		484	0.50	—		263	0.54
						350	0.15
						361	0.30
						410	0.10

TABLE IV (continued)

Emission spectra				*Excitation spectra*			
P		*P-TFA*		*P*		*P-TFA*	
λ	$MM \cdot T$	λ	$MM \cdot T$	λ	$MM \cdot T$	λ	$MM \cdot T$
DL = 50		*Indeno(1,2,3-ij)isoquinoline ($10^{-5}M$)*				$K_Q^{475} = 0.3$	
Exc. λ 368		*Exc. λ 313*		*Emiss. λ 475*		*Emiss. λ 450*	
450	0.70	432	0.09	250	0.28	313	0.10
475	0.75	450	0.10	290	0.63	350s	0.04
				350	0.60	402s	0.01
				368	0.74	426	0.02
						448	0.03
DL = 30		*Acenaphtho(1,2-b)pyridine ($10^{-5}M$)*				$K_Q^{450} = 0.5$	
Exc. λ 292		*Exc. λ 305*[e]		*Emiss. λ 450*		*Emiss. λ 450*[d]	
430s	1.2	430	0.84	233	0.2	305	0.92
450	1.5	450	0.90	292	1.5	358	0.38
~460s	1.2			320	0.75		
				330	0.7		
				340	0.7		
				347	0.75		
DL = 20		*Benzo(lmn)phenanthridine ($10^{-6}M$)*				$K_Q^{370} = 4$	
Exc. λ 328		*Exc. λ 394*		*Emiss. λ 370*		*Emiss. λ 470*	
370	0.64	470	1.10	240	0.09	243	0.10
380	0.43			270	0.28	298	0.80
390	0.23			328	0.61	394	1.10
410	0.12			338	0.56		
				364	0.10		
DL = 25		*8,12-Dimethylbenz(a)acridine ($10^{-6}M$)*				$K_Q^{390} = 0.8$	
Exc. λ 287		*Exc. λ 290*		*Emiss. λ 390*		*Emiss. λ 460*	
390	0.28	460	1.8	287	0.32	290	1.8
412	0.21			335s	0.12	402	1.4
434	0.09			348	0.15		
				364	0.17		
				387	0.24		
DL = 25		*9,12-Dimethylbenz(a)acridine ($10^{-6}M$)*				$K_Q^{390} = 0.4$	
Exc. λ 286		*Exc. λ 403*		*Emiss. λ 390*		*Emiss. λ 457*	
390	0.14	457	2.3	286	0.16	290	2.0
410	0.12			335s	0.07	403	2.0
430	0.08			348	0.08		
				364	0.09		
				388	0.08		
DL = 10		*Benz(c)acridine ($10^{-6}M$)*				$K_Q^{384} = 3$	
Exc. λ 280		*Exc. λ 284*		*Emiss. λ 384*		*Emiss. λ 475*	
384	1.0	475	1.3	273	1.1	284	0.90
408	0.65			280	1.2	330s	0.20
430	0.20			300s	0.30	378	0.60
458	0.04			340	0.40	405	0.40
				356	0.50	420	0.33
				376	0.70		
DL = 20		*Benz(a)acridine ($10^{-6}M$)*				$K_Q^{382} = 0.7$	
Exc. λ 284		*Exc. λ 294*		*Emiss. λ 382*		*Emiss. λ 460*	
382	0.25	460	2.0	284	0.25	262	0.6
401	0.18			330s	0.10	294	2.0

TABLE IV (continued)

Emission spectra				*Excitation spectra*			
P		*P-TFA*		*P*		*P-TFA*	
λ	$MM \cdot T$	λ	$MM \cdot T$	λ	$MM \cdot T$	λ	$MM \cdot T$
425	0.06			345	0.12	410	1.5
				360	0.14		
				380	0.20		
DL = 80		*12-Methylbenz(a)acridine ($10^{-5}M$)*				$K_Q^{390} = 2$	
Exc. λ 382		*Exc. λ 286*[t]		*Emiss. λ 410*		*Emiss. λ 460*[t]	
390	0.60	460	2.2	290	0.60	286	2.0
410	0.52			335s	0.25	394	2.0
430	0.24			350	0.33		
				364	0.40		
				384	0.36		
DL = 4		*8,10-Dimethylbenz(c)acridine ($10^{-6}M$)*				$K_Q^{382} = 6$	
Exc. λ 290		*Exc. λ 294*		*Emiss. λ 382*		*Emiss. λ 468*	
382	2.5	468	6.4	280	2.2	294	6.5
406	1.7			290	2.3	330	0.7
430	0.57			334s	0.6	390	2.8
458s	0.18			348	0.87	408s	2.0
				360	1.02	424	1.6
				382	0.87		
DL = 20		*7-Methylbenz(c)acridine ($10^{-6}M$)*				$K_Q^{388} = 2$	
Exc. λ 280		*Exc. λ 290*		*Emiss. λ 388*		*Emiss. λ 460*	
388	0.66	460	2.3	274	0.58	290	0.60
408	0.43			280	0.65	334s	0.06
430	0.14			330s	0.17	378	0.19
455s	0.03			340	0.22	406	0.14
				360	0.24	424	0.10
				380	0.26		
DL = 5		*7,9-Dimethylbenz(c)acridine ($10^{-6}M$)*				$K_Q^{388} = 9$	
Exc. λ 286		*Exc. λ 290*		*Emiss. λ 387*		*Emiss. λ 465*	
388	2.9	465	2.2	275	2.5	290	2.1
409	1.8			286	2.6	334s	0.2
430	0.6			330s	0.7	390	1.1
455s	0.07			340	1.0	424s	0.6
				360	1.1		
				380	1.1		
DL = 5		*7,10-Dimethylbenz(c)acridine ($10^{-6}M$)*				$K_Q^{390} = 5$	
Exc. λ 288		*Exc. λ 290*		*Emiss. λ 390*		*Emiss. λ 460*	
390	1.8	460	2.6	278	1.6	290	2.4
412	1.2			288	1.8	334s	0.4
437	0.5			335s	1.3	372	1.1
460s	0.1			347	0.50	403	0.9
				363	0.57	427	0.8
				386	0.55		
DL = 1.4		*Dibenz(a,h)acridine ($10^{-7}M$)*				$K_Q^{390} = 30$	
Exc. λ 290		*Exc. λ 310*		*Emiss. λ 390*		*Emiss. λ 447*	
390	1.4	447	1.1	290	1.3	260	0.18
400	0.40	471s	0.74	318	0.32	300	1.0
410	0.66			330	0.27	310	1.1
438	0.17			344	0.17	430	0.70

TABLE IV (continued)

Emission spectra				*Excitation spectra*			
P		*P-TFA*		*P*		*P-TFA*	
λ	*MM · T*	λ	*MM · T*	λ	*MM · T*	λ	*MM · T*
~460s	0.06			350	0.17		
				370	0.33		
				380	0.15		
DL = 1.4		*Dibenz(a,j)acridine (10⁻⁷M)*				K_Q^{390} = *20*	
Exc. λ 290		*Exc. λ 300*		*Emiss. λ 390*		*Emiss. λ 440*	
390	0.78	440	2.6	264	0.13	300	2.6
400s	0.20			290	0.64	402	1.3
410	0.40			320	0.17	425	2.0
434	0.15			330	0.17		
460s	0.05			352	0.12		
				370	0.21		
				388	0.30		
DL = 7		*14-Phenyldibenz(a,j)acridine (10⁻⁶M)*				K_Q^{404} = *5*	
Exc. λ 300		*Exc. λ 300*		*Emiss. λ 404*		*Emiss. λ 450*	
404	2.7	450	2.1	250	0.4	300	2.0
428	1.5			295s	2.6	410	1.3
450s	0.5			300	2.8	430	1.9
				325	0.8		
				340	0.8		
				355	0.5		
				375	0.5		
				395	0.7		
DL = 1.2		*7-Phenyldibenz(c,h)acridine (10⁻⁷M)*				K_Q^{397} = *40*	
Exc. λ 302		*Exc. λ 310*		*Emiss. λ 397*		*Emiss. λ 470*	
397	2.2	470	0.86	290	2.0	270	0.12
420	1.2			302	2.2	310	0.84
440	0.33			325	0.50	345	0.09
				340	0.66	410	0.30
				352	0.63	430	0.39
				372	0.50		
				395	0.80		
DL = 25		*Pyrenoline (10⁻⁶M)*				K_Q^{410} = *2*	
Exc. λ 290[g]		*Exc. λ 320*		*Emiss. λ 410*[g]		*Emiss. λ 548*	
410	0.60	548	0.74	265s	0.30	275	0.09
430	0.34			290	0.65	320	0.73
462	0.10			297	0.65	370	0.09
				315s	0.20	390	0.04
				340s	0.20	455s	0.10
				354	0.40	464	0.13
				368	0.60	475s	0.10
				384	0.52		
				408	0.52		

[a] DL = Determination limit in nanograms of material per 0.1 ml of solvent.
[b] K_Q is the relative fluorescence intensity of a compound compared to quinine[3].
[c] s = Shoulder.
[d] — = No spectra found.
[e] Concentration is $2 \cdot 10^{-6}$ *M*.
[f] Concentration is 10^{-6} *M*.
[g] Concentration is 10^{-7} *M*.

larger differences in intensity are apparent. These differences indicate that a standard should be run whenever an unknown is to be characterized or analyzed.

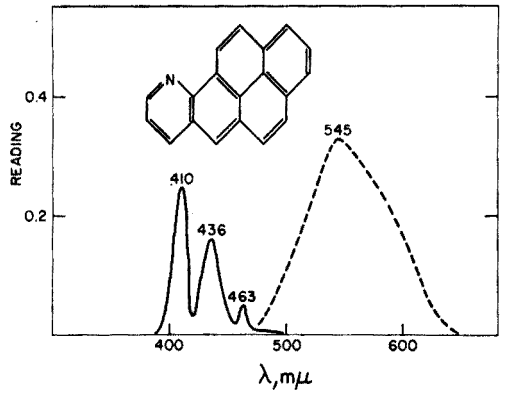


Fig. 4. Fluorescence emission spectra of pyrenoline in pentane (10^{-7} *M*) with excitation wavelength 364 mμ (———) and in pentane–trifluoroacetic acid (10^{-6} *M*) with excitation wavelength 312 mμ (— — —).

Phosphorescence spectra

Among the different spectral techniques the poorest reproducibility is obtained with the spectrophotophosphorimetric method. The difficulty lies in reproducing the band intensities. Tenfold or greater changes in concentration can sometimes affect the excitation spectrum by changing its shape. Another disadvantage is that a compound with a long lifetime must be scanned at a slower speed, otherwise bands in the excitation spectrum may shift, disappear, or lose intensity.

The sensitivities of the spectral methods in the ultraviolet-visible region are usually in the order fluorimetric > phosphorimetric > absorption. The definition of the detection limit is the same as that for spectrophotofluorimetry. The detection limits range from 9 for benzo(f)quinoline and benzo(h)quinoline to over 20,000 for indeno(1,2,3-ij)isoquinoline. Pyrenoline yields negligible phosphorescence.

On the basis of phosphorescence spectra only, the following isomers can be differentiated: phenanthridine and acridine, indeno(1,2,3-ij)isoquinoline and acenaphtho-(1,2-b)pyridine, benz(a)acridine and benz(c)acridine, dibenz(a,h)acridine and dibenz-(a,j)acridine, and 14-phenyldibenz(a,j)acridine and 7-phenyldibenz(c,h)acridine. Benz(a)acridine, benz(c)acridine and many of their methylated derivatives could probably be differentiated from one another.

The phosphorescence excitation and emission spectra of benzo(h)quinoline in EPA are given in Fig. 5.

Three factors give the phosphorimetric technique great selectivity as compared to absorption spectral methods. These 3 factors are: (*1*) the very large differences in intensities between some compounds, as shown by the detection limits; (*2*) the fairly large differences in the positions of the wavelength maxima of the excitation and emission spectra (so that one compound could be characterized or determined in the presence of the other by selective excitation or emission as in fluorimetric[3–5] or phosphorimetric[6] analysis); and (*3*) the differences in the mean lifetime by means of which a com-

pound with a long lifetime could be determined in the presence of other compounds with much shorter lifetimes[6].

Some of these factors were considered in the analysis of a mixture of benzo(f)-quinoline and benz(c)acridine, each 10^{-4} M. At the excitation wavelength maximum of benz(f)quinoline (272 mμ), the pure emission spectrum of benzo(f)quinoline was obtained, while at the excitation wavelength maximum of benz(c)acridine (380 mμ) the pure emission spectrum of benz(c)acridine was obtained.

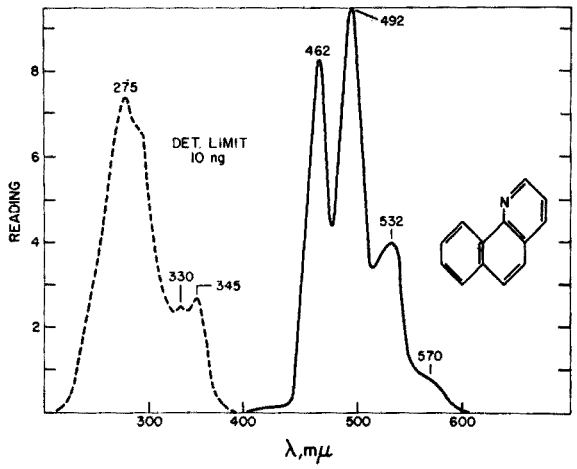


Fig. 5. Phosphorescence excitation (— — —) and emission (———) spectra obtained at emission wavelength maximum 492 mμ and excitation wavelength maximum 275 mμ, respectively, in EPA containing 10% chloroform.

