THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society, organised by the Microchemistry Group, was held at 7.15 p.m. on Friday, January 25th, 1957, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. K. A. Williams, A.Inst.P., M.Inst.Pet., F.R.I.C.

The subject of the meeting was "Micro-volumetric Analysis," and the following papers were presented and discussed: "Apparatus and Technique," by D. W. Wilson, M.Sc., F.R.I.C.; "Primary Standards," by R. Belcher, Ph.D., D.Sc., F.R.I.C. (presented on his behalf by J. H. Thompson, B.Sc., Ph.D., A.R.I.C.); "End-point Location," by E. Bishop, B.Sc., A.R.T.C., A.R.I.C.

NEW MEMBERS

ORDINARY MEMBERS

Thomas Edmund Alston, A.M.C.S.T.; Henry Reason Ambler, O.B.E., Ph.D. (Lond.), F.R.I.C.; John Granville Baber, B.Sc. (Vict.), A.R.I.C.; Desmond Goble Brown, M.Sc. (N.Z.); Florence Emma Calladine, B.Sc. (Lond.); Alfred Lorraine Cochrane, A.R.I.C.; William Douglas Duffield; Leslie Ernest Harrison, B.Sc. (Birm.), A.R.I.C.; Ronald Arthur Neale, B.Sc. (Lond.), A.R.I.C.

DEATHS

WE record with regret the deaths of

Nelson Trafalgar Foley
William Herbert Miles.

NORTH OF ENGLAND SECTION

AN Ordinary Meeting of the Section was held at 2.15 p.m. on Saturday, December 8th, 1956, at the City Laboratories, Mount Pleasant, Liverpool 3. The Chair was taken by the Chairman of the Section, Mr. J. R. Walmsley, A.M.C.T., F.R.I.C., F.P.S.

A lecture on "Some Applications of the Weisz Ring-oven" was given by W. I. Stephen, B.Sc., Ph.D., A.R.I.C.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Monday, December 10th, 1956, at the Department of Forensic Medicine, University of Glasgow. The Chair was taken by the Chairman of the Section, Dr. F. J. Elliott, M.Sc., F.R.I.C., F.R.S.E.

A lecture on "Problems and Techniques in Forensic Analysis" was given by Edgar Rentoul, M.B., Ch.B., LL.B.

WESTERN SECTION

THE second Annual General Meeting of the Section was held at 12 noon on Saturday, December 15th, 1956, at the Royal Albert Grill, Newport, Mon. The Chairman of the Section, Mr. P. J. C. Haywood, B.Sc., F.R.I.C., presided. The following appointments

were made for the ensuing year:—*Chairman*—Mr. P. J. C. Haywood. *Vice-Chairman*—Mr. S. Dixon. *Honorary Secretary and Treasurer*—Dr. G. V. James, Western Counties Laboratory, 45 Colston Street, Bristol, 1. *Members of Committee*—Messrs. R. G. H. Boddy, H. J. Evans, R. S. Morris, A. Pickard, G. F. Price and R. Stephens. Mr. R. E. Coulson and Dr. Z. Hybs were appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section and the following paper was presented and discussed: "The Co-ordination of Analytical Techniques in Industrial Research."

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, December 11th, 1956, in the Gas Showrooms, Nottingham. The Chair was taken by the Chairman of the Section, Mr. J. R. Leech, J.P.

The following paper was presented and discussed: "Aspects of the Application of Chromatography to the Quantitative Analysis of Inorganic Substances," by F. H. Pollard, B.Sc., Ph.d.

AN Ordinary Meeting of the Section was held at 7 p.m. on Thursday, January 10th, 1957, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Vice-Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F.

The following paper was presented and discussed: "The Analytical Chemistry of Some Newer Insecticides and Herbicides," by K. Gardner, B.Sc., F.R.I.C.

MICROCHEMISTRY GROUP

THE eighth London Discussion Meeting of the Microchemistry Group was held at 6.30 p.m. on Wednesday, January 9th, 1957, in the restaurant room of "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Chairman of the Group, Dr. G. F. Hodsman, A.Inst.P.

This was a review meeting, and an informal discussion took place on each of the subjects covered in previous meetings.

PHYSICAL METHODS GROUP

THE twelfth Annual General Meeting of the Group was held at 6.30 p.m. on Wednesday, November 28th, 1956, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Dr. J. E. Page, F.R.I.C. The following appointments were made for the ensuing year:—*Chairman*—Dr. J. E. Page. *Vice-Chairman*—Mr. R. A. C. Isbell. *Hon. Secretary and Treasurer*—Mr. L. Brealey, Boots Pure Drug Co. Ltd., Standards Department, Station Street, Nottingham. *Members of Committee*—Dr. Bella B. Bauminger, Messrs. R. A. Chalmers, H. J. Cluley, A. G. Jones, H. Liebmann and G. W. C. Milner. Dr. D. C. Garratt and Mr. C. A. Bassett were re-appointed as Hon. Auditors.

The Annual General Meeting was followed at 6.50 p.m. by an Ordinary Meeting of the Group. A lecture on "Optical Rotations in the Study of Organic Structures" was given by W. Klyne, M.A., B.Sc., Ph.D. This was followed by a brief description and demonstration of a prototype model of the Bellingham and Stanley Photo-electric Polarimeter.

The Determination, by Radioactivation, of Small Quantities of Nickel, Cobalt and Copper in Rocks, Marine Sediments and Meteorites*

By A. A. SMALES, D. MAPPER and A. J. WOOD

Methods are described for the determination of microgram or smaller quantities of nickel, cobalt and copper; these methods involve neutron activation of the samples in the Harwell Pile, followed by radiochemical separation of the individual elements using carriers and, finally, comparison of the radioactivity due to these elements from the samples with that from known amounts of the elements that were irradiated simultaneously. The accuracy of the method has been checked for each element by analysing standard samples of steels, and the method can, in fact, be very useful for steel analysis when the levels of the three elements are lower than is convenient for existing methods.

The results for terrestrial and oceanic rocks, marine sediments and meteorites are briefly examined with a view to the information they can give on the possibility of meteoritic origin of the nickel in marine sediments. Results are also quoted for the standard granite G1 and diabase W1, for some other rocks, commercial pure irons and, finally, for seaweeds.

In a recent communication Smales and Wiseman[1] discussed the significance of the nickel to cobalt, nickel to copper and copper to cobalt ratios determined in a number of samples of deep-sea sediments, oceanic rocks and meteorites as a means of yielding information on Pettersson and Rotschi's suggestion[2] of the possible meteoritic origin of the nickel found in Pacific deep-sea cores. It was apparent that the method of radioactivation analysis was very suitable for the simultaneous determination of nickel, cobalt and copper, especially as methods for these elements had already been developed in this laboratory. As was stated by Smales,[3] "the particular advantage of the radioactivation method in this case was its sensitivity, which made it possible to determine all three elements at the level of a few parts per million and upwards with adequate precision on no more than a few milligrams of sample —the sample being particularly limited in the case of the marine sediments." In all, 73 samples, including 5 igneous rocks and 3 manganese nodules were analysed for nickel, cobalt and copper, and in addition the cobalt contents of seven samples of seaweeds were determined. A number of standard steels was also analysed with a view to giving information on the accuracy of the method. In this paper the analytical methods used, and the detailed results obtained, are described.

A number of other rock samples, from the Skaergaard intrusion, East Greenland, was also analysed, and the results, which are of more geological interest in showing the distribution of the three elements in rocks formed at different stages in the crystallisation from the original magma, will be published in a separate paper.

The principles, methods and limitations of the radioactivation method have been fully discussed by numerous workers[4,5,6,7,8] and will not be dealt with here.

Nuclear data—

The nuclear characteristics of importance in this work for cobalt, nickel and copper are shown in Table I.

Because of their short half-lives, 11-minute ^{60m}Co and 5·2-minute ^{66}Cu were not used for determinations of cobalt and copper.

The fact that the half-lives of ^{65}Ni, ^{64}Cu and ^{60}Co are 2·56 hours, 12·8 hours and 5·2 years, respectively, made it possible to deal adequately with the successive radiochemical separations in that order. After the separation of nickel and copper from cobalt, the cobalt can be determined at any convenient time. The nickel separation must be done as soon after the irradiation as possible, because of the comparatively short half-life of ^{65}Ni and the already

* Presented at the XVth International Congress on Pure and Applied Chemistry (Analytical Chemistry), Lisbon, September 8th to 16th, 1956.

rather poor sensitivity, for radioactivation, for that element. Much more latitude in respect of speed is available with copper.

TABLE I

NUCLEAR DATA FOR COBALT, NICKEL AND COPPER

Target nuclide	Abundance in natural element, %	Isotopic activation cross-section, barns	Product on neutron irradiation	Radiation and energy, MeV	Half-life
^{59}Co	100	14	^{60m}Co	β^- 1·5	11 minutes
		20	^{60}Co	β^- 0·32 γ 1·17, 1·33	5·2 years
^{64}Ni	1	3·0	^{65}Ni	β^- 2·1 γ	2·56 hours
^{63}Cu	69	4·3	^{64}Cu	β^+ 0·66 β^- 0·57 γ	12·8 hours
^{65}Cu	31	2·1	^{66}Cu	β^- 2·6 γ	5·2 minutes

In Table II are shown the activities due to each radionuclide after irradiations of 1 μg of each element for 15 hours, 2 days and 5 days, respectively, in a flux of 10^{12} neutrons per sq. cm per second.

TABLE II

ACTIVITIES FOR 1 μg OF ^{65}Ni, ^{64}Cu AND ^{60}Co AFTER VARIOUS IRRADIATION TIMES

Irradiation time	Activity of ^{65}Ni, disintegrations per minute	Activity of ^{64}Cu, disintegrations per minute	Activity of ^{60}Co, disintegrations per minute
15 hours	$1·7 \times 10^4$	$9·2 \times 10^5$	$2·7 \times 10^3$
2 days	$1·7 \times 10^4$	$1·6 \times 10^6$	$8·5 \times 10^3$
5 days	$1·7 \times 10^4$	$1·7 \times 10^6$	$2·2 \times 10^4$

For most of the samples dealt with in this paper, a 15-hour irradiation was adequate, but sometimes, when this gave inadequate sensitivity for cobalt, longer times were necessary. Working sensitivity limits that allow reasonable time for the radiochemical separation of nickel (2 to 3 half-lives) and for sample irradiation for cobalt (up to a week), can be put at 10^{-7} g for nickel, 10^{-8} g for cobalt and 10^{-10} g for copper.

EXPERIMENTAL

IRRADIATION—

The samples were sealed in short lengths of polythene tubing, which were packed, together with the standards, in a 3-inch × 1-inch aluminium can and irradiated in the "self-serve" position in the Harwell Pile (except for some of the cobalt determinations in steels, when the thermal column was used, see p. 88).

STANDARDS—

For nickel and copper, 10-mg strips of the "pure" and AnalaR metal foils, respectively, were used as standards, but for cobalt a mild steel, sample "A," was used containing 0·016 per cent. of cobalt, accurately determined by neutron activation by standardisation against a variety of standard cobalt-containing materials (see Table III), and also by an absorptiometric method.[9] It was considered advisable from the aspects of safety, disposal and contamination to avoid unduly large quantities of 5·2-year ^{60}Co.

The nickel and copper were dissolved after irradiation, and suitable aliquots at the 1 to 100-μg level were taken. As 50 mg of the steel sample contained about 10 μg of cobalt, it was unnecessary to take an aliquot. All duplicate standards were irradiated and checked against each other. As relatively very large quantities of the elements to be determined were used as standards, it was essential to avoid any possibility of cross-contamination of the samples, and all the chemical operations were performed with this consideration in mind.

Neutron self-shielding was unimportant, *i.e.*, it caused errors of less than 5 per cent., in this work, as would be expected for nickel and copper from a consideration of the total

neutron cross-sections concerned,[10] although it was also proved experimentally for the standards used for all three elements, by measuring the specific activities produced by irradiation of 10 to 20-mg quantities of the solid standards simultaneously with aqueous solutions containing only microgram amounts of the same elements. The samples had, as major constituents, elements of low neutron-absorption cross-section, such as calcium, aluminium and iron, and therefore self-shielding was unimportant for them also.

TABLE III

COBALT CONTENT OF THE MILD STEEL "A"

Standard used	Amount of standard irradiated	Approximate mass of cobalt in the standard	Sample weight, mg	Cobalt content of steel "A," p.p.m.
Cobalt anthranilate	70 mg	10 mg	100	163, 172
	50 mg	7 mg	30	151, 167
B.C.S. steel No. 233 (23·7% of Co) ..	20 mg	5 mg	1000	156
			500	151, 156, 161
	100 mg	25 mg	100	165, 175
B.C.S. steel No. 241 (5·84% of Co) ..	100 mg	6 mg	100	152, 162
Cobalt sulphate solution	0·1 ml	5 μg	30	158, 142
			Average cobalt content =	159 ± 8

DISSOLUTION OF SAMPLES—

Between 10 and 100 mg of the samples were used for the determinations, depending primarily on the quantity of sample available, and after irradiation the samples were dissolved, after addition of carriers, in suitable acid mixtures in glass beakers. Most of the samples, such as the sediments, dissolved readily in hydrochloric acid, but for the meteorites and some of the rocks it was necessary to use a mixture of hydrochloric, nitric and perchloric acids, and a few drops of hydrofluoric acid to obtain complete solution. The excess of hydrofluoric acid was removed by boiling and the solution was evaporated to fumes of perchloric acid.

OUTLINE OF THE RADIOCHEMICAL SEPARATIONS—

The nickel was first precipitated from the ammoniacal solution as the dimethylglyoxime complex, the copper and cobalt remaining in solution. The copper was then separated as the copper thionalide, the cobalt remaining in solution for later determination. The nickel and copper compounds were dissolved separately and, after radiochemical purification, the metals were precipitated as the nickel - dimethylglyoxime complex and cuprous thiocyanate, in which forms they were counted. The cobalt was later purified, re-precipitated as potassium cobaltinitrite and counted. The details of the chemistry involved are described later, but it might be mentioned here that, with most of the samples handled, the initial level of radioactivity was sufficient to necessitate shielding precautions being taken by the operators.