Collaborative use of the various techniques

The first step in the separation and concentration of the aza heterocyclic compounds is extraction of the basic fraction with aqueous acid solution or application of one of the described general thin-layer chromatographic methods. The next step is the separation of the various aza compounds through one of the more selective thin-layer chromatographic methods. The possible presence of an aza heterocyclic compound can be ascertained on the plate by the trifluoroacetic acid test. In this test the fluorescence color of a spot containing an azo hydrocarbon changes dramatically on the addition of the acid. The identification limits for the aza hydrocarbons ranged from 0.5 to 10 ng; the majority of the compounds yielded values of 1 ng.

Once a spot is formed for which the R_F value is close to that of a known aza hydrocarbon and the fluorescence color test is positive, then the spot is extracted and the fluorescence and phosphorescence spectra of the solution are determined. For further characterization 20 to 60 spots are combined into one spot containing a larger quantity of material than in the absorption spectral detection limit; then another thin-layer chromatographic run is made, and the absorption spectra of the neutral and acid solutions of the appropriate spot are determined.

Such a procedure, modified by further advances and by the vicissitudes of applied analysis, will be necessary in the analysis of complex mixtures for the aza compounds.

SUMMARY

Many thin-layer chromatographic systems can be used for the general separation of aza heterocyclic compounds from polynuclear aromatic hydrocarbons. Several can be used for the separation of the aza compounds from each other. With alumina as the adsorbent the sterically hindered aza heterocyclic compounds can be readily separated from the non-hindered aza compounds.

The use of the trifluoroacetic acid spot test on the thin-layer plate is discussed. Spectral data for the aza compounds are presented and discussed.

RÉSUMÉ

Plusieurs systèmes de chromatographie sur couche mince peuvent être utilisés pour la séparation de composés aza hétérocycliques d'avec des hydrocarbures aromatiques polynucléaires ou pour la séparation de composés aza les uns des autres. Les spectres d'absorption, les spectres de fluorescence et les spectres de phosphorescence de ces composés sont examinés.

ZUSAMMENFASSUNG

Es wird die Analyse von aza heterocyclischen Verbindungen und ihre Trennung untereinander und von mehrkernigen Kohlenwasserstoffen und anderen aromatischen Verbindungen mittels der Dünnschichtchromatographie beschrieben. Absorptions-, Fluoreszenz- und Phosphoreszenzspektren werden aufgeführt und diskutiert.

REFERENCES

1 J. L. HARTWELL, *Survey of Compounds which have been tested for Carcinogenic Activity*, U. S. Government Printing Office, Washington, D. C.

2 B. L. VAN DUUREN, *J. Natl. Cancer Inst.*, 25 (1960) 53.

3 E. SAWICKI, T. R. HAUSER AND T. W. STANLEY, *Intern. J. Air Pollution*, 2 (1960) 253.

4 E. SAWICKI, W. C. ELBERT, T. W. STANLEY, T. R. HAUSER AND F. T. FOX, *Intern. J. Air Pollution*, 2 (1960) 273.

5 E. SAWICKI, W. C. ELBERT, T. W. STANLEY, T. R. HAUSER AND F. T. FOX, *Anal. Chem.*, 32 (1960) 810.

6 R. J. KEIRS, R. D. BRITT, JR. AND W. E. WENTWORTH, *Anal. Chem.*, 29 (1957) 202.

THE PAPER CHROMATOGRAPHIC ISOLATION OF NUCLIDES IN AIR SAMPLES

G. A. WELFORD AND E. L. CHIOTIS

Health and Safety Laboratory, U.S. Atomic Energy Commission, New York, N.Y. (U.S.A.)

(Received February 23rd, 1964)

Many published methods[1-4] describe the separation of fission products from air dust samples and other collection devices. These primarily consist of classical chemical separations and several specific purification procedures. In general, milligram quantities of inactive carriers are added and equilibrated with nuclide activities. The presence of this amount of stable element provides media for chemical operations and recovery correction.

Paper chromatographic methods[5,6] for the separation of fission products have recently been reported in the literature. Generally high activity levels of radionuclides are spotted or streaked on a paper and separated chromatographically. The spotting technique is limited to application of microliter volumes, while streaking has been reported valid up to 0.1 ml. The presence of inert salts in environmental samples prevents concentration to such low volumes while the detection limits for environmental collections require analysis of the entire sample.

The method presented collects the total concentration of various nuclides from environmental samples thus increasing the minimum activity detection limit.

Milligram quantities of iron are used as a carrier for nuclides of strontium, barium, cerium, yttrium, lanthanum, zirconium, and niobium. Iron is precipitated as the hydroxide from a carbonate solution and filtered directly onto the chromatographic paper. Hydrogen chloride gas is used to dissolve the precipitate and convert the salts to the chlorides. In the first solvent iron chloride migrates close to the solvent front while leaving the nuclides distributed near the origin. A second dimensional development separates the nuclides quantitatively so that specific and precise areas are available for radiometric measurements.

EXPERIMENTAL

Reagents

Iron carrier	— 5 mg/ml: 5 g of iron wire/l in 5% HCl.
Strontium carrier	— 40 μg/ml: 48.2 mg of $Sr(NO_3)_2$/l in 1% HCl.
Cerium carrier	— 40 μg/ml: 124 mg of $Ce(NO_3)_3 \cdot 6H_2O$/l, in 1% HCl.
Barium carrier	— 40 μg/ml: 60.8 mg of $BaCl_2$/l in 1% HCl.
Zirconium carrier	— 40 μg/ml: 121.2 mg of $ZrOCl_2 \cdot 5H_2O$/l in 1% HCl.

Niobium carrier — 40 μg/ml: 740.0 mg of Nb metal/l in saturated $H_2C_2O_4$.
Yttrium carrier — 40 μg/ml: 172.8 mg of $Y(NO_3)_3 \cdot 6H_2O$/l in H_2O.
Lanthanum carrier — 40 μg/ml: 96.8 mg of $La(NO_3)_3$/l in 1% HCl.
HCl gas — Lecture bottle.
Solvents — I = 40% Ethanol, 40% methanol, 20% 6 *N* HCl and 2.5% acetyl acetone.
II = 40% Ethanol, 40% methanol, 20% 2 *N* HCl and 2.5% acetyl acetone.
III = 87% Acetone, 8% HCl and 5% water.
Sprays — Alizarin red S (Alizarin sulfonic acid) — alcoholic 0.1% solution of sodium alizarin sulfonate.
Oxine (8-hydroxyquinoline) — 0.5 g in 100 ml of 60% (v/v) alcohol.

Apparatus

Chromatography paper. Whatman #42, 57 × 46 cm.

Millipore filter apparatus. Available from the Millipore Filter Corporation, P.O. Box F, Bedford, Massachusetts. Two sizes are in present use, having filtering areas of 3 and 10 cm², respectively.

Developing tanks. Chromatocab from the Research Specialties Co., 200 S. Garred Boulevard, Richmond, California.

Glass racks. See Fig. 1.

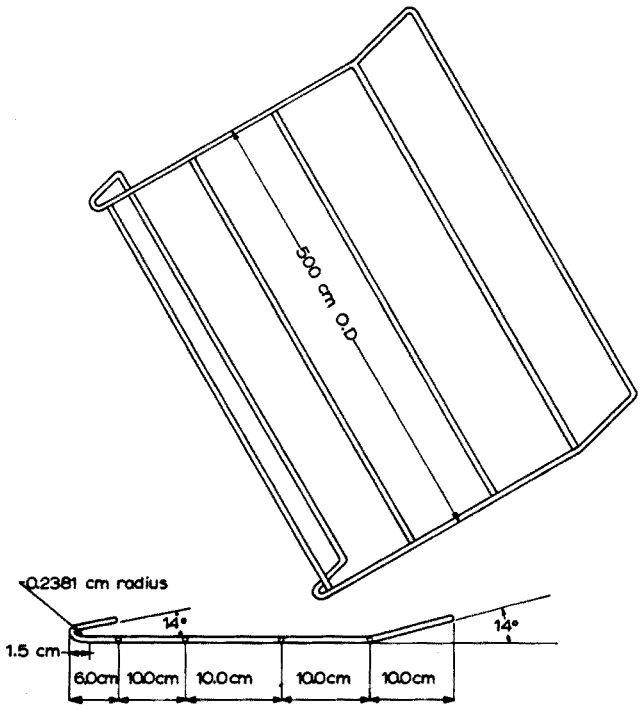


Fig. 1. Glass rack. Material: Pyrex 0.7144 cm solid rod or nearest standard Pyrex rod.

Chemical procedure

Transfer the air dust sample to an appropriate size of beaker and wet with 1 : 10 nitric acid. Add 1 ml of each carrier solution and *ca.* 20 ml of hydrochloric acid. Wet-ash with repeated additions of nitric acid and 30% hydrogen peroxide until the organic material is completely destroyed. Evaporate to *ca.* 25 ml and transfer with nitric acid to a 100-ml platinum dish. Evaporate on a sand bath to a small volume and expel silica with 3 additions of 10 ml of hydrofluoric acid and 10 ml of nitric acid. Add 20 ml of nitric acid and evaporate to near dryness.

Transfer to a 150-ml beaker, add 25 ml of hydrochloric acid, and boil gently. Cool and adjust the pH to *ca.* 5 with ammonia solution. Add 3 ml of saturated sodium carbonate solution. Warm gently and adjust the pH to 9 with ammonia solution. Digest for 5 min at *ca.* 90°. Cool.

Set up the Millipore filter apparatus with a 2.8-cm glass fiber filter disc on the filter stick. Arrange the Whatman #42 paper so that the filtering apparatus is in the right-hand corner, 12 cm from the adjacent sides. Filter the cooled digest using gentle suction. Remove the paper and introduce a small stream of hydrogen chloride gas directly onto the area containing the precipitate. Attach the paper to the glass rack with the 47-cm length along the vertical axis, and allow to air-dry for approximately 15 min.

Place the rack into the Chromatocab previously saturated for 1 h with solvent I. Develop the chromatogram with solvent I until the solvent front is approximately 2 cm from the end of the paper. Air-dry and cut the paper into 2 parts as illustrated in Fig. 2.

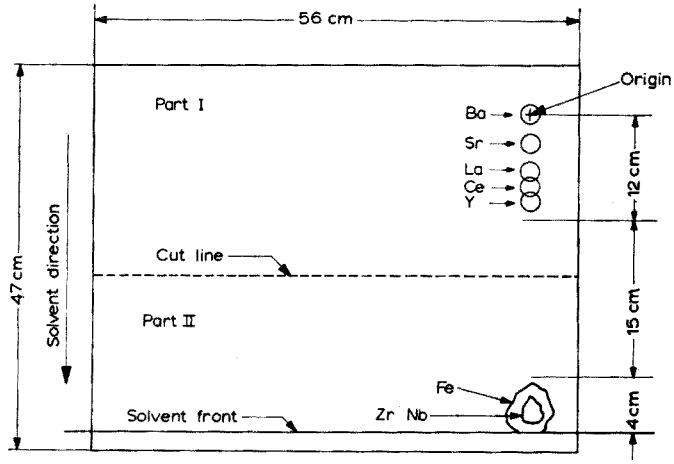
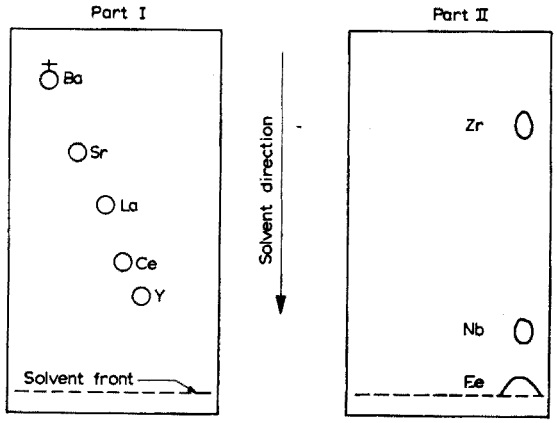


Fig. 2. First dimension chromatography.

Assemble each part on separate glass racks. Develop part I for 16 h in a presaturated Chromatocab using solvent II. Remove, air-dry, and continue as under *Radiometric procedure.* Develop part II for 5 h in a presaturated Chromatocab using solvent III. Remove, air-dry, and continue as under *Radiometric procedure.*

Location of nuclides

Part I. Figure 3a illustrates the relative location of the nuclides of barium, strontium, cerium, yttrium, and lanthanum after the chromatographic separation using solvent II. Continue as follows. Spray with 8-hydroxyquinoline and suspend the chromatogram for 2 min in a glass tank previously saturated with vapors of ammonia. Expose the treated chromatogram to an ultraviolet light source and lightly circle the



Figs. 3a and 3b. Second dimension chromatography.

fluorescent areas. This identifies the above-mentioned nuclides. Spray the lower part of the paper with alizarin red S and circle the persistent purple area. This identifies lanthanum.

Part II. Figure 3b indicates the relative location of iron and the nuclides of zirconium and niobium. Spray with alizarin red S and suspend in a glass tank previously saturated with ammonia vapors. Circle the persistent purple areas.

Radiometric procedure

The γ-emitting nuclides are cut from the chromatogram sealed in Mylar, and counted directly on a NaI(Tl) crystal.

The β-emitting nuclides are measured by the following procedure. Carefully cut out the indicated area and place in a 150-ml beaker. Add 50 ml of nitric acid and 5 ml of perchloric acid. Wet-ash with additions of nitric acid, if required, and evaporate to a volume of 1–2 ml. Transfer to a 40-ml centrifuge tube and add 10 mg of the stable element for each nuclide. Continue as follows.

Strontium. Store for 14 days to allow growth of the yttrium daughter. Follow the standard procedure for yttrium milking to determine ^{90}Sr. For other strontium nuclides, collect strontium as the carbonate, filter, mount, and resolve radiometrically.