MEASUREMENT OF RADIOACTIVITY—

As carriers, 10 mg of nickel, 20 mg of copper and 10 mg of cobalt in solution form were used, and the final yields, determined on the precipitates prepared for end-window beta counting, were usually about 50 to 90 per cent. The maximum β-energy of ^{65}Ni is 2·1 MeV, so that no correction for self-absorption was necessary. Radiochemical purity was confirmed by plotting decay curves, automatic equipment now available being used. Similarly the copper, as cuprous thiocyanate, was checked for radiochemical purity by plotting a decay curve and by measuring the maximum beta-particle energy. A correction for self-absorption is necessary in this case and values for a correction curve were obtained in the conventional manner by precipitating different amounts of cuprous thiocyanate with a fixed amount of radioactive copper. A further test on the final purified copper precipitate was provided by the gamma-ray spectrometer, ^{64}Cu being identified by the peak at 0·51 MeV due to the annihilation radiation produced by the 0·65-MeV positron (β^+) emission. For cobalt, however, it was generally possible to check the radiochemical purity only by measurement of the maximum β-energy of 0·32 MeV, although with steel samples the gamma-ray spectrometer

was also used for measuring the 1·17 and 1·33-MeV gamma-ray emissions of ^{60}Co. As with copper, it was necessary to prepare a self-absorption correction curve when counting beta particles from ^{60}Co.

METHOD

PROCEDURE—

After irradiation, transfer the samples to 150-ml beakers and add 10·0 mg of nickel in the form of nickel nitrate solution, 20·0 mg of copper as copper sulphate solution, 10·0 mg of cobalt as cobalt nitrate solution and 5 ml of hydrochloric acid, sp.gr. 1·18. Heat gently on a hot-plate until the solids have dissolved. As mentioned earlier, this treatment is adequate for the dissolution of the marine sediments, but for meteorites and rocks more drastic methods are required. To these samples add 5 ml of nitric acid, sp.gr. 1·42, and 10 ml of perchloric acid, sp.gr. 1·70, and evaporate to fumes of perchloric acid. Allow to cool, and then add a further 5 ml of nitric acid and 10 to 20 drops of 40 per cent. w/w hydrofluoric acid, evaporate to fumes of perchloric acid, cool, and add 5 ml of aqua regia, and again evaporate to fumes of perchloric acid; if necessary, repeat this treatment should any insoluble residue remain at this stage.

Allow the solution to cool and then dilute it to 80 ml with water; add 100 mg of ferric iron, as ferric ammonium sulphate solution, 10 ml of a 10 per cent. solution of sodium nitrate, 5 ml of 40 per cent. w/v solution of ammonium citrate and ammonium hydroxide in slight excess. Precipitate the nickel by adding slowly 10 ml of a 1 per cent. w/v solution of dimethylglyoxime in methanol, and collect the precipitate on a Whatman No. 541 filter-paper. The nickel precipitate is dealt with as described below. To the filtrate add dilute nitric acid until the solution is just acid, and then heat to 80° C and add 10 ml of a freshly prepared 1 per cent. solution of thionalide in methanol. Place the beaker on a hot-plate, stirring until coagulation of the precipitate is complete, and then collect it on a Whatman No. 541 filter-paper. The copper precipitate and the filtrate containing cobalt are treated as described below.

Procedure for nickel—Dissolve the precipitate of the nickel - dimethylglyoxime complex by the dropwise addition of 5 ml of hydrochloric acid, sp.gr. 1·18, and to the solution add 1 mg of copper, in solution form, and 1 ml of nitric acid, sp.gr. 1·42, heating gently on the hot-plate. After cooling the solution, just neutralise it with ammonium hydroxide solution, re-acidify with hydrochloric acid, add 2 ml of 10 per cent. sodium nitrate solution and precipitate the copper with 2 ml of the thionalide solution, as described above, removing the precipitate by means of a 9-cm Whatman No. 541 filter-paper. To the filtrate add 5 mg of bismuth in the form of bismuth nitrate solution and then a slight excess of ammonium hydroxide, and remove the precipitate by means of a Whatman No. 541 filter-paper, again retaining the filtrate. Now add 5 ml of 1 per cent. dimethylglyoxime solution in methanol and precipitate the nickel as described previously. Filter off the precipitate, wash it well and dissolve it in hydrochloric acid. To the solution add 1 ml of nitric acid, sp.gr. 1·42, and boil it gently on a hot-plate. Add a further 10 mg of copper as nitrate or chloride solution and add ammonium hydroxide until the solution is just acid. Then add 2 ml of a saturated solution of sodium sulphite, followed by 2 ml of a 10 per cent. aqueous potassium thiocyanate solution. Filter off the precipitate of cuprous thiocyanate and discard it, but retain the filtrate. After making the filtrate just ammoniacal, add 5 ml of the 1 per cent. dimethylglyoxime solution and filter off the nickel precipitate. Re-dissolve the precipitate in hydrochloric acid and to the solution add 10 mg of manganese, as manganous chloride solution, solutions of calcium, strontium and barium carriers equivalent to 2 mg of each element, 2 mg of ferric iron, 2 ml of nitric acid and finally 5 ml of saturated bromine water, and boil the solution until the excess of bromine is removed. Add ammonium hydroxide in slight excess, and then 5 ml of a 20 per cent. w/v aqueous solution of ammonium carbonate, boil and, using a 9-cm Whatman No. 40 filter-paper, remove the precipitate and retain the filtrate only. Finally, to the filtrate add 5 ml of the dimethylglyoxime solution, spin in a centrifuge, and wash the precipitate thoroughly twice with hot water, discarding the washings. Then add a few drops of ethanol and transfer the precipitate to a weighed aluminium counting tray; dry it under an infra-red lamp and, when it has cooled, determine the chemical yield by weighing the nickel - dimethylglyoxime complex.

For the standards dissolve the irradiated nickel foil in nitric acid solution, take a suitable aliquot (containing about 100 μg of nickel), add 10·0 mg of nickel as nickel nitrate solution and proceed as described for the samples.

Procedure for copper—Wash the precipitate of copper thionalide into a 150-ml beaker, using the minimum amount of water. Add 10 ml of nitric acid, sp.gr. 1·42, and 3 drops of sulphuric acid, sp.gr. 1·84, and boil on a hot-plate until nitrous fumes are no longer evolved. Cool the solution, add 20 ml of perchloric acid, sp.gr. 1·7, and heat until the perchloric acid begins to fume strongly. After cooling, dilute with water to 40 ml and add ammonium hydroxide until the solution just turns blue. Add sufficient hydrochloric acid to discharge the blue colour and warm the solution on a hot-plate before adding 2 ml of saturated sodium sulphite solution and 2 ml of 10 per cent. potassium thiocyanate solution. Spin in a centrifuge, and discard the supernatant liquid. Dissolve the cuprous thiocyanate in 2 to 3 ml of nitric acid, sp.gr. 1·42, and heat until nitrous fumes cease to be evolved. Then transfer the solution to a 50-ml beaker, add 10 mg of ferric iron and 10 mg of manganese, in solution, and 5 ml of saturated bromine water, and then boil until the excess of bromine is removed. Now make the cooled solution slightly ammoniacal, boil and filter through a Whatman No. 541 filter-paper, retaining the blue filtrate. Discharge the blue colour by adding dilute hydrochloric acid and from the slightly acid solution re-precipitate the cuprous thiocyanate as before. Spin in a centrifuge, washing the precipitate twice with water, very thoroughly. Then, by slurrying the precipitate with a little water, transfer it to a weighed aluminium counting tray, dry it under an infra-red lamp and determine the chemical yield by weighing the cuprous thiocyanate.

For the standards, dissolve the foil in nitric acid, take a suitable aliquot, add 20·0 mg of copper, in solution, and precipitate the cuprous thiocyanate as for the last stage of the sample treatment.

Procedure for cobalt—To the filtrate from the copper thionalide precipitation add 20 ml of nitric acid, sp.gr. 1·42, and evaporate to dryness. Then add 10 ml of sulphuric acid, sp.gr. 1·84, and evaporate to fumes of sulphuric acid. Add further quantities of nitric acid and heat strongly to destroy completely all the organic matter. After cooling the solution, dilute it to 250 ml with water, add a suspension of zinc oxide in water until precipitation occurs and then a slight excess, and set aside for 10 minutes before removing the precipitate by means of a 15-cm Whatman No. 541 filter-paper. Heat the filtrate, and add dropwise 5 ml of a 10 per cent. solution of 1-nitroso-2-naphthol in glacial acetic acid, and boil for 2 minutes. Collect the precipitate on an 11-cm Whatman No. 31 filter-paper, and wash it thoroughly with hot water.

Transfer the precipitate to a silica crucible, and discard the filtrate. Ignite the crucible and its contents at 800° C (in a muffle furnace) and dissolve the cooled oxide residue in 5 ml of hydrochloric acid, sp.gr. 1·18, warming if necessary. Make a "scavenging" precipitation by adding a few milligrams of ferric iron, precipitating with ammonium hydroxide and filtering. To the filtrate add 10 ml of a 40 per cent. w/v solution of potassium hydroxide, and carefully boil the solution until all the ammonia is expelled. Spin in a centrifuge and discard the supernatant liquid. Dissolve the precipitate in 5 ml of 3 *M* hydrochloric acid solution, transfer it to a 150-ml beaker, and add 15 ml of water, 5 ml of glacial acetic acid and 5 ml of 60 per cent. w/v solution of potassium nitrite. Set the solution aside for 5 minutes and then spin it in a centrifuge. Wash the precipitate well, first with water and then with ethanol, and, by slurrying it with a small quantity of ethanol, transfer it to a weighed aluminium counting tray, dry it under an infra-red lamp and determine the chemical yield by weighing the potassium cobaltinitrite.

Dissolve the irradiated steel, used as standards, in hydrochloric and nitric acids. To the solution add 10·0 mg of cobalt in solution and treat as for the samples.

Count all samples and standards on suitable radiometric equipment, make any corrections necessary for background, self-absorption and chemical yield, and calculate the nickel, copper and cobalt content. Check the radiochemical purity of the samples either by decay, by beta-absorption curves, by gamma-ray spectrometry, or by all the methods or any pair.

Accuracy and precision—

The precision of the methods described in this paper has already been illustrated to some extent in Table III, from which the cobalt content of mild steel "A" was found to

Table IV

Nickel results on standard steels

B.C.S. No. and steel type	B.C.S. average nickel content, %	B.C.S. range for nickel content, %	Nickel content by activation analysis, %	Average nickel content by activation analysis, %
256, low-alloy steel	0·18	0·165 to 0·210	0·149, 0·151	0·150
258, low-alloy steel	0·048	0·042 to 0·055	0·048, 0·047	0·048
218, carbon steel	0·170	0·162 to 0·190	0·174, 0·182	0·178
212, alloy steel	0·04	0·035 to 0·065	0·031, 0·035	0·033
220, alloy steel	0·15	0·13 to 0·16	0·134, 0·139	0·137

Table V

Copper results on standard steels

B.C.S. No. and steel type	B.C.S. average copper content, %	B.C.S. range for copper content, %	Copper content by activation analysis, %	Average copper content by activation analysis, %
241, alloy steel (Cr, V, W, Co, Mo) ..	0·15	0·13 to 0·165	0·151, 0·160, 0·154	0·155
220, alloy steel (W, 7%; Mo, 4%) ..	0·14	0·125 to 0·145	0·135, 0·144, 0·140	0·140
218, carbon steel	0·16	0·148 to 0·163	0·156, 0·159	0·157
212, alloy steel	0·10	0·095 to 0·101	0·108, 0·109, 0·109	0·109
211, alloy steel (Cr, 13%)	0·08	0·07 to 0·084	0·081, 0·081	0·081
150, carbon steel	0·07	0·054 to 0·079	0·072, 0·066	0·069
206, cast iron (high Si and P) ..	0·02	0·010 to 0·023	0·025, 0·023	0·024
251, low-alloy steel	0·090	0·083 to 0·096	0·098, 0·099, 0·095, 0·094	0·096
252, low-alloy steel	0·110	0·105 to 0·120	0·115, 0·113	0·114
253, low-alloy steel	0·495	0·48 to 0·508	0·521, 0·515	0·518
254, low-alloy steel	0·110	0·100 to 0·112	0·117, 0·113	0·115
255, low-alloy steel	0·240	0·23 to 0·244	0·238, 0·244	0·242
256, low-alloy steel	0·230	0·215 to 0·240	0·249, 0·250	0·250
257, low-alloy steel	0·305	0·295 to 0·320	0·310, 0·315	0·313
258, low-alloy steel	0·185	0·165 to 0·195	0·190, 0·186	0·188

Table VI

Cobalt results on standard steels

B.C.S. No. and steel type	B.C.S. average cobalt content formerly, %	B.C.S. average cobalt content revised, %	Cobalt content by activation analysis, %	Cobalt content by tetraphenyl-arsonium chloride absorptiometric method, %
218, carbon steel	< 0·01	—	0·014	—
212, alloy steel	0·02	—	0·021	—
251, low-alloy steel (Ni, 5·15%) ..	0·018	0·06	0·070	0·073
252, low-alloy steel (Ni, 4·1%) ..	0·015	0·02(5)	0·043	0·043
253, low-alloy steel (Ni, 2·9%) ..	0·012	0·02(5)	0·031	0·030
254, low-alloy steel (Ni, 2·1%) ..	0·010	0·02	0·027	0·027
255, low-alloy steel (Ni, 0·57%) ..	0·006	0·01(5)	0·019	0·020
256, low-alloy steel (Ni, 0·18%) ..	0·016	0·02(5)	0·031	0·031
257, low-alloy steel (Ni, 0·84%) ..	0·010	0·02	0·023	0·023
258, low-alloy steel (Ni, 0·05%) ..	0·012	0·02	0·028	0·032
Steel "A" used as standard in radio-activation work	—	—	—	0·016, 0·016, 0·014

be 159 p.p.m. with a standard deviation of ± 8 p.p.m. In fact such precision is much better than is required for many of the samples dealt with as some of these were rather heterogeneous, particularly the meteorites. A further idea of the precision for all three elements in finely ground rocks can be obtained from the information in Table XVII (p. 86), in which results on the granite G1 and diabase W1 are given. Even here there is a sampling problem, as the mass of sample used in the determination is only 10 to 100 mg.