Barium. Store for 10 days to allow growth of ^{140}La. Follow the standard procedures for lanthanum milking to determine the concentration of barium-140. Collect lanthanum as the oxalate.

Cerium. Cerium is precipitated directly as the iodate or oxalate, filtered, mounted, and β-counted. Nuclides of cerium are resolved radiometrically.

Yttrium. Yttrium is precipitated as the oxalate and stored for decay of ^{90}Y.

Lanthanum. Lanthanum contains primarily ^{140}La and is used to estimate the ^{140}Ba content when it is not expedient to wait for the 10-day growth period. It is precipitated as the oxalate.

Zirconium. Zirconium is precipitated as the mandelate and ignited to the oxide for β-counting.

Niobium. Niobium is precipitated as the hydroxide and ignited to the oxide for γ-counting.

RESULTS AND DISCUSSION

The final location of the 7 nuclides is shown in Figs. 3a and 3b. The procedure outlined for location of the nuclides is only required for preliminary experimentation. The areas are reproducible for a given chromatographic system and a template is used to extract quantitatively the portion of the finished chromatogram containing each nuclide. Recovery studies were performed using β- and γ-emitting nuclides of the 7 elements. Table I reports the results from a series of experiments carried through the entire chemical procedure. Within the range of counting statistics, better than 95% of the added activity was consistently recovered for all nuclides. These reported results were obtained using a template previously constructed from runs containing amounts of the elements detectable with the spraying agents. Gamma-spectrometry and β-absorption measurements were made on each extracted area and indicated no cross-contamination of activities.

The second dimension development is required for quantitative isolation of the elements. Figure 4 illustrates the distribution of activity after development with the first solvent. As illustrated, there are only 2 areas of activity. The major area spreads 12 cm from the origin and is followed by a 15-cm length containing no activity. A second activity region is located 27 cm from the origin and is 4 cm in length. This second region overlaps the iron band. From Fig. 4 it is clear that an initial separation has occurred, as activity peaks for each nuclide are clearly discernible. Prior to the second dimension development the paper is cut as illustrated in Fig. 2 and the final development distributes the nuclides as shown in Figs. 3a and 3b.

TABLE I

RECOVERY STUDIES

Sample no.	^{133}Ba	^{89}Sr	^{85}Sr	^{144}Ce	^{95}Zr
246A	94.2	99.3	—	98.6	95.3
B	99.3	100.9	—	lost	96.7
C	105.2	99.0	—	95.8	97.4
247A	96.1	99.6	—	95.8	91.5
B	100.6	99.2	—	94.4	lost
C	92.8	100.3	—	98.8	98.5
D	98.1	98.3	—	99.8	94.8
248A	97.9	—	98.4	100.0	97.7
B	97.7	—	93.2	98.9	93.9
C	100.6	—	97.2	97.3	98.5
D	98.9	—	99.5	100.0	95.5
Average	98.3	99.5	97.0	97.9	96.0

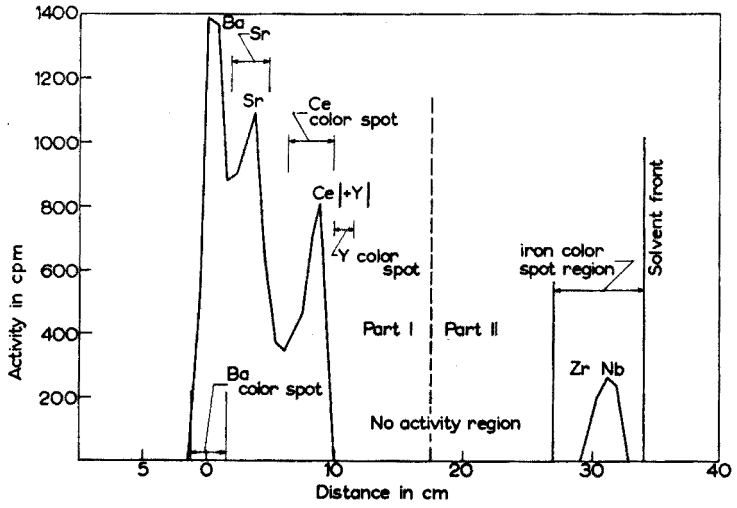


Fig. 4. Activity after first-dimension development.

SUMMARY

A method is outlined for the quantitative transfer of nuclides of strontium, barium, cerium, zirconium, lanthanum, yttrium, and niobium from solution to a paper for chromatographic separation. Carrier quantities of iron are precipitated as the hydroxide from a carbonate solution and filtered directly onto the chromatographic paper. A two-dimensional development is utilized to isolate the individual nuclides.

This separation procedure is applied to air dust samples containing nuclear debris. Radiometric determinations may be conveniently made by γ-spectrometric or conventional low-background β-measurements.

RÉSUMÉ

On décrit une méthode pour le transfert quantitatif de nuclides de strontium, baryum, cérium, zirconium, lanthane, yttrium et niobium d'une solution sur un papier pour séparation chromatographique. Ce procédé est appliqué à des échantillons de poussières atmosphériques, renfermant des débris nucléaires. Des déterminations radiométriques (spectrométrie gamma ou mesures de rayonnement bêta) peuvent être effectuées.

ZUSAMMENFASSUNG

Zur papierchromatographischen Bestimmung von Spaltprodukten aus der Luft wird eine Methode beschrieben, mit der die Nuklide des Sr, Ba, Ce, Zr, La, Y und Nb aus einer Lösung quantitativ auf das Papier übertragen werden. Dazu wird als Träger Eisen als Hydroxid gefällt und direkt auf das chromatographische Papier filtriert. Zur Isolierung der einzelnen Nuklide wird eine zweidimensionale Entwicklung angewandt. Zur Bestimmung der Nuklide werden die γ- und β-Aktivitäten gemessen.

REFERENCES

1 G. A. WELFORD, W. R. COLLINS, Jr., R. S. MORSE AND D. C. SUTTON, *Talanta*, 5 (1960) 168.
2 C. CORYELL AND N. SUGARMAN, *Radiochemical Studies. The Fission Products*, McGraw-Hill, New York, 1951.
3 LOS ALAMOS SCIENTIFIC LABORATORY, *Collected Radiochemical Procedures*, LA-1721, 2nd Ed.
4 HEALTH AND SAFETY LABORATORY, *Manual of Standard Procedures*, USAEC Report-NYO-4700 (Rev.).
5 H. GOTTE AND D. PATZE, *Angew. Chem.*, 69 (1957) 608.
6 H. IMAI, *Technol. Rept. Kansai Univ.*, 35 (1960) 143.

PRECIPITATION OF ZINC SULFIDE BY HYDROLYSIS OF THIOACETAMIDE IN THE PRESENCE OF HYDRAZINE HYDROCHLORIDE

RICHARD B. HAHN AND DAVID LEE PRINGLE

Department of Chemistry, Wayne State University, Detroit 2, Mich. (U.S.A.)

(Received January 28th, 1964)

Zinc can be separated from iron, manganese, chromium, aluminum, nickel and cobalt by precipitation of the sulfide from solutions maintained at pH 2. Various buffer mixtures have been recommended and these include: formic acid–formate[1], bisulfate–sulfate[2], chloracetic acid–acetate[3], and citric acid–citrate mixtures[4]. When hydrogen sulfide gas is used as precipitant, some cobalt sulfide is coprecipitated with the zinc sulfide. This interference can be minimized by the addition of ammonium thiocyanate which complexes the cobalt ion and thereby reduces the amount of coprecipitation.

Thioacetamide was used as a source of hydrogen sulfide by HAHN AND SHELLINGTON[5]. Precipitations were carried out in pressure bottles since the rate of hydrolysis of thioacetamide is extremely slow even at 100° in solutions of pH 2. Complete precipitation of zinc sulfide was attained by heating the samples under pressure at 120° for a period of 3 h. Bisulfate–sulfate and citric acid–citrate buffers were employed. KING AND ANSON[6] studied the kinetics of the hydrolysis of thioacetamide and found the rate of reaction greatly increased by the addition of hydrazine hydrochloride. The following study was undertaken to determine if zinc sulfide can be quantitatively precipitated using thioacetamide–hydrazine hydrochloride mixtures and to determine the extent of coprecipitation of cobalt in this process.

EXPERIMENTAL

Reagents

Citric acid–citrate buffer. Add 6 *M* ammonium hydroxide, with stirring, to a 1 *M* solution of citric acid until a pH of 2.0 is obtained.

Cobalt solution (10 mg of Co per ml). Dissolve 49.4 g of $Co(NO_3)_2 \cdot 6H_2O$ in distilled water and dilute to 1 l.

Hydrazine hydrochloride (1 M adjusted to pH = 2). Dissolve 105 g of hydrazine dihydrochloride in about 800 ml of water, then add hydrazine hydrate (85%) dropwise with stirring until a pH of 2 is obtained. Dilute the resulting solution to 1 l with distilled water.

Sulfate–bisulfate buffer. Dissolve 100 g of $Na_2SO_4 \cdot 10H_2O$ in 900 ml of water. Dissolve 13 g of sodium bisulfate in 100 ml of water. Add this with stirring to the sodium sulfate solution until a pH of 2 is obtained.

Thioacetamide (1 M). Dissolve 75 g of CH_3CSNH_2 in enough distilled water to make 1 l of solution, and then filter.

Zinc solution. Dissolve 10.00 g of chemically pure zinc metal in 50 ml of 8 *M* nitric acid and dilute to 1 l. This solution was standardized by precipitation of zinc ammonium phosphate and by precipitation as zinc tetrathiocyanatomercurate(II)[7].

General procedure

The following general procedure was used in all experiments. A known quantity of standard zinc nitrate solution was pipetted into a 40-ml, round-bottom centrifuge tube and the desired buffer mixture was added. Known amounts of hydrazine hydrochloride and thioacetamide then were added and the pH of the mixture was checked. If necessary it was adjusted to a pH of 2 by the addition of dilute ammonium hydroxide or hydrochloric acid. The tubes were heated at 100° in a boiling water bath. At this temperature complete precipitation of zinc sulfide was achieved in about 1 h. The precipitates were coarse and easily manipulated.

The precipitates were centrifuged down, then washed 2 times with cold 0.1 *M* ammonium nitrate solution. The zinc sulfide was dissolved by adding 5 ml of concentrated hydrochloric acid and 2 ml of concentrated nitric acid and then heating in a boiling water bath until all oxides of nitrogen were expelled. Zinc was then determined as the pyrophosphate or as zinc tetrathiocyanatomercurate(II)[7].

Effect of thioacetamide–hydrazine hydrochloride ratio

In this study known amounts of zinc nitrate solution were buffered at pH 2 using a sulfate–bisulfate buffer and varying amounts of thioacetamide and hydrazine hydrochloride were added. These solutions were treated as described above in the general procedure and the amount of precipitated zinc sulfide was determined in each sample. The results are presented in Table I.

TABLE I

EFFECT OF THIOACETAMIDE–HYDRAZINE HYDROCHLORIDE RATIOS[a]

CH_3CSNH_2 (ml)	$N_2H_4 \cdot HCl$ (ml)	Zn taken (mg)	Zn recovered (mg)
5	0.5	30.00	26.87
5	0.5	30.00	28.43
5	7.5	30.00	29.72
5	7.5	30.00	29.55
5	12.0	30.00	29.56
5	12.0	30.00	29.51
5	1.0	50.00	45.51
5	1.0	50.00	46.68
5	3.0	50.00	48.89
5	3.0	50.00	49.03
5	5.0	50.00	49.50
5	5.0	50.00	49.94

[a] Sulfate–bisulfate buffer used.

These data indicate that optimum results are obtained with approximately equimolar quantities of thioacetamide and hydrazine hydrochloride. Incomplete precipitation occurs when an insufficient quantity of either reagent is present.

Effect of buffer system

Precipitations were carried out using a citric acid–citrate buffer at pH 2. The results are comparable to those obtained with sulfate–bisulfate buffers as indicated in Table III.

TABLE II

PRECIPITATION OF VARYING AMOUNTS OF ZINC[a]

CH_3CSNH_2 (ml)	$N_2H_4 \cdot HCl$ (ml)	Zn taken (mg)	Zn recovered (mg)
7.5	5.0	100.00	99.16
7.5	5.0	100.00	99.79
5	5.0	30.00	29.36
5	5.0	30.00	29.22
5	5.0	10.00	9.67
5	5.0	10.00	9.76

[a] Sulfate–bisulfate buffer used.

TABLE III

PRECIPITATION FROM CITRIC ACID–CITRATE BUFFER

CH_3CSNH_2 (ml)	$N_2H_4 \cdot HCl$ (ml)	Zn taken (mg)	Zn recovered (mg)
5	5.0	30.00	29.92
5	5.0	30.00	29.72
5	5.0	30.00	29.73

TABLE IV

SEPARATION FROM COBALT

Zn taken (mg)	CH_3CSNH_3 (ml)	$N_2H_4 \cdot HCl$ (ml)	Co(II) added (mg)	Wt. of Co copptd. (mg)	% of cobalt copptd.
Sulfate–bisulfate buffer					
30.00	5	5.0	10.3	2.38	23.10
30.00	5	5.0	10.3	3.09	29.98
30.00[a]	5	5.0	10.3	0.06	0.58
30.00[a]	5	5.0	10.3	0.05	0.48
30.00[a]	5	5.0	30.9	0.48	1.53
30.00[a]	5	5.0	30.9	0.21	0.70
100.00[a]	7.5	5.0	10.3	0.06	0.56
100.00[a]	7.5	5.0	10.3	0.09	0.87
Citrate–citric acid buffer					
30.00	5	5.0	10.3	0.12	1.14
30.00	5	5.0	10.3	0.11	1.10
30.00	5	5.0	30.9	0.27	0.90
30.00	5	5.0	30.9	0.45	1.44
100.00	7.5	5.0	10.3	0.07	0.72
100.00	7.5	5.0	10.3	0.09	0.86
30.00[a]	5	5.0	10.3	No precipitation of ZnS	
30.00[a]	5	5.0	10.3	No precipitation of ZnS	

[a] NH_4SCN added.

Separation from cobalt ion

In this study known amounts of cobalt ion in the form of cobalt nitrate and known activities of cobalt-60 tracer were added to each sample containing zinc ion. Zinc sulfide was precipitated both from sulfate–bisulfate and citrate–citric acid buffered solutions using the above procedure. The zinc sulfide precipitates were centrifuged down, washed, and the amount of coprecipitated cobalt was determined by gamma counting using a well-type scintillation counter.

A second series was run in the same manner, but 20 ml of a 20% solution of ammonium thiocyanate was added along with the other reagents. The results are given in Table IV. Ammonium thiocyanate effectively reduces the coprecipitation of cobalt sulfide in sulfate–bisulfate buffer systems. In citrate–citric acid buffers, however, no precipitation of zinc sulfide was obtained in the presence of ammonium thiocyanate. Optimum results were attained using a sulfate–bisulfate buffer in the presence of ammonium thiocyanate.

SUMMARY

Zinc sulfide can be precipitated quantitatively from solutions buffered at pH 2 using thioacetamide–hydrazine hydrochloride mixtures as precipitants. Optimum results are obtained using equimolar quantities of thioacetamide and hydrazine hydrochloride. Coprecipitation of cobalt sulfide was studied using citrate–citric acid, and sulfate–bisulfate buffers. Optimum separations are achieved using a sulfate–bisulfate buffer containing ammonium thiocyanate.

RÉSUMÉ

Le sulfure de zinc peut être précipité quantitativement en solutions tamponnées au pH 2, en utilisant des mélanges thioacétamide–chlorhydrate d'hydrazine. Les auteurs ont examiné la coprécipitation du sulfure de cobalt avec tampons citrate–acide citrique et sulfate–bisulfate; les meilleures séparations ont été obtenues avec tampon sulfate–bisulfate, en présence de thiocyanate d'ammonium.

ZUSAMMENFASSUNG

Zinksulfid lässt sich quantitativ aus gepufferten Lösungen beim pH-Wert 2 mit einer Mischung aus Thioacetamid und Hydraziniumchlorid fällen. Mit äquimolaren Mengen werden die besten Ergebnisse erzielt. Die Mitfällung von Kobaltsulfid wurde bei Anwendung von Citrat–Citronensäure- und Sulfat–Bisulfat-Puffern untersucht. Die besten Trennungen wurden mit einem Sulfat–Bisulfat-Puffer, der Ammoniumthiocyanat enthielt, erzielt.

REFERENCES

1 H. A. Fales and G. M. Ware, *J. Am. Chem. Soc.*, 41 (1919) 487.
2 C. E. P. Jeffereys and E. H. Swift, *J. Am. Chem. Soc.*, 54 (1932) 3219.
3 C. Mayr, *Z. Anal. Chem.*, 96 (1934) 273.
4 S. A. Coleman and G. B. L. Smith, *Anal. Chem.*, 13 (1941) 377.
5 R. B. Hahn and F. M. Shellington, *Anal. Chim. Acta*, 19 (1958) 238.
6 D. M. King and F. C. Anson, *Anal. Chem.*, 33 (1961) 572.
7 W. C. Vosburgh, G. Cooper, W. V. Clayton and H. Pfann, *Anal. Chem.*, 10 (1938) 393.

DETERMINATION OF SERUM LACTIC DEHYDROGENASE BY AN AUTOMATIC REACTION-RATE METHOD

T. P. HADJIIOANNOU AND P. L. SANTOS

Department of Chemistry, College of Pharmacy, University of Illinois, Chicago, Ill. (U.S.A.)

(Received February 13th, 1964)

The usefulness of lactic dehydrogenase determination in blood serum for the diagnosis of acute myocardial infarction has resulted in the development of various analytical methods. Lactic dehydrogenase* is an enzyme that catalyzes the reversible reaction:

$$\text{lactate} + \text{DPN} \overset{\text{LDH}}{\rightleftharpoons} \text{pyruvate} + \text{DPNH} + \text{H}^+ \qquad (1)$$

Ultraviolet spectrophotometric procedures for LDH measure the rate of oxidation[1,2] of DPNH or the rate of reduction[3,4] of DPN. DPNH has an absorbance peak at 340 mμ, whereas DPN does not absorb at this wavelength. Hence, the reaction rate is determined from the change in absorbance at 340 mμ. Colorimetric methods utilize either the decrease in pyruvate concentration as measured with 2,4-dinitrophenylhydrazine[5,6] or the color change upon reduction of oxidation–reduction dyes[7,8] by DPNH. One instrument which permits continuous measurement of the rate of enzyme-catalyzed reactions, and its application to LDH determination has been described[9,10].

To simplify and automate the LDH determination, a new method that provides a digital readout value within a few sec after starting the reaction has been developed. The method utilizes the type of automatic spectrophotometric reaction-rate system recently used for the determination of glucose[11], alcohol[12], and iodine[13]. Briefly, the operation of the automatic system is as follows. When the output voltage from the photoconductive circuit in the Sargent Spectro unit reaches a predetermined value the system starts a timer. The timer continues to operate until a second predetermined value of output voltage is reached, at which point it is stopped by the control system. The method is based on the reduction of DPN in the presence of lactate. The time required for reaction 1 to produce a small fixed amount of DPNH, and therefore for the absorbance to change by a preselected amount (about 0.06 unit) and consequently for the output voltage to change by 1 mV is measured automatically and related directly to the LDH concentration.

Speed, precision, and simplicity are distinctive advantages of the automatic

* The following abbreviations are used: DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; LDH, lactic dehydrogenase.

procedure. The serum is injected into the reaction cell, a composite reagent is added, DPN is injected to start the reaction, the start button is pressed, and the data read off a dial about 1 to 2 min after the start for normal sera or in a shorter time for abnormal sera. Automatic results obtained on samples containing 0.05 to 0.2 ml of serum show good proportionality and a coefficient of variation of about 1%.

GENERAL CONSIDERATIONS

There are two possible directions in which one may follow reaction 1. In the "backward" reaction—reduction of pyruvate to lactate—a 20-min waiting period is necessary to ensure that the endogenous serum keto and diketo acids will be enzymatically reduced[14] by DPNH. Since an uncertain amount of DPNH is expended in the endogenous reaction, the concentration of DPNH just before the addition of pyruvate may not be optimum and this may result in decrease of the reaction rate during the customary 4–6-min recording period. Moreover, the DPNH solution is not very stable and should be prepared fresh daily. In the "forward" reaction —oxidation of lactate to pyruvate—no waiting period is necessary, the DPN solution is stable for several weeks if kept frozen and linear rates are the almost invariable rule[3,14]. The "forward" reaction is used in the proposed method.

The DPN reagent is added to a solution containing serum sample and lactate reagent. In this way the zero adjustment on the Spectro unit can be made before starting the reaction. Any small change in initial absorbance, A_1 (before the injection of DPN), is compensated by adjusting the balance control in the Spectro unit. As soon as the DPN is added, the absorbance changes to a new value A_2. The instrument measures only the time t required for a small preset change in absorbance (A_4–A_3, equal to about 0.06) to occur during the early part of the reaction. It is only the absorbance change (A_4–A_3) and not the absolute values of A_3 and A_4 that are important for the measurement. The time required for the absorbance to reach the value A_3 (premeasurement time) is not measured and can vary from sample to sample. To calibrate the instrument, a control serum is used. After a simple calibration with 2 different dilutions of reconstituted serum of known LDH content, unknown sample values are read directly from a working curve. The working curve is obtained by plotting reciprocals of measurement times against conventional WROBLEWSKI–LADUE[1] LDH units.

After initiation of the reaction a minimum premeasurement time of 10 sec is desirable to ensure thorough mixing of the reagents. The premeasurement time can be controlled by appropriate setting of the comparator zero adjust. For example, the zero adjust was set at 5.80 to ensure that the zero adjust was in the range 5.25 to 5.40 *immediately* after the addition of DPN. Larger zero adjust settings for the same LDH sample result in longer premeasurement and measurement times, but they do not affect the accuracy and precision of the results. The zero adjust setting may be varied, but that decided upon must be duplicated from sample to sample.

The time required for a fixed change in absorbance depends not only on the LDH concentration but also on pH, temperature, and the concentrations of lactate and DPN. By running the reaction under controlled pH and temperature conditions and by making the concentrations of lactate and DPN sufficiently high, the rate of the reaction depends only on the LDH concentration.

The rate of the reaction has a large temperature coefficient and therefore the reaction takes place in a thermostatted cell at 40° ± 0.1°.

EXPERIMENTAL

Instrumentation

The basic instrumental components are the same as those used for determination of glucose except that a 100 K Helipot is substituted for the 5 K Helipot previously used[11].

Although DPNH has its absorption maximum at 340 mμ, a narrow transmittance band at 350 mμ is selected by a combination of filters because of the higher instrumental sensitivity for the tungsten source and photoconductive detector in the Spectro unit[12].

Reagents

All reagents are prepared in deionized water.

Buffer solution. 24.8 g of sodium pyrophosphate, 8.4 g of semicarbazide hydrochloride and 8 ml of lactic acid (86.9%; J. T. Baker, Phillipsburg, N. J.) are dissolved in 700 ml of water, the pH is adjusted to 9.0 with approximately 2 *N* sodium hydroxide, and the solution is diluted to the mark in a 1-l volumetric flask and refrigerated when not in use.

DPN solution. 30 mg of DPN (Sigma Chemical Co., St. Louis, Mo.) per ml of water. This solution is stored frozen.

Reconstituted serum. Enza-tiol (Dade Reagents, Inc., Miami, Florida) is reconstituted by adding water, divided into aliquots, and frozen. The LDH values can be varied according to the reconstitution volume. Aliquots may be kept frozen for several days[10].

The buffer solution is kept immersed in the water bath supplying the thermostatted cell when used. The serum is brought to the same temperature when analyzed.

Procedure

Preparation of equipment. Switch the Spectro-Electro titrator to the Spectro position about 2 h before the measurements are started to ensure good stability from the light source. Dial the 500 position on the filter wheel and place the Corning No. 5970 visible cutoff filter in the auxiliary holder; throw the polarity switch to position 1. A few min before the measurements are started, turn the comparator unit to *on* and the range selector switch to PNP.

Measurement step. Set the comparator Zero Adjust control at 5.80. Inject 0.200 ml of reconstituted control serum or sample into the reaction cell and pipet *quickly* 3.00 ml of buffer solution into the cell. Throw the comparator reagent selector switch to Position 1 to start the stirring and adjust the Spectro balance control so that the meter needle is at the center. Set the comparator Zero Adjust at 4.50, and 1 min after the start of the stirring, inject 0.100 ml of the DPN solution using a 0.1-ml Hamilton Microliter Syringe and press *at once* the start button on the Model Q-RR reaction-rate adapter. The analysis is completed automatically and the number on the readout dial is recorded. Empty the cell by inserting an aspirator tube and rinse with water. Repeat the procedure for each analysis.

Calculations. Working curves are made to read directly LDH units by plotting reciprocal of readout *vs.* the known LDH values of different dilutions of reconstituted serum.

RESULTS AND DISCUSSION

The relationship between the rate of formation of DPNH and the amount of LDH is linear up to at least 2000 LDH units. This has been demonstrated repeatedly with control sera of elevated LDH content. The linear relationship can be seen in Fig. 1 where a working curve prepared with different dilutions of reconstituted serum (Enza-tiol no. 211, 2000 LDH units) is shown. The curve passes through zero, confirming the absence of a blank. Since there is no blank, one standard is sufficient for the preparation of the calibration curve. The unknown LDH concentration C_u can also be calculated from equation 2:

$$C_u = C_s \frac{t_s}{t_u} \tag{2}$$

where C_s = LDH concentration of the standard (reconstituted serum), t_s = measurement time for the standard, t_u = measurement time for the unknown.

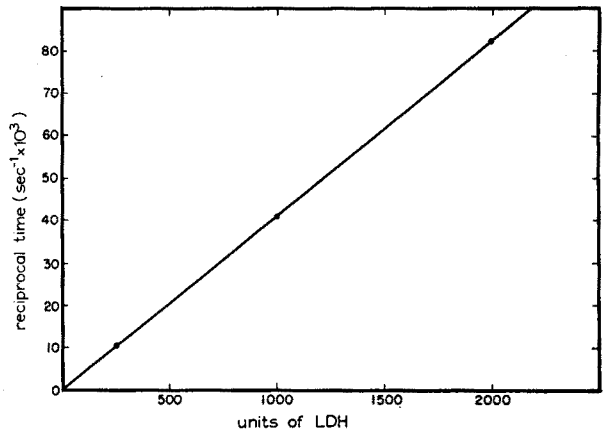


Fig. 1. LDH working curve prepared by quantitative dilution of reconstituted serum.

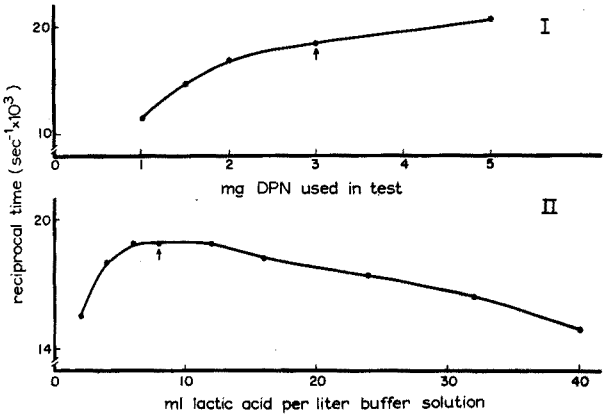


Fig. 2. Effect of variation of concentration of reactants on reaction rate in LDH assay. Conditions as under procedure. Arrows indicate concentration selected for the standard assay. I, variation of DPN; II, variation of lactate.