Much more important than precision, in this work on trace elements, is the accuracy of the methods. It is not easy to determine this for meteorites and rocks, because of the lack of reliable analysed samples, and the best we have been able to do is to analyse so called "standard" steels. Even here there is some difficulty and, although satisfactory agreement has been obtained for nickel and copper figures with the published values, as can be seen from Tables IV and V, there was much more difficulty with cobalt. In fact, on a number of steel samples cobalt results by the radioactivation method were at complete variance with the originally published British Chemical Standard values and, although as a result of our work the latter have been re-determined and brought more nearly into line with our own figures, there is still a lack of agreement. Nevertheless, we have satisfied ourselves of the general accuracy of our cobalt figures on these steels by carrying out not only extensive experiments on possible interferences (see p. 87), but also absorptiometric determinations by the tetraphenylarsonium chloride method of Pepkowitz and Shirley.[9] For B.C.S. pure iron No. 149 one independent absorptiometric result is available,[11] of value of 0·010 ± 0·001 per cent., and this is in agreement with figures obtained by radioactivation of 0·011 per cent. (see Table XVIII, p. 86).

TABLE VII

NICKEL, COPPER AND COBALT IN GLOBIGERINA OOZE

Samples from a short pilot core, collected by Dr. B. Kullenberg on Prof. Pettersson's Swedish deep-sea expedition (1947–1948), and separated into slices ½ cm thick

Sample No.	Depth of mid-point, cm	Carbon dioxide content corrected for sea-salt, %	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
P2	1·09	38·89	11·0	27	4·3
			11·9	25	4·1
P8	4·89	39·29	9·1	31	3·5
			12·7	30	3·2
P28	15·27	39·53	13·7	36	4·9
			14·0	36	5·0
P30	16·43	39·56	15	37	4·6
			15	39	4·6
P37	20·20	38·01	28		7·6
			26		7·4
P38	20·78	37·77	24	49	6·0
			26	39	8·2
P39	21·37	37·74	27		7·1
			28		6·9
P44	24·13	36·23	33	40	8·0
			26	47	6·8
P45	24·67	36·17	23		5·9
			25		6·0
P46	25·21	35·58	30		7·4
			31		7·3
P49	26·87	33·27	65	58	10·0
			62	56	10·1
P50	27·46	32·02	55		14·6
			57		11·7
P54	29·65	31·96	63	79	14·6
			61	74	15·3
P57	31·30	31·91	62	66	12·5
				70	12·7
P58	31·85	31·74	68	65	12·0
			66	66	11·8

The comparison of our values for cobalt with the official B.C.S. results can be seen in Table VI.

TABLE VIII

NICKEL, COBALT AND COPPER IN GLOBIGERINA OOZE

Samples from a long core number 241, station 343, collected by Dr. B. Kullenberg on Prof. Pettersson's Swedish deep-sea expedition (1947–1948). The core, of length 14·402 metres, was collected in the Equatorial Atlantic Ocean on the north-east side of the Mid-atlantic Ridge (1° 10′ N, 19° 50′ W) at a depth of approximately 4350 metres

Sample No.	Depth, cm	Carbon dioxide content, uncorrected for sea-salt, %	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
13	59·7	28·57	34	98	33
			34	91	33
42	204·7	35·14	21	53	5·0
			21	54	4·9
82	404·7	26·29	45		8·4
			48		8·4
107	529·1	24·3	34	48	10·8
			37	61	10·9
109	539·7	19·84	48		10·4
			43		10·1
115	569·7	22·94	55		10·0
			51		11·7
142	704·7	37·16	15	17	2·6
			11·6	15	2·3
152	749·7	20·64	91		20
			90		19
210	1044·7	37·19	11·7	22	6·5
			11·6	20	6·5
269	1334·7	35·18	25	44	13·3
			24	53	13·3
288	1429·7	7·74	82	75	17·7
			75	75	18·1
289	1434·7	35·51	12·8		4·0
			13·9		3·1

TABLE IX

NICKEL, COPPER AND COBALT IN MISCELLANEOUS SAMPLES OF GLOBIGERINA OOZE AND IN A SAMPLE OF RADIOLARIAN OOZE

Sample No. (British Museum of Natural History No.)	Depth	Location	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
M367, St. 296 ..	1825 fathoms	S.E. Pacific, 38° 6′ S, 88° 2′ W	286	168	39
			270	178	41
M355, St. 283 ..	2075 fathoms	S. Pacific, 26° 9′ S, 145° 17′ W	247	243	122
			255		
M352, St. 280 ..	1940 fathoms	S. Pacific, 18° 40′ S, 149° 52′ W	76	65	27
			77		26
M275, St. 216a ..	2000 fathoms	N. Pacific, 2° 56′ N, 134° 11′ E	112	117	22
			110	117	22
St. 167 ..	4042 metres	Indian Ocean, 7° 11′ 48″ to 7° 11′ 24″ N, 63° 05′ 36″ to 62° 59′ 30″ E	67	54	9·9
			68	53	9·4
BM 1953, 164 (6) (rich in cocco-lithophoride)	1710 fathoms	N. Atlantic, 51° 55′ N, 23° 03′ W	16	28	7·7
			21	30	13·2
St. 128 ..	—	Indian Ocean, 5° 31′ 42″ to 5° 30′ 0″ S	44	59	8·8
			38	60	8·8
Radiolarian ooze—					
M334, St. 266 ..	2750 fathoms	N. Pacific, 11° 7′ N, 152° 3′ W	351	329	226
			343	319	222

Results and discussion

Marine sediments and rocks—

Results obtained for samples of globigerina ooze are shown in Tables VII, VIII and IX; for a single sample of radiolarian ooze also in Table IX; for red clays in Tables X and XI; for manganese nodules in Table XII and for some oceanic rocks in Table XIII.

Table X

Nickel, copper and cobalt in Atlantic red clay

Clay from core No. 230, collected by the Swedish deep-sea expedition

Sample No.	Depth, cm	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
2	371·5 to 374·5	90	128	38
		88	128	36
3	621·5 to 624·5	73	143	28
		72	135	28
4	896·5 to 899·5	111	120	22
		116	105	21
6	1496·5 to 1499·5	133	115	38
		131	114	39

Table XI

Nickel, copper and cobalt in miscellaneous red clays

Sample No. (British Museum of Natural History No.)	Depth	Location	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
M343, St. 275 ..	2610 fathoms	S.E. Pacific, 11° 20′ S, 150° 30′ W	458	409	308
			445	409	304
M309, St. 244 .. (sounding tube)	2900 fathoms	N.E. Pacific, 35° 22′ N, 169° 53′ W	264	311	56
			268	324	
M309, St. 244 .. (pumice)	2900 fathoms	N.E. Pacific, 35° 22′ N, 169° 53′ E	63	144	27
			61	138	
M315, St. 251 ..	2950 fathoms	N.E. Pacific, 37° 37′ N, 163° 26′ W	77	149	51
			70	154	50
M344, St. 276 ..	2350 fathoms	S.E. Pacific, 13° 28′ S, 149° 30′ W	305	361	182
			293	376	188
St. 166 ..	4793 metres	Indian Ocean, 6° 55′ 17″ to 6° 48′ 54″ N, 67° 11′ 18″ to 67° 14′ 0″ E	188	140	30
			203	149	25
M230, St. 178 ..	1525 fathoms	N. Pacific, 16° 47′ S, 165° 20′ E	59	150	43
			60		42
M273, St. 215 ..	2550 fathoms	N. Pacific, 4° 19′ N, 130° 15′ E	209	241	33
			199	253	34
M285, St. 221 ..	2650 fathoms	S. Pacific, 0° 40′ N, 148° 41′ E	172	304	48
			176	301	38