However, for better accuracy a working curve obtained with 2 different standards (usually in the range 250–1000 LDH units) prepared by different dilutions of the same reconstituted serum was always used in this work. Working curves should be rechecked every 2 h. If the measurement time for samples is smaller than 20 sec, for better accuracy such a sample should be appropriately diluted.

The data in Fig. 2 show the effect on the reaction rate of variation in DPN and lactic acid concentrations. For each study, all conditions were as described under procedure but the concentration of the component under study was varied. Reconstituted serum or pooled serum from different patients was used. Variation of the concentration of DPN or lactic acid has little effect on the reaction rate in the range chosen for the analysis.

Semicarbazide was used as a trapping agent for the pyruvate formed. Variation in semicarbazide concentration showed that the reaction rate was slightly increased when the concentration of the semicarbazide in the buffer was increased from zero to 0.02 *M*, but no further increase in rate was observed with concentrations up to 0.11 *M*. A 0.075 *M* semicarbazide concentration was chosen for the assay.

Figure 3 illustrates the variation of activity with pH for 2 pooled sera with all other conditions the same as described under procedure. The optimum lies in the range of pH 8.5 to 10.0. A pH of 9.0 was chosen for the assay.

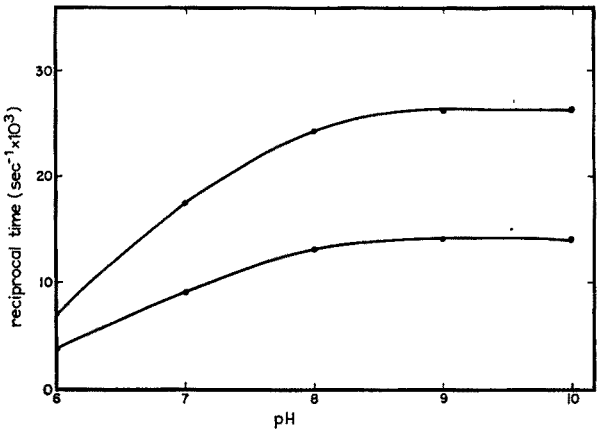


Fig. 3. Variation of enzyme activity with pH. Other conditions as under procedure.

The enzyme activities of several sera were determined at 30°, 34°, 37° and 40°. Arrhenius plots of log $1/t$ *vs.* reciprocal of absolute temperature were made. All samples gave straight lines over this temperature range, indicating that heat denaturation of the enzyme does not occur up to 40°. Higher temperatures were not tried because of possible enzyme denaturation[2]. A temperature of 40° was chosen to ensure shorter measurement times.

Table I shows results obtained for 7 control sera covering the range 200 to 1200 LDH units. Sample 8 was made from freshly obtained serum fortified with purified LDH from rabbit muscle; a mean value of 1800 was obtained from 16 individual ultraviolet and colorimetric values. There is good agreement—within ±3% or

TABLE I

RESULTS FOR LDH IN CONTROL SERA USING AUTOMATIC REACTION-RATE METHOD

Sample no.	*LDH units*	
	Reported	*Found*
1	815	845
2	408	432
3	438	435
4	1140	1120
5	1200	1165
6	775	805
7	215	230
8	1800	1840

TABLE II

COMPARISON OF AUTOMATIC AND SPECTROPHOTOMETRIC RESULTS FOR THE DETERMINATION OF SERUM LACTIC DEHYDROGENASE

Serum no.	*Units of lactic dehydrogenase activity*		
	Automatic (A)	*Spectrophotometric (S)*	*Difference (%)*[a]
1	676	650	+ 3.9
2	256	280	− 9.4
3	526	530	− 0.8
4	395	430	− 7.3
5	411	420	− 2.2
6	270	280	− 3.7
7	380	375	+ 1.3
8	668	650	+ 2.7
9	564	555	+ 1.6
10	589	560	+ 4.9
11	350	350	0
12	627	565	+ 9.9
13	6285[b]	6200	+ 1.4
14	513	530	− 1.4
15	595	580	+ 2.5
16	589	610	− 3.6
17	803	790	+ 1.6
18	311	325	− 5.5
19	356	405	+13.8
20	312	315	− 1.0

[a] ((A−S)/A) 100.
[b] This sample was analyzed after a 1:9 dilution.

30 LDH units, whichever is larger—between the results obtained automatically and the values reported by the manufacturer (Dade Reagents Inc., Miami, Florida).

LDH values for a number of normal and pathological sera obtained by the present method and by an independent ultraviolet method using the "backward reaction" are shown in Table II. The data in Table II are typical of about 60 serum samples that have been compared by the 2 methods. The agreement between the 2 methods is in accord with other studies that compare LDH values[10].

The accuracy of the automatic method depends on the stability of the dried

control serum before reconstitution and on how accurately the value of the control serum is known. To check the accuracy of the control sera, 4 control sera (Enza-trol no. 205, 208, 210 and 212) were analyzed at 25° by an independent ultraviolet method[2]. The results obtained agreed within ±4% with the reported values. This close agreement and the results on Table I show that the accuracy of the control sera is good enough to satisfy the clinician's demand for reliable standards.

TABLE III

REPRODUCIBILITY OF AUTOMATIC PROCEDURE

Sample no.	*Measurement time (sec)*
1	39.7
2	38.6
3	38.4
4	37.7
5	38.4
6	38.3
7	38.4
8	38.3
9	38.9
10	38.5
Average	38.5
Standard deviation	0.5

To check the reproducibility of the proposed procedure for serum LDH determination, a pooled serum with an activity of about 580 units was analyzed. The measurement times for 10 replicate LDH assays are tabulated in Table III; the reproducibility is 1.3%. Better precision is obtained when the light source is kept *on* continuously even when not in use. The high precision of the automatic method ensures more reliable results than existing spectrophotometric and colorimetric procedures.

The average total time per determination is about 3 to 4 min, and serum samples can be analyzed at a rate of 15 to 20 per hour.

We wish to express our thanks to Dr. M. RAFELSON for making fresh serum samples available and to Miss M. TURNER and Mr. G. TAKANO of Presbyterian–St. Luke's Hospital for technical assistance with the ultraviolet procedure.

This work was supported in part by the National Institutes of Health, Public Health Service, Research Grant GM 11039-01.

SUMMARY

An automatic spectrophotometric reaction-rate method is described for the determination of lactic dehydrogenase (LDH) in blood serum. The method is based on the oxidation of lactic acid in the presence of LDH and diphosphopyridine nucleotide (DPN) to form an absorbing species DPNH. The time required for a small fixed (about 0.06 unit) change in absorbance is measured automatically and related to the LDH concentration. Automatic results obtained on samples containing 0.05 to 0.2 ml of serum show good proportionality and a coefficient of variation of 1.3%. Serum samples (0.2 ml) are analyzed at a rate of 15 to 20 per hour.

RÉSUMÉ

Une méthode spectrophotométrique automatique est décrite pour le dosage de déhydrogénase lactique (LDH) dans le sérum sanguin. Elle est basée sur l'oxydation de l'acide lactique en pré-

sence de LDH et de diphosphopyridinenucléotide. On mesure automatiquement le temps nécessaire pour une petite variation déterminée de l'absorption (environ 0.06 unité), par rapport à la concentration en LDH. On peut ainsi analyser 15 à 20 échantillons de 0.2 ml de sérum en une heure.

ZUSAMMENFASSUNG

Es wird eine automatische spektralphotometrische Reaktionsgeschwindigkeitsmethode zur Bestimmung von Lactatdehydrogenase (LDH) in Blutserum beschrieben. Die Methode beruht auf der Oxydation von Milchsäure in Gegenwart von LDH und Diphosphopyridinnucleotid (DPN) um absorbierendes DPNH zu bilden. Die Zeit, die für einen kleinen bestimmten Betrag (*ca.* 0.06 Einheiten) im Wechsel der Extinktion benötigt wird, wird automatisch gemessen und auf die LDH-Konzentration bezogen. Automatische Ergebnisse, die mit 0.05–0.2 ml des Serums erhalten wurden, zeigen eine gute Proportionalität und einen Variationskoeffizienten von 1.3%. 15–20 Serumproben können in der Stunde analysiert werden.

REFERENCES

1 F. WROBLEWSKI AND J. S. LADUE, *Proc. Soc. Exptl. Biol. Med.*, 90 (1955) 210.
2 R. J. HENRY, N. CHIAMORI, O. J. GOLUB AND S. BERKMAN, *Am. J. Clin. Pathol.*, 34 (1960) 381.
3 E. AMADOR, L. E. DORFMAN AND W. E. C. WACKER, *Clin. Chem.*, 9 (1963) 391.
4 W. E. C. WACKER, D. D. ULMER AND B. L. VALLEE, *New Engl. J. Med.*, 255 (1956) 449.
5 P. G. CABAND AND F. WROBLEWSKI, *Am. J. Clin. Pathol.*, 30 (1958) 234.
6 L. BERGER AND D. BROIDA, *Tech. Bull. No. 500*, Sigma Chemical Company, 1960.
7 M. M. NACHLAS, S. I. MARGULIES, J. D. GOLDBERG AND A. M. SELIGMAN, *Anal. Biochem.*, 1 (1960) 317.
8 H. A. ELLS, *Clin. Chem.*, 7 (1961) 265.
9 W. J. BLAEDEL AND G. P. HICKS, *Anal. Chem.*, 34 (1962) 388.
10 W. J. BLAEDEL AND G. P. HICKS, *Anal. Biochem.*, 4 (1962) 476.
11 H. V. MALMSTADT AND S. I. HADJIIOANNOU, *Anal. Chem.*, 34 (1962) 452.
12 H. V. MALMSTADT AND T. P. HADJIIOANNOU, *Anal. Chem.*, 34 (1962) 455.
13 H. V. MALMSTADT AND T. P. HADJIIOANNOU, *Anal. Chem.*, 35 (1963) 2157.
14 R. E. THIERS AND B. L. VALLEE, *Ann. N. Y. Acad. Sci.*, 75 (1958) 214.

Short Communications

Dispositif adaptable sur une cuve pour les titrations spectrophotométriques

Nous avons cherché à mettre au point un dispositif nous permettant d'effectuer des titrations spectrophotométriques sans devoir modifier les spectrophotomètres eux-mêmes. Dans son livre traitant de ces méthodes de dosage HEADRIDGE[1] indique les modifications à apporter aux spectrophotomètres et aux cuves, mais ces modifications sont souvent complexes, onéreuses ou difficiles à mettre en oeuvre. Nous nous sommes efforcés de trouver un système assurant une parfaite étanchéité en partant de cuvettes cylindriques à faces parallèles en quartz. Dans ce but nous avons utilisé le principe d'une circulation du liquide en circuit fermé qui présente en outre l'avantage d'obtenir une bonne homogénéisation des solutions avant mesure des densités optiques.

Ce dispositif nécessite pour son adaptation une cuve à deux entrées (type commercial des cuves de 5 ou 10 cm ou cuves spécialement fabriquées avec deux ouvertures munies de rodage sur la paroi cylindrique). L'encombrement du système mis au point

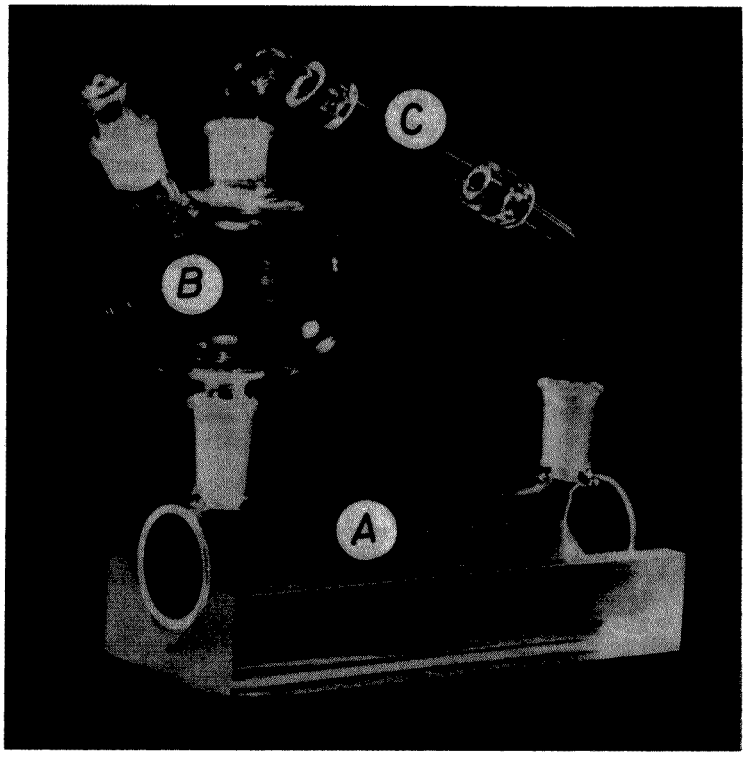


Fig. 1.

est tel qu'il permet de le loger dans le compartiment des cuves de la plupart des spectrophotomètres (Cary Modèle 11–50 et Zeiss PMQ 11 par exemple).

Le dispositif ainsi réalisé est représenté sur la Fig. 1*.

Il comporte une cuve de 10 cm d'épaisseur, de capacité 30 ml et une ampoule de capacité environ 30 ml. Le volume de l'ampoule peut être réduit ou augmenté selon la capacité de la cuve ou selon le volume total de réactif à ajouter.