Table XII

Nickel, copper and cobalt in manganese nodules

Sample No. (British Museum of Natural History No.)	Depth	Location	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
M313, St. 248 ...	2900 fathoms	N. Pacific, 37° 41′ N, 177° 4′ W	7020	4190	1125
			7200	4200	1050
				4580	
				4330	
M344, St. 176 ..	2350 fathoms	S. Pacific, 13° 28′ S, 149° 30′ W	4310	1924	3780
			4520	2392	3660
St. 166 (41A)	4793 metres	Arabian Sea, 6° 55′ 18″ N, 67° 11′ 18″ E	6630	2635	1140
			6590	2800	1110

TABLE XIII

NICKEL, COPPER AND COBALT IN OCEANIC ROCKS

Sample No.	Depth	Location	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
R1, St. 133 (8), basalt with augite and oligoclase	3385 metres	1° 25′ 54″ S, 66° 34′ 12″ E	87 90	60 60	47 44
R2, St. 133 (15), hornblende augite dolerite	3385 metres	1° 25′ 54″ S, 66° 34′ 12″ E	74 72	24	34 33
R3, St. 133 (5), variolitic basalt ..	3385 metres	1° 25′ 54″ S, 65° 34′ 12″ E	55	102 64	35 34
R4, St. 133 (12), variolitic augite basalt	3385 metres	1° 25′ 54″ S, 65° 34′ 12″ E	81 86	101 100	45 42
R5, St. 166 (6), variolitic basalt ..	4793 metres	6° 55′ 18″ N, 67° 11′ 18″ E	53 44	208 214	30 31
R6, olivine augite basalt ..	2270 metres	8° 32′ N, 94° 10′ E	138 136	57 57	40 42
R7, basaltic agglomerate ..	1361 metres	Near Providence Reef	174 176	60 55 55	50 48
B.M. 1954 (128) basalt	—	11° 25′ N, 162° 10′ E (Eniwetok Atoll, E. Pacific)	266 269	95 101	60 63
B.M. D.T.	367 fathoms	42° 05′ S, 0° 06′ 2″ E (Discovery Table Mount, S. Atlantic)	67 70	84 84	 48

TABLE XIV

NICKEL, COPPER AND COBALT IN STONE AND STONY-IRON METEORITES

Sample	Location	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
M1 Khor Temiki. White chondrite .. Fell 8 April, 1932 [1934, 280]	approximately 16° N, 36° E	68 103 109 109	8·4 7·9 7·8 7·6 8·3 7·8	3·3 4·4 4·6 4·8
M2 Merua. Grey bronzite chondrite .. Fell 30 August, 1920 [1924, 134]	25° 29′ N, 81° 59′ E	18,600 18,900		847 961
M3 Gilgoin. Crystalline bronzite chondrite Found 1889 [1927, 1279]	30° 23′ S, 147° 20′ E	8920 8920 12,100 9800	 109 101	730 511 539 527
M4 Futtehpur. Veined white chondrite .. Fell 30 November, 1822 [33757]	25° 57′ N, 80° 49′ E	10,350 12,800	88 86	376 582
M5 Ochansk. Brecciated spheroidal bronzite chondrite Fell 30 August, 1887 [63549]	57° 47′ N, 55° 16′ E	12,400 13,500	99 96	576 785
M6 Bremervöde. Brecciated spherical bronzite chondrite Fell 13 May, 1855 [33739]	53° 4′ N, 9° 1′ E	8680 7830	111 79 88 99	433 318
M7 Crumlia. Grey chondrite .. .. Fell 13 September, 1902 [86115]	54° 37′ N, 6° 13′ W	5420 7420 5720 7660	 72 76	104 198 203 255
M8 Estherville. Stony-iron mesosiderite .. Fell 10 May, 1879 [53764]	43° 25′ N, 94° 50′ W	17,000 20,500	225 231	1100 1135
M9 Mangwendi. Intermediate hypersthene chondrite Fell 7 March, 1934 [1934, 839]	17° 39′ S, 31° 36′ E	9340 8450	80 82	490 465

METEORITES—

Although there is a good deal of information about the nickel, copper and cobalt content of iron meteorites, in which the levels are comparatively high, less information is available

about the stony meteorites, so it was thought worth while to examine a few of these to supplement the existing data. It should be pointed out that such samples are rather heterogeneous and, with the small sample weight used in the radioactivation method, agreement between replicates is not expected to be very good. Nevertheless, as the nickel, copper and cobalt were determined on the same replicate sample, values for the ratios of these elements may still be useful. Results for nine such samples are given in Table XIV.

From the results in Tables VII to XIV, the ratios of nickel to cobalt, nickel to copper and copper to cobalt have in each case been calculated and are summarised in Table XV, together with average values for igneous rocks and for meteorites taken from Rankama and Sahama.[12] As stated by Smales and Wiseman,[1] "it would appear that there is little significant contribution of meteorite material to the nickel content of deep sea sediments, unless some remarkable differential behaviour of the three elements nickel, copper and cobalt has taken place." This statement must not, of course, be taken to rule out local deposition of meteoritic matter.

TABLE XV

RATIOS INVOLVING NICKEL, COPPER AND COBALT

	Ratio of nickel to cobalt			Ratio of nickel to copper			Ratio of copper to cobalt		
Material	Number of samples	Average ratio	Range	Number of samples	Average ratio	Range	Number of samples	Average ratio	Range
Red clay ..	13	3·4	1·4 to 7·1	13	0·8	0·4 to 1·4	13	4·4	1·3 to 7·3
Globigerina ooze	34	4·0	1·0 to 7·0	24	0·8	0·4 to 1·6	24	5·4	2·0 to 10·9
Manganese nodules ..	3	4·5	1·2 to 6·5	3	2·0	1·6 to 2·4	3	2·3	0·6 to 4·0
Oceanic rocks..	9	2·4	1·4 to 4·3	9	1·7	0·2 to 3·1	9	2·2	0·7 to 6·9
Average for igneous rocks[12]		3·5			1·1			3·0	
Meteorites (stones and stony-irons)..	9	21·8	16·8 to 34·5	7	104	82 to 133*	7	0·22	0·14 to 0·39*
Average for meteorites[12]..		13·1			92			0·14	

* Omitting Khor Temiki, which gave a nickel to copper ratio of 12·2 and a copper to cobalt ratio of 1·9.

The nickel to copper and copper to cobalt ratios for the Khor Temiki meteorite have been omitted from Table XV and are clearly quite unusual. It has been pointed out by Mr. E. P. Henderson[13] that some stone meteorite specimens may have been treated with copper sulphate solution by geologists. Whether the particular specimen of Khor Temiki analysed has received such treatment is not known, but it is the unexpectedly high copper figure that upsets the ratios mentioned, and it might be interesting to analyse other specimens of this meteorite.

TABLE XVI

NICKEL, COPPER AND COBALT IN MISCELLANEOUS SPECIMENS

Sample	Location	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
B.M. 44434, iron from basalt	Ovifak, Greenland	15,850	1074	4670
		16,100	1095	4230
		16,200	1141	4540
		23,000	1181	4340
B.M. 1927, 1248 (3), werlite - dunite ..	1° 0′ N, 29° 30′ W (St. Paul's Rocks, Equatorial Atlantic)	1750	13	118
		1790	14	113
28, quartz dolerite	Knob Head, South Victoria Land	88	206	40
		88	249	45

MISCELLANEOUS GEOLOGICAL MATERIALS—

Three specimens of some general interest are reported in Table XVI, while in Table XVII are given the results on a granite G1 and diabase W1, some of which had already previously been reported briefly.[14] Included in Table XVIII are the figures obtained by other methods, chemical and spectrographic, summarised by Ahrens,[15] and polarographic, used by Smythe and Gatehouse.[16] The accumulation of values by a number of different techniques on these two rocks is very desirable.

TABLE XVII

NICKEL, COPPER AND COBALT IN "STANDARD" GRANITE AND DIABASE

	Proposed radioactivation method			Spectrographic and chemical results quoted by Ahrens[15]				Polarographic results[16]		
Sample	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.	Analyst No.	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
W1 diabase from Centerville, Virginia	76	111·5	54	1	47	130	35	64 ± 8	100 ± 11	23 ± 1
	71	111	52	2	80	90	25			
	73	111	46	3	—	44	30			
	78	108	45	4	80	—	50			
	78	113	50	5	140	140	55			
	63	119	46	6	60	—	26			
		110		7	70	100	—			
		109		8	150	80	20			
				9	80	130	40			
			"Recommended" value		90	110	36			
G1 granite from Westerly, Rhode Island	1·5	8·7	1·8	1	5	< 8	n.d.*			
	1·3	11·1	2·4	2	n.d.	15	3	2 ± 2	17 ± 16	0
	1·0	8·8	2·1	3	—	5	n.d.			
	1·0	9·1	2·1	4	n.d.	—	—			
		9·5		5	< 2·5	6	< 2			
		9·6		6	< 10	—	< 5			
				7	—	trace	—			
				8	60	10	10			
				9	—	20	3			
			"Recommended" value		(5)	11	(4)			

* n.d. = not determined.

PURE IRON—

In addition to the steel samples mentioned earlier, some commercially available samples of pure iron were examined, the results being given in Table XVIII. The sensitivity of the method for cobalt is of value for some of these samples in which the cobalt content is as low as 1 p.p.m.

TABLE XVIII

NICKEL, COPPER AND COBALT IN "PURE" IRON

Sample	Nickel found, p.p.m.	Copper found, p.p.m.	Cobalt found, p.p.m.
B.C.S. No. 149, pure iron ..	32, 37, 39, 39	3·3, 5·1, 3·4	107, 113, 113, 115
B.C.S. No. 260, pure iron ..	96, 94, 101, 103	49, 49	26, 27, 26, 28
Specpure iron, batch 5092 ..	71, 70	2·9	1·1, 1·2
Specpure iron, batch 5123 ..	79, 81	2·7, 2·7	0·8, 0·8

SEAWEEDS—

The cobalt contents of several samples of dried seaweed were determined and results are given in Table XIX. Again the sensitivity of the method for cobalt is valuable.

TABLE XIX

COBALT IN SEAWEED

Seaweed	Wet weight / Dry weight	Ash as percentage of dry weight	Sample weight, g	Cobalt found, μg	Dry weed, p.p.m.	Wet weed, p.p.m.
Ascophyllum nodosum	4·78	25·1	0·1043	0·132	1·26	0·26
			0·1097	0·122	1·11	0·23
Fucus serratus	4·13	19·7	0·1039	0·308	2·97	0·72
			0·1006	0·249	2·48	0·60
Fucus vesiculosus ..	4·48	20·3	0·1009	0·185	1·83	0·41
			0·1008	0·160	1·59	0·36
Laminaria digitata ..	8·64	34·6	0·1023	0·023	0·22	0·026
			0·1077	0·019	0·18	0·021
Laminaria saccarina ..	11·31	36·4	0·1019	0·026	0·26	0·023
			0·1098	0·026	0·24	0·021
Porphyra umbilicalis ..	6·42	20·8	0·1122	0·059	0·53	0·082
			0·1023	0·050	0·49	0·076
Rhodymenia palmata ..	10·62	38·7	0·1022	0·053	0·52	0·049
			0·1010	0·048	0·48	0·045

POSSIBLE INTERFERING ELEMENTS

As with other applications of radioactivation, the possibility of conflicting nuclear processes must be borne in mind. Hence, ^{65}Ni can be formed by an n,p reaction on ^{65}Cu or by an n,α reaction on ^{68}Zn, as well as by the n,γ reaction on ^{64}Ni used for the determination. Fortunately, for the samples under consideration in this paper, these conflicting reactions are unlikely to be troublesome, as the nickel content was of the same order as, or much greater than, the copper and zinc contents, and the n,p and n,α cross-sections are likely to be much lower than the n,γ cross-section. Similar arguments apply to the possible formation of ^{64}Cu from ^{64}Zn by an n,p reaction. The presence of significant amounts of radionuclides of nickel and copper other than ^{65}Ni and ^{64}Cu, respectively, would be recognised when measuring the decay curve and could readily be allowed for.

The picture is not quite so clear cut with cobalt, as a decay curve cannot readily be drawn, owing to the long (5·2-year) half-life of ^{60}Co.

Although "spurious" formation of ^{60}Co from an n,p reaction on ^{60}Ni or from an n,α reaction on ^{63}Cu is unlikely to be important in the samples dealt with, particularly as the n,γ cross-section for formation of ^{60}Co from ^{59}Co is so high, it is known that ^{58}Co can be formed from ^{58}Ni in surprisingly high yield by neutron irradiation in a Pile.[17] If counting is done without energy discrimination, *e.g.*, by end-window beta counting, then any ^{58}Co will of course be counted as if it were ^{60}Co and give rise to a spuriously high cobalt figure. From the known cross-section and Pile fluxes the extent of the error can be calculated as follows—

Assume beta counting with the same efficiency for both ^{60}Co and ^{58}Co, and irradiation for periods of not more than 70 days, *i.e.*, where the rate of growth of the two radionuclides is linear with time. Then the ratio of measured beta activity from ^{58}Co and ^{60}Co from equal masses of nickel and cobalt, respectively, irradiated in the Harwell Pile for the same time, will be—

$$\frac{A_{^{58}\mathrm{Co}}}{A_{^{60}\mathrm{Co}}} = \frac{\sigma_{^{58}\mathrm{Co}} \times f_{\mathrm{fast}} \times R_{^{58}\mathrm{Co}} \times T_{^{60}\mathrm{Co}}}{\sigma_{^{60}\mathrm{Co}} \times f_{\mathrm{thermal}} \times R_{^{60}\mathrm{Co}} \times T_{^{58}\mathrm{Co}}},$$

where A = measured beta activity,
σ = activation cross-section,
f_{fast} = fast neutron flux,
f_{thermal} = thermal neutron flux,
R = beta branching ratio,
T = half-life,

$$= \frac{0{\cdot}03 \times 2 \times 10^{11} \times 0{\cdot}15 \times 5{\cdot}2 \times 365}{34 \times 1 \times 10^{12} \times 1 \times 70}$$

$$= 0{\cdot}0007.$$

This means that for equal quantities of nickel and cobalt in a sample, the error involved in the ordinary radioactivation method for determining cobalt, assuming irradiation in the

Harwell Pile and adequate radiochemical separation of the cobalt, would amount to 0·07 per cent. This is clearly negligible, and even for one meteorite sample in which the ratio of nickel to cobalt is 34·5 (Sample M7, Table XIV), the error is 2·5 per cent. and can be neglected owing to the heterogeneity of the sample.