La boucle C peut comporter un tube en plastique ou être entièrement en verre; dans ce dernier cas elle sera plus difficile à ajuster sur les rodages de la cuve par suite de sa rigidité.

La solution à titrer est introduite par l'ouverture ménagée à cet effet dans l'ampoule B; elle s'écoule ensuite dans la cuve A. Le réactif est ajouté en utilisant la même technique et l'on procède à un lent mouvement d'oscillation pendant lequel le liquide passe de la cuve A dans l'ampoule B en empruntant la boucle C. Après homogénéisation on fait rentrer à nouveau la solution dans la cuve et on répète ce processus 3 à 4 fois avant de faire la mesure colorimétrique. On procède à une nouvelle addition de réactif et on applique la même technique avant la mesure suivante.

Remarque

Une deuxième entrée peut être éventuellement prévue sur l'ampoule B afin de permettre l'introduction du réactif sous atmosphère inerte.

Nous remercions M. GEERTS pour l'exécution de la partie en verre du dispositif.

Euratom,
Bureau Central de Mesures Nucléaires,
Geel (Belgique)

Y. LE DUIGOU
A. BRÜCK

[1] J. B. HEADRIDGE, *Photometric Titrations*, Pergamon, Oxford, 1961.

(Reçu le 3 mars, 1964)

* La demande de brevet a été déposée.

Electrogravimetric determination of lead as lead dioxide

Prompted by a suggestion of LINGANE[1], we have carried out an electrogravimetric determination of lead by depositing it as lead dioxide which was then weighed under water to circumvent the necessity of drying.

Procedure

Known amounts of reagent-grade lead, in the neighborhood of 40 mg were dissolved in 15 ml of 6 *M* nitric acid. The solution was diluted to about 100 ml and one ml of 2 *M* sulfuric acid added to retard flaking of lead dioxide at the anode; 10 ml of 0.1 *M* copper sulfate was added to prevent deposition of lead at the cathode. The depositions

were performed at 90° for 20–30 min at 1 A with platinum gauze electrodes. At the end of the electrolysis the anode coated with lead dioxide was washed in distilled water and hung in a container of distilled water.

A platform was constructed to support this container above, and without touching, one pan of an analytical balance. The platinum wire used to hang the electrode in solution was marked so that on each successive weighing the liquid level was the same, *i.e.*, the electrode and wire were submerged to the same depth for reproducibility. The water temperature was measured and the weighing performed in the usual manner. The electrode was removed, cleaned in nitric acid–hydrogen peroxide mixture and reweighed. The difference in weights after a correction for buoyancy effects gave the weight of lead dioxide.

Calculation

Where W = true weight, W' = apparent weight, F = buoyant force, ϱ = density of water, $\eth$ = density of lead dioxide, and V = volume of lead dioxide = volume of water displaced, then obviously:

$$W = W' + F, \quad W = W' + \varrho V, \quad W = W' + \varrho W/\eth \quad W = W'/(1 - \varrho/\eth)$$

Results

The results of several tests are shown in Table I. Although these results are not quite as good as those reported by SCHRENK AND DELANO[2], there is a 3–5-fold saving in time, due to both shorter time of electrolysis and to circumventing the necessity of drying.

TABLE I

Pb taken (mg)	*Pb found (mg)*	*Deviation (mg)*
41.0	40.7	−0.3
43.8	44.0	+0.2
37.8	38.0	+0.2
39.8	39.6	−0.2
53.7	53.9	+0.2

Besides saving time this procedure also avoids undesirable changes in the composition of the deposits with the result that the theoretical gravimetric factor rather than the empirical factor commonly recommended may be used.

Chemistry Department,
Villanova University,
Pennsylvania (U.S.A.)

R. L. CARMAN III
J. J. MARKHAM

1 J. J. LINGANE, *Electroanalytical Chemistry*, Interscience, New York, 1958, p. 391.
2 W. T. SCHRENK AND P. H. DELANO, *Ind. Eng. Chem., Anal. Ed.*, 3 (1931) 27.

(Received March 31st, 1964)

Low-temperature differential thermal analysis

The bulk of existing differential thermal analysis (DTA) data and equipment refer to temperature ranges extending from ambient to the upper limits of the available furnaces; by comparison, DTA studies below ambient temperatures are rather few in number[1,2]. Thus, the present work is aimed at detailing a method for performing low-temperature DTA experiments and at presenting some representative results. Applications of this technique should prove useful in studying a wide variety of thermal phenomena at depressed temperatures including second-order or glass transitions in polymeric materials[3,4].

Experimental procedure

The refrigeration of the sample was achieved through use of a heat-transfer fluid of low freezing point in conjunction with a dry ice bath (−78.5°). Heptane[5] (b. p., 98.4°, f. p., −90.6°) was used. A schematic diagram of the cooling and heating assembly is shown in Fig. 1. The attendant electronic instrumentation and arrangement of chromel–alumel thermocouples have been detailed elsewhere[6]. Because of the reversal in sign of the e.m.f. from the sample temperature (T) sensing thermocouple below 0°, it was found expedient to incorporate a double-pole, double-throw switch at the X–Y recorder input for the T signal. In addition, a zero-shifter was used in series with the T signal in order to acquire complete and continuous breaks when these occurred near 0°.

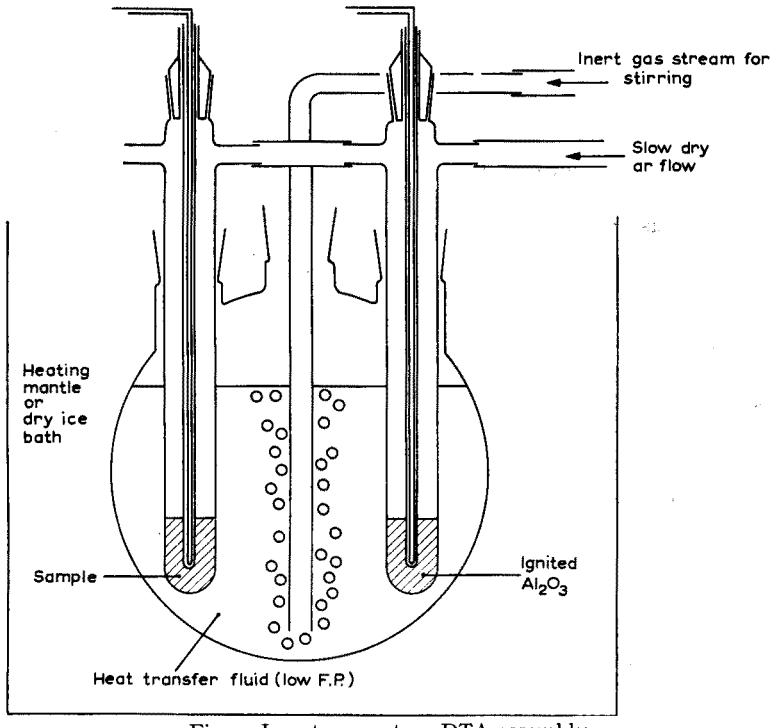


Fig. 1. Low temperature DTA assembly.

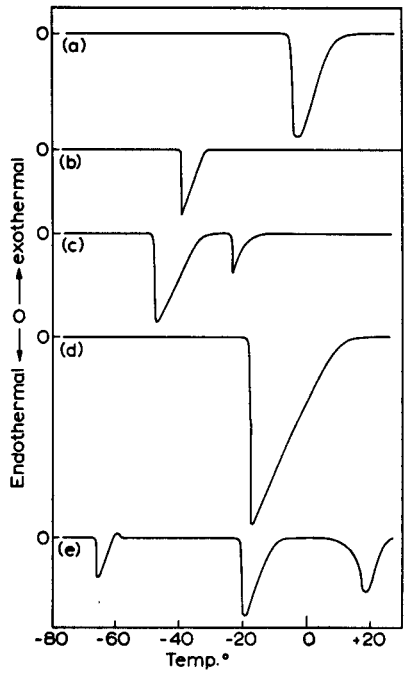


Fig. 2. Low-temperature DTA curves extending from −78°. (a) Br_2; (b) Hg; (c) CCl_4; (d) 17.5 mole % $NaNO_3$, 82.5 mole % H_2O; (e) 28.6 mole % LiCl, 71.4 mole % H_2O.

TABLE I

LOW-TEMPERATURE DTA RESULTS

Sample	*Phase reaction*	*DTA temperature (°)*	*Literature temperature (°)*
Br_2	Fusion	−6.8	−7.3 (ref. 8)
Hg	Fusion	−40.0	−38.9 (ref. 9)
CCl_4	Crystallographic transition	−47.0	−47.5 (ref. 10)
	Fusion	−23.8	−22.8 (ref. 11)
17.5% $NaNO_3$ 82.5% H_2O (mole %)	Eutectic fusion	−18.0	−17.5 (ref. 12)
28.6% LiCl 71.4% H_2O (mole %)	Hydrate transition[a] $LiCl \cdot 5\,H_2O \rightarrow LiCl \cdot 3\,H_2O + 2\,H_2O$	−68.0	−68 (ref. 13)
	Hydrate transition $LiCl \cdot 3\,H_2O \rightarrow LiCl \cdot 2\,H_2O + H_2O$	−20.5	−20 (ref. 13)
	Hydrate transition $LiCl \cdot 2\,H_2O \rightarrow LiCl \cdot H_2O + H_2O$	+18.8	+12.5 (ref. 13)

[a] The existence of $LiCl \cdot 5\,H_2O$ at this sample composition indicates a metastable state.

After a suitably depressed sample temperature had been reached, the entire unit of Fig. 1 was placed on an electric heating mantle to obtain the desired DTA heating curve. In the studies reported here a linear heating rate of 1.1°/min was secured with a motorized variable transformer[7]. The resultant DTA curves pertaining to various condensed-phase, invariant equilibria are presented in Fig. 2 and the numerical data are summarized in Table I.

Discussion of results

Inspection of Table I shows that the simple technique described is capable of yielding accurate and significant information relative to a broad range of low temperature processes accompanied by enthalpy changes.

Double compounds of the type $CCl_4 \cdot (1 \text{ or } 2)EtR$ have been reported in the systems CCl_4–Et_2O, CCl_4–EtOH, and CCl_4–EtOAc as gauged from freezing point determinations[14]; the corresponding transition temperatures for these addition compounds are reported as −48.2°, −47.6°, and −47.8°, respectively. However, without additional information concerning the nature of the solid phases in these systems it is possible that the low temperature crystallographic transition of carbon tetrachloride (−47.0°) might have been misinterpreted as being indicative of compound formation.

In general the cooling curves obtained with the samples each showed some degree of supercooling. Therefore, it is felt that the heating curves are more representative of the various equilibria. Because of the absence of stirring and the fairly rapid cooling rate (1.5°/min), the LiCl–H_2O sample appears not to have been equilibrated, so that the presence of some $LiCl \cdot 5H_2O$ is indicated by its transition temperature at −68°.

The use of heat-transfer fluids other than heptane can be used to extend the operating temperature range of the apparatus. In this regard, the mixed isomers of decahydronaphthalene (b. p., 192°; f. p., −124°)[5] appear particularly suitable.

Foote Mineral Company,
Research Center,
Exton, Pa., (U.S.A.)

MEYER M. MARKOWITZ
DANIEL A. BORYTA

1 W. J. SMOTHERS AND Y. CHIANG, *Differential Thermal Analysis. Theory and Practice*, Chemical Publishing Co., New York, 1958, p. 42, 294.
2 C. B. MURPHY, *Anal. Chem.*, 34 (1962) 298R.
3 H. MARK AND A. V. TOBOLSKY, *Physical Chemistry of High Polymeric Systems*, 2nd Ed., Interscience, New York, 1950, p. 345.
4 G. O. JONES, *Glass*, Methuen, London, 1956, p. 4, 46.
5 A. WEISSBERGER, E. S. PROSKAUER, J. A. RIDDICK AND E. E. TOOPS, JR., *Organic Solvents, Physical Properties and Methods of Purification*, Interscience, New York, 1955.
6 M. M. MARKOWITZ, *J. Phys. Chem.*, 62 (1958) 827.
7 M. M. MARKOWITZ, D. A. BORYTA AND G. CAPRIOLA, *J. Chem. Educ.*, 38 (1961) 96.
8 F. D. ROSSINI, D. D. WAGMAN, W. H. EVANS, S. LEVINE AND I. JAFFE, *Selected Values of Chemical Thermodynamic Properties*, National Bureau of Standards Circular 500, U. S. Government Printing Office, Washington, D. C., 1952.
9 V. N. KOSTRYNKOV AND P. G. STRELKOV, *Zh. Fiz. Khim.*, 28 (1954) 1825.
10 H. SACKMANN, *Z. Physik. Chem. (Leipzig)*, 204 (1955) 299.
11 A. K. DUNLOP, *J. Am. Chem. Soc.*, 77 (1955) 2016.
12 W. H. RODEBUSH, *J. Am. Chem. Soc.*, 40 (1918) 1204.
13 G. F. HUTTIG AND W. STEUDEMANN, *Z. Physik. Chem. (Leipzig)*, 126 (1927) 105.
14 W. F. WYATT, *Trans. Faraday Soc.*, 24 (1928) 429; 25 (1929) 43.

(Received March 2nd, 1964)

Spectrophotometric determination of catechols with 4-aminoantipyrine*

Phenols, oxidized with hydrogen peroxide or potassium ferricyanide in alkaline medium, give a color reaction with 4-aminopyrazoles[1,2]. This reaction with 4-aminoantipyrine (Emerson's reagent) is the basis of a sensitive and specific analytical method for phenols[3]. We have observed that when atmospheric oxygen acts as the oxidant, only catechols respond to the test. This permits the determination of catechols in the presence of other phenols.

Apparatus

Optical densities were determined with 1-cm cells in a Beckman DU spectrophotometer.

Reagent

Prepare daily a solution containing 100 mg of 4-aminoantipyrine (4-AAP), 10 ml of 20% sodium carbonate and 2.0 ml of 1 *N* sodium hydroxide, diluted to 100 ml with water.

Procedure

To 1 ml of sample containing 0–0.5 μmole of a catechol, add 3.0 ml of 4-AAP reagent. Mix thoroughly several times to ensure absorption of oxygen. After 20 min, read the optical density of the resulting red color at the wavelength of maximum absorbance.

Results

Under the conditions described most catechols form a red color exhibiting a maximum absorbance in the range 500–540 mμ (Fig. 1). No color is formed if the reaction mixture is de-oxygenated by passing a stream of nitrogen through the solutions before and during the addition of 4-AAP.

The relationship of optical density to concentration obeys Beer's law. Of 20 *o*-dihydroxybenzene compounds tested, 16 yield the red color while 4 give yellow-brown oxidation products similar to those noted when these compounds react with alkali in the absence of 4-AAP (Table I). The addition of 0.1 ml of concentrated hydrochloric acid to the red pigment produces a more intense, but transient, color absorbing at 550 mμ. Under controlled conditions this might serve as a more sensitive test for the presence of catechols.

Table II lists 63 compounds tested at a level of 0.5 μmole. None give the red color characteristic of catechols. Most give no color while a few give brown or yellow oxidation products. The presence of phenols which produce no color does not interfere in the determination of catechols (Table III). The yellow-brown color of most of the others can be discharged by adding 0.1 ml of concentrated hydrochloric acid.

4-Aminopyrazole and 4-amino-3,5-dimethylpyrazole react similarly with catechols. However, the use of these compounds presents no obvious advantage over the com-

* Issued as N.R.C. No. 8138.

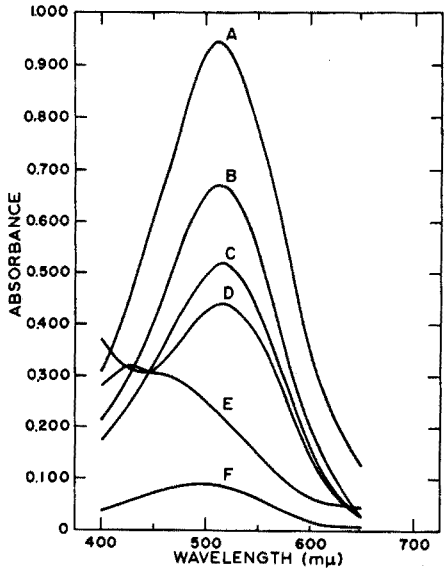


Fig. 1. Typical absorbance curves of catechols and 4-aminoantipyrine. One ml of water containing 0.5 μmole of catechol shaken with 3 ml of 4-AAP reagent. A, 3-methylcatechol; B, 3,4-dihydroxyphenylacetic acid; C, 3,4-dihydroxynorephedrine (nordefrin); D, noradrenalin; E, 3,4-dihydroxycinnamic acid; F, 3-*tert.*-butyl-5-methylcatechol.

TABLE I

o-DIHYDROXY COMPOUNDS

Compounds giving a red color with 4-AAP	*λmax*	$E^{M}_{1cm} \cdot 10^{-3}$
Catechol	515	7.2
3-Methylcatechol	515	7.5
4-Methylcatechol	505	5.8
4-*tert.*-Butylcatechol	515	2.1
3-*tert.*-Butyl-5-methylcatechol	500	0.7
5-*tert.*-Butyl-3-methylcatechol	520	1.0
4,5-Dichlorocatechol	540	2.2
2,3-Dihydroxybenzoic acid	520	1.4
3,4-Dihydroxybenzoic acid	515	2.5
3,4-Dihydroxyphenylacetic acid	515	5.4
3,4-Dihydroxyphenylpropionic acid	510	4.5
3,4-Dihydroxyphenylalanine (DOPA)	505	5.0
3,4-Dihydroxyphenylethylamine (dopamine)	500	6.8
3,4-Dihydroxynorephedrine (nordefrin)	515	4.2
Noradrenalin	520	3.5
DL-N-Isopropyl noradrenalin	510	2.5
Compounds that produce a yellow color		
2,3-Dihydroxybenzaldehyde		
2,3-Dihydroxyacetophenone		
3,4-Dihydroxyacetophenone		
3,4-Dihydroxycinnamic acid		

TABLE II

COMPOUNDS WHICH DO NOT PRODUCE A RED COMPOUND WITH 4-AMINOANTIPYRINE

Monohydroxy compounds		
Phenol	2,6-Dimethoxyphenol	4-Hydroxyphenylacetic acid
Cyclohexanol	2-Hydroxybenzoic acid	2-Hydroxycinnamic acid[a]
3-Bromophenol[a]	Cyclohexanol-2-carboxylic acid	3-Hydroxycinnamic acid
2-Aminophenol[b]	3-Hydroxybenzoic acid	4-Hydroxycinnamic acid[a]
3-Aminophenol	4-Hydroxybenzoic acid	Coumarin
2-Ethylphenol[a]	2-Hydroxybenzaldehyde[b]	3-Hydroxyphenylpropionic acid
3-Ethylphenol	3-Hydroxybenzaldehyde[a]	2-Hydroxyacetophenone[a]
4-Ethylphenol	4-Hydroxybenzaldehyde	3-Hydroxyacetophenone[a]
2,3-Dimethylphenol	2-Hydroxybenzyl alcohol[a]	Vanillin[b]
2,4-Dimethylphenol	4-Hydroxybenzyl alcohol	*o*-Vanillin
2,5-Dimethylphenol	2-Hydroxyphenylacetic acid	Vanillyl alcohol
3,4-Dimethylphenol	3-Hydroxyphenylacetic acid	DL-Tyrosine
3,5-Dimethylphenol		
m-*Dihydroxy compounds*		
Resorcinol	2,4-Dihydroxybenzoic acid	3,5-Dihydroxybenzoic acid
Orcinol	2,6-Dihydroxybenzoic acid	2,4-Dihydroxyacetophenone
p-*Dihydroxy compounds*		
2,5-Dihydroxybenzoic acid	2,5-Dihydroxyphenylacetic lactone[a]	2,5-Dihydroxybenzaldehyde[b]
		2,5-Dihydroxyacetophenone[a]
Trihydroxy compounds		
Pyrogallol[b]	Phloroglucinol	Phloroglucinol carboxylic acid
Miscellaneous		
2,3-Dimethoxybenzaldehyde	2,5-Dimethoxybenzaldehyde	5,6-Dihydroxy-2-methylindole
2,3-Dimethoxyphenylpropionic acid	2,5-Dimethoxycinnamic acid	2,3-Naphthalenediol
2,3-Dimethoxycinnamic acid	Quinic acid	Adrenolutin
3,4-Dimethoxycinnamic acid	5,6-Dihydroxy-N-methylindole	
p-Dimethoxybenzene		

[a] These compounds form a yellow or brown pigment in alkali which is discharged by adding acid.
[b] These compounds form a yellow or brown pigment in alkali which is not discharged by adding acid.

TABLE III

THE ABSORBANCE OF CATECHOLS ALONE AND IN THE PRESENCE OF PHENOLS

	Absorbance 515 mμ
0.25 μmole catechol	0.45
5.0 μmole phenol	0.00
0.25 μmole catechol + 5.0 μmole phenol	0.45
0.25 μmole 3,4-dihydroxyphenylacetic acid (3,4-DHP)	0.36
5.0 μmole 2-hydroxyphenylacetic acid (2-HP)	0.00
5.0 μmole 4-hydroxyphenylacetic acid (4-HP)	0.00
0.25 μmole 3,4-DHP + 5.0 μmole 2-HP	0.36
0.25 μmole 3,4-DHP + 5.0 μmole 4-HP	0.36

mercially available 4-aminoantipyrine. Preliminary experiments demonstrated that 1% 4-aminoantipyrine in 10% sodium carbonate forms colored compounds with catechols on paper chromatograms. A subsequent overspray with 1% ammonium persulfate or 1% potassium ferricyanide produces colors with other phenols.

Prairie Regional Laboratory,
National Research Council,
Saskatoon, Saskatchewan (Canada)

T. A. LaRue
E. R. Blakley

[1] G. T. Morgan and J. Reily, *J. Chem. Soc.*, 105 (1914) 435.
[2] E. Emerson, *J. Org. Chem.*, 8 (1943) 417.
[3] E. F. Tohler and L. N. Jacobs, *Anal. Chem.*, 29 (1957) 1369.

(Received January 7th, 1964)

Book Reviews

The Application of Mathematical Statistics to Chemical Analysis, by V. V. Nalimov, Translated by Prasenjit Basu, English translation editor: M. Williams, Pergamon Press, Oxford–London–Paris–Frankfurt, 1963, ix + 294 pp., price 84 s.

Mathematical statistics becomes more and more important for analytical chemists, and therefore the publication of the present book must be welcomed. After a short "Publisher's Note" and a Preface, chapter I deals with the problems of mathematical statistics in general and especially from the view of analytical chemists. The next chapter, entitled "Classification of errors of chemical analysis" is of an introductory nature, but chapter III, presenting the problems of random variables, gives a detailed discussion of the matter. Although distribution functions are mentioned here already, separate chapters deal with the most important ones, namely normal (chapter IV), Poisson and binomial distributions (chapter V). The next chapter has the title "Estimation of results of chemical analysis" and contains mainly comparison tests between results, means and variances, including the t and F functions. A short discussion of the Tschebyscheff inequality and its use is added here. A separate chapter deals with analysis of variance, indicating the growing importance of this matter. Statistics of linear relations together with regression analysis and correlation calculus are described relatively briefly, while regression analysis in the case of non-linear correlations is omitted completely. The last chapter is perhaps the best one of the book and presents some working methods connected with the statistical design of experiments. Research workers will profit particularly from this part of the book. The bibliography is presented in a valuable manner; full titles of textbooks and original papers are given, and short abstracts (or notes) are added to each title. The 172

references cover the field in which analytical chemists would be interested, although it is not quite clear why some of them have no separate numbers. There is also an appendix containing tabulated values of some important functions, and a subject index.

The mathematical standard of the book is relatively high; this allows a more exact treatment of the matter than is customary. It must be pointed out that from the very beginning of the discussions, numerical calculations are presented based on examples familiar to analytical chemists; this makes the problems easier to understand for the reader. The examples cover various classical and instrumental methods, with regard to both error calculation and experimental design.

Translation and translation editing can be difficult, but these, together with the layout of the book, are of a high standard. This new book can be recommended to all analytical chemists working in industry and research.

G. SVEHLA (Budapest)

Anal. Chim. Acta, 31 (1964) 403–404

M. ST. C. FLETT, *Characteristic Frequencies of Chemical Groups in the Infra-red*, Elsevier Publishing Company, Amsterdam–London–New York, 1963, ix + 98 pp., price 25 s; DM 14.—; D.fl. 12.50.

The wide application of infrared methods to qualitative and quantitative analysis is largely dependent on the ready availability of data on the frequencies and intensities of absorption bands. This book collects data for characteristic group frequencies in a convenient form. After a series of correlation charts there follow sections, arranged alphabetically, dealing with each functional group in turn; some sections are supplemented by a list of references of variable value as a key to further information. The brevity of the treatment excludes from the main text any discussion of factors likely to interfere with the correct assignment of bands and it is unfortunate in this respect that the cautionary comments in the Introduction cannot be displayed more prominently. As an adjunct to Bellamy's bible this book performs a useful function.

K. J. MORGAN (Birmingham)

Anal. Chim. Acta, 31 (1964) 404

Eastman Organic Chemicals around the world

Workers in the countries served by these distributors of Eastman Organic Chemicals can avoid the paperwork involved in importing. Some of these distributors maintain their own rather substantial stocks from which they can fill orders immediately. All can give you the benefit of the consolidated orders they place.

Among the 4000 Eastman Organic Chemicals these firms distribute are uncommon ones as well as uncommonly pure ones—organics that are often more economical to purchase than to make. These firms make it easier to purchase them. Get in touch with the one best located to serve you.

Distillation Products Industries
Eastman Organic Chemicals Department
Rochester 3, N. Y., U.S.A.

DPi

Distributors

AUSTRALIA

H. B. Selby & Co. Pty. Ltd.
35 York Street
Sydney, N.S.W.

H. B. Selby & Co. Pty. Ltd.
393 Swanston Street
P. O. Box 409G
Melbourne, C.1

H. B. Selby & Co. Pty. Ltd.
Box 606J, G.P.O.
Brisbane, Queensland

AUSTRIA

Heilmittelwerke Wien G.m.b.H.
III/40, Rennweg 12
Vienna

W. J. Rohrbecks Nachf.
Weihrgasse 18
Vienna V

BELGIUM

Anails, S. A.
14, Rue Dewez
Namur

DENMARK

H. Struers Chemiske Laboratorium
38, Skindergade
Copenhagen K

FINLAND

G. W. Berg & Co.
Fabiansgatan 14
Helsinki

Havulinna Oy
P. O. Box 468
Helsinki

FRANCE

Touzart & Matignon
3, Rue Amyot
Paris V°

Substantial stocks maintained

GERMANY

Serva-Entwicklungslabor
Romerstrasse 45
Heidelberg

GREAT BRITAIN

Kodak Limited
Kirkby
Liverpool

Substantial stocks maintained

HOLLAND

N. V. Holland Indonesia Agenturen Mij.
HIAM
139 De Ruyterkade
Amsterdam C

INDIA

The Andhra Scientific Co., Ltd.
4, Blacker's Road,
Mount Road
Madras

Raj-Der-Kar & Co.
Sadhana Rayon House
Dr. D. Naoroji Road
Bombay

INDONESIA

N. V. Chemicalienhandel Rathkamp and Co.
18 Djalan Madja Pahit
Djakarta

ITALY

Prodotti Gianni, s.r.l.
Via Mecenate, 30/14
Milano

JAPAN

Tokyo Tsusho Kaisha, Ltd.
New Kaijo Building
6, Marunouchi 1-chome,
Chiyoda-ku
Tokyo

NEW ZEALAND

Kempthorne, Prosser and Co's.
New Zealand Drug Co., Ltd.
P. O. Box 1495
Wellington, C.1

Kempthorne, Prosser and Co's.
New Zealand Drug Co., Ltd.
P. O. Box 319
Dunedin, C.1

NORWAY

Norlems Kemisk-Tekniske Aktieselskap
Tollbodgt. 32
Oslo

PORTUGAL

Soquimica, Sociedade de Representacoes de Quimica, Lda.
Rua dos Correeiros, 113-3°
Lisbon

SOUTH AFRICA

Kodak (South Africa) (Pty.) Limited
"Kodak House"
45 Shortmarket Street
Cape Town

SWEDEN

Rudolph Grave A/B
Pyramidvagen 9, Fack Solna 1
Stockholm 3

SWITZERLAND

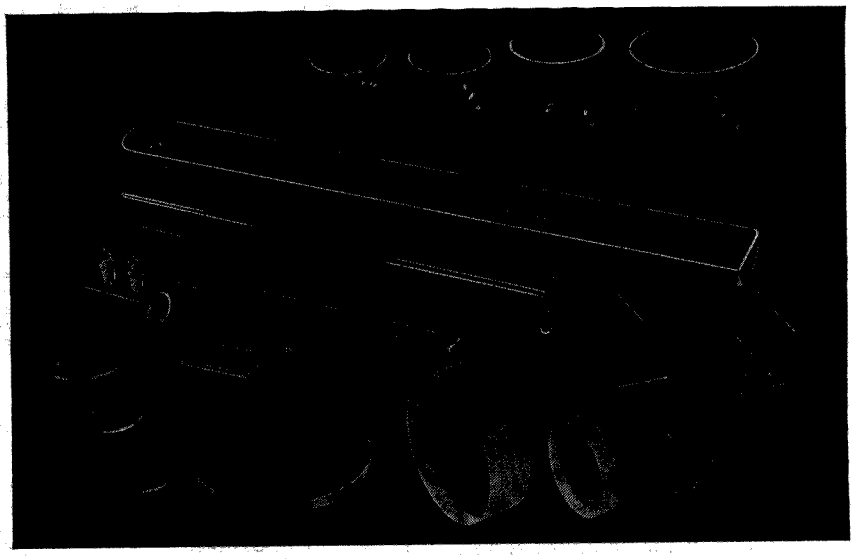
Dr. Bender & Dr. Hobein AG.
Riedtlistrasse 15a
Zurich 6

Distillation Products Industries *is a division of* **Eastman Kodak Company**

SPECTROSIL . . . *the purest fused silica*

REGD. TRADE MARK

SPECTROSIL, synthetic fused silica, is readily available as crucibles, basins, beakers and optical components; tubing is in a limited range of sizes. Synthetic fused silica extends the range of commercially available prism spectrophotometers down to 1850A. Total metallic impurities are less than 0.2 parts per million.



all BRITISH T S L ...the BEST

THERMAL SYNDICATE LTD.

P.O. BOX No. 6, WALLSEND, NORTHUMBERLAND. *Tel: Wallsend 62-3242/3*
LONDON: 9 BERKELEY STREET, W.1. *Tel: Hyde Park 1711/2*

519

STEROID CHROMATOGRAPHY

BY ROBERT NEHER

Pharmaceutical Research Department
CIBA Ltd., Basel, Switzerland

Chromatographic methods are today indispensable aids in the isolation, separation and determination of the various classes of steroids. In this English Edition of his highly successful "Chromatographie von Sterinen, Steroiden und verwandten Verbindungen" (Elsevier, 1958), the author has completely revised the original material and taken into account the most recently published results in the field. He provides a detailed exposition of the techniques best suited to the analysis of steroids, sterols and related compounds, describes apparatus specially adapted for this purpose, presents typical examples of applications, and discusses the relationship between chemical structure and chromatographic behaviour. Reference is also made to paper electrophoresis of ionized steroids and derivatives, in the development of which success has been recently noted.

CONTENTS

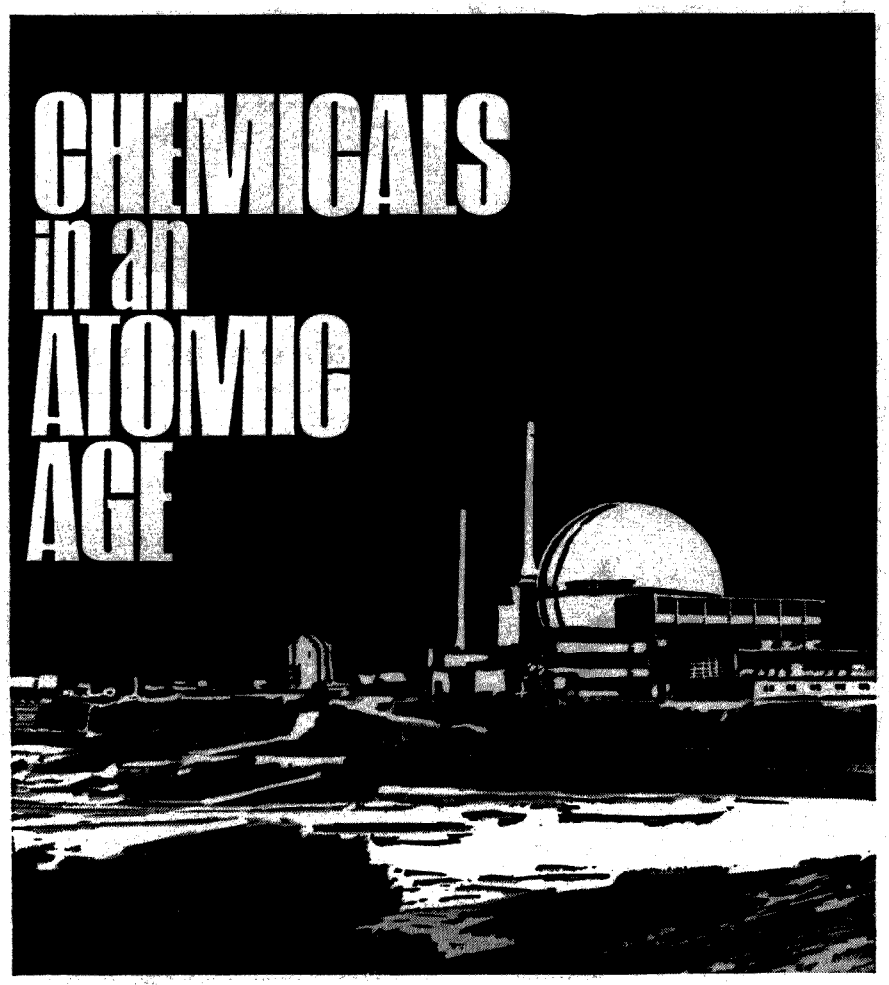
1. Introduction. 2. Nomenclature, Stereoisomerism, and Structural Formulas of Typical Steroids. 3. Chromatography on Columns: a. Adsorption Chromatography. b. Partition Chromatography. 4. Paper Chromatography (PC). 5. Thin Layer Chromatography (TLC). 6. Gas Chromatography (GC).

xiv + 389 pages, 78 tables, 31 illus., 920 refs., May 1964, 60s.

ELSEVIER PUBLISHING COMPANY

AMSTERDAM LONDON NEW YORK

592 E



B.D.H. make fine chemicals in bulk for today's new industries—the industries that represent the era of atomic energy, man-made fibres, plastics, advanced metallurgy, electronics and artificial satellites.

For the new processes of the new industries, and many new processes in older industries, B.D.H. produce by the ton chemical raw materials of the highest laboratory purity, and welcome opportunities of supplying more and more of them.

Equally B.D.H. are a major source of chemicals for all scientific purposes: analytical and micro-analytical reagents, biochemicals, indicators and stains, ion-exchangers and molecular sieves and thousands of other products reach the world's laboratories from B.D.H. at Poole.

B.D.H. Biochemicals **B.D.H. Laboratory Chemicals**
B.D.H. Fine Chemicals for Industry

BDH

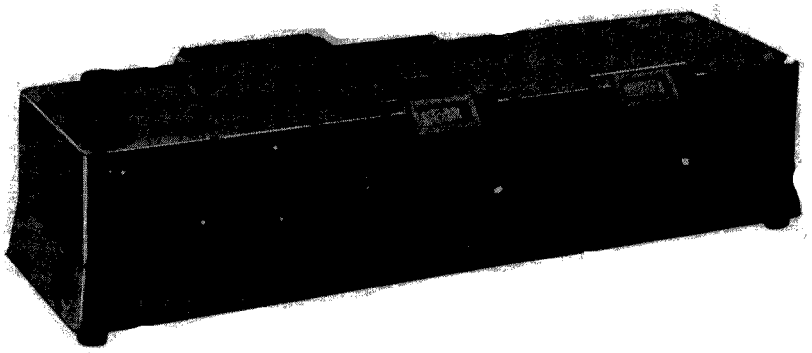
are all supplied by
THE BRITISH DRUG HOUSES LTD.
B.D.H. LABORATORY CHEMICALS DIVISION · POOLE · ENGLAND

SPECTROPHOTOMÈTRE

Jean et Constant

UV

220 à 800 Nanomètres



Instrument robuste, stable, fidèle, d'emploi sûr, pour dosages absorptiométriques précis en lumière monochromatique entre 200 et 800 nm (mμ).

- Monochromateur à prisme de quartz avec fente de largeur réglable.
- Lampe à incandescence et lampe à vapeurs de deutérium.
- Alimentation complète sur le secteur 110/220 V (deux possibilités).
- Appareil effectuant les mesures par compensation optique d'où son excellente stabilité.
- Graduation linéaire en densité optique. Mesures entre 0 et 2.

Notices détaillées et démonstrations sur simple demande.

PROLABO

12, Rue Pelée-PARIS 11e * Tél. : VOL. 11-90 et 41-19

CONTENTS

All rights reserved

ELSEVIER PUBLISHING COMPANY, AMSTERDAM
Printed in The Netherlands by
NEDERLANDSE BOEKDRUK INRICHTING N.V., 'S-HERTOGENBOSCH

RODD'S CHEMISTRY OF CARBON COMPOUNDS

Second Edition

edited by S. COFFEY, M.Sc. (London), D.Sc. (Leyden). Formerly of I.C.I. Dyestuffs Division, Blackley, Manchester. Assisted by an Advisory Board of prominent scientists.

More than 12 years have passed since the appearance of the first volume of CHEMISTRY OF CARBON COMPOUNDS. During this period great advances have been made in both theoretical and experimental organic chemistry, and these are being recorded in a completely new and revised edition.

In all important respects the second edition will be the same as the first, although some minor changes will be made. The large volume of new material will certainly cause an increase in total size and thus make it necessary to produce a greater number of sub-volumes. However, to reduce the delay between the appearance of these sub-volumes, each will be of smaller size than was the case for the first edition.

VOLUME IA - GENERAL INTRODUCTION, HYDROCARBONS, HALOGEN DERIVATIVES

General Introduction

1. The saturated or paraffin hydrocarbons. Alkanes. 2. Unsaturated acyclic hydrocarbons. 3. Halogen derivatives of the aliphatic hydrocarbons.

6 × 9″ xx + 569 pages subscription price £7.0.0.
non-subscription price £8.0.0.

VOLUME IB - MONOHYDRIC ALCOHOLS, THEIR ETHERS AND ESTERS, SULPHUR ANALOGUES; NITROGEN DERIVATIVES; ORGANOMETALLIC COMPOUNDS

4. Monohydric alcohols, their ethers and esters. 5. Sulphur analogues of the alcohols and their derivatives. 6. Nitrogen derivatives of the aliphatic hydrocarbons. 7. Aliphatic organometallic and organometalloidal compounds.

6 × 9″ xx + 277 pages + index, 16 tables; to appear late in 1964

VOLUME IC - ACYCLIC ALDEHYDES, KETONES AND MONOCARBOXYLIC ACIDS; CARBON MONOXIDE, ISOCYANIDES AND FULMINIC ACID; CARBONIC ACID AND ITS DERIVATIVES

VOLUME ID - DIHYDRIC ALCOHOLS, THEIR OXIDATION PRODUCTS AND DERIVATIVES

VOLUME IE - TRIHYDRIC ALCOHOLS, THEIR OXIDATION PRODUCTS AND DERIVATIVES

VOLUME IF - TETRA-, PENTA- AND HIGHER POLYHYDRIC ALCOHOLS, THEIR OXIDATION PRODUCTS AND DERIVATIVES; SACCHARIDES

VOLUME IG - ENZYMES; MACROMOLECULES; CUMULATIVE INDEX TO VOLUME I

To follow

Volume II Alicyclic Compounds
Volume III Aromatic Compounds
Volume IV Heterocyclic Compounds
Volume V Miscellaneous; General Index

Multi-volume works such as Rodd's Chemistry of Carbon Compounds represent a considerable outlay for the purchaser. In order to alleviate this to a certain extent, the publishers offer a discount of 15% on the publication price. Subscribers to the complete series will thus be able to acquire the work for only 26s. (approx.) per 100 pages.

ELSEVIER PUBLISHING COMPANY
AMSTERDAM LONDON NEW YORK