In the case of some materials, however, the error could be serious, as for example, in steels for which the ratios of nickel to cobalt are often as high as 100 or more. Fortunately, any serious error would be apparent from the beta-absorption curve as the maximum beta energy of ^{58}Co is 0·47 MeV (positron) and that of ^{60}Co is 0·32 MeV (β-). Nevertheless, an accurate correction cannot readily be made from a beta-absorption curve and methods of avoiding the error are desirable. Fortunately, there are at least two ways of overcoming the difficulty—

(*a*) instead of using end-window beta counting, by comparing the activities of the cobalt isolated from sample and standard by measuring the areas under the 1·17 and 1·33-MeV peaks of ^{60}Co on a gamma-ray spectrometer; and

(*b*) by irradiating the samples in the thermal column of the Harwell Pile instead of in the usual irradiation position. Some sensitivity is lost by such an irradiation, as the thermal flux is lower possibly by a factor of about ten. (This could be offset by increasing the irradiation time.) The fast neutron flux is very considerably lower still, and as can be seen from the above calculation this decreases the relative contribution of ^{58}Co.

The results for the cobalt content of steels in Table VI were obtained by using both the above methods. Results not quoted here, obtained by the usual procedure of irradiation in the Pile in the normal place, and final activity measurement of the isolated cobalt activity determined by end-window beta counting, confirmed the calculation made above.

Summarising the position on possible interfering elements, particular care has to be taken in attempting to use neutron-activation methods to determine small amounts of one element in the presence of major amounts of another element of mass number differing only by one or two. Specifically for elements dealt with in this paper, large amounts of zinc or copper might interfere with the nickel determination, of zinc with the copper determination, and of nickel and copper with the cobalt determination. The techniques described to overcome the interference of nickel in the determination of cobalt may also be applicable to the other metals, but these have not been considered in detail here, as they do not occur in the samples examined.

The specimens of marine sediment, rocks, meteorites, etc., mentioned in Tables VII to XIV were kindly provided by Dr. J. D. H. Wiseman of the Department of Mineralogy, British Museum (Natural History), and he also supplied the figures for the carbon dioxide content of the marine sediments described in Tables VII and VIII. We thank Mr. L. Salmon for considerable assistance in the gamma-ray spectrometry work.

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The Absorptiometric Determination of Microgram Quantities of Uranium with the Thoronol Complex of Quadrivalent Uranium*

By J. K. FOREMAN, C. J. RILEY and T. D. SMITH

In dilute acid solution, quadrivalent uranium forms a red complex with thoronol [the red sodium salt of 1-(*o*-arsonophenylazo)-2-naphthol-3 : 6-disulphonic acid]. The complex is stable in aqueous acetone solution and affords a sensitive and precise method of determining microgram quantities of uranium. Several elements interfere and to overcome this a method of separation has been developed in which uranium is extracted as its sodium diethyldithiocarbamate complex with chloroform. Cupferron and ethylenediaminetetra-acetic acid are used to hold back impurities.

Coloured complexes formed by the uranyl ion with a number of reagents, for example, 8-hydroxyquinoline,[1] dibenzoylmethane,[2] resacetophenone[3] and thiocyanate ion,[4] have been used for determining small amounts of uranium. However, for the accurate and precise absorptiometric assay of uranium at the microgram level, a method of greater sensitivity than those at present available is required. To this end, complexes of quadrivalent uranium were examined as an alternative to those of sexavalent uranium, which have already received extensive study. The uranyl ion is conveniently and quantitatively reduced to uranium^{IV} by using a lead reductor,[5] a procedure having the advantage that further reduction to uranium^{III} does not occur.

Thomason, Perry and Byerly[6] have described a sensitive method for the determination of thorium by means of the reagent thoronol [the red sodium salt of 1-(*o*-arsonophenylazo)-2-naphthol-3 : 6-disulphonic acid] and noted that uranium^{IV} reacted with it to yield an unstable deep red complex. The properties of this complex have now been studied in greater detail, and, based on its formation, a simple, precise and sensitive method for determining uranium is described. Details are also given of a separation that is specific for uranium and can be used in conjunction with the proposed method.

Experimental

Reagents used in the investigation included the following—

Uranium solution—Standard uranium solutions were prepared at the mg per ml level by dissolving weighed amounts of pure uranoso-uranic oxide, U_3O_8, to a final acidity of 3 *N* hydrochloric acid. Sub-standards in the μg per ml region were prepared from these solutions by appropriate dilution with 3 *N* hydrochloric acid.

Thoronol solution—The commercial product obtained from L. Light & Co. Ltd. was recrystallised from dilute acetic acid, washed and dried at 110° C for 15 minutes. The material was used as a 0·1 per cent. aqueous solution.

Lead shot—Lead shot obtained from The British Drug Houses Ltd. was cleaned thoroughly before use by vigorous shaking, first with concentrated hydrochloric acid and then with 3 *N* hydrochloric acid, until a high metallic lustre was observed. When not in use, the material was stored in 3 *N* hydrochloric acid.

The uranium^{IV} - thoronol complex—

According to Cooke, Hazel and McNabb,[5] the reduction of uranium^{VI} to uranium^{IV} proceeds most efficiently in 3 *N* hydrochloric acid. For studies of the stability of the complex, it was prepared as follows—

Fifteen grams of freshly cleaned lead shot were introduced into a 10-ml stoppered flask, and then a suitable aliquot (containing about 20 μg of uranium) of uranium solution in 3 *N* hydrochloric acid was added. These were shaken together for 1 minute and the uranium solution was transferred to a 25-ml calibrated flask and diluted to 5 ml

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with distilled-water washings of the lead shot. Then 1 ml of thoronol solution was added, and the solution was diluted to the mark with water. Optical-density readings were taken by means of a Spekker photo-electric absorptiometer, with use of Ilford No. 605 filters, the absorption peak of the complex being at 535 mμ. Acidity conditions for the formation of the complex are not critical; a final acidity of up to 0·2 *N* hydrochloric acid may be used for full development of the colour.

Although reproducible results could be achieved by making the optical-density measurements immediately after development of the colour, the complex is nevertheless unstable and over a period of 15 minutes the optical density decreased by about 5 per cent. It was shown that uranium^{IV} is unstable under the conditions used by preparing a number of uranium^{IV} solutions of identical concentration and developing the thoronol complex after various intervals of time. Various reducing agents, for example, stannous chloride, sodium hypophosphite, sodium sulphite and hydrazine hydrochloride, were incorporated in an attempt to stabilise the complex, but these either interfered with the colour development or did not prevent fading. Liquid zinc amalgam and solid cadmium amalgam were investigated, but they offered no advantage over the lead reductor.

The development of coloured complexes in non-aqueous or mixed aqueous - non-aqueous media to impart stability and improve sensitivity has frequently been exploited; in particular acetone has been used by Crouthamel and Johnson[7] in the determination of uranium as the thiocyanate complex, by Marriott and Wolf[8] and Winsor[9] in the thiocyanate determination of iron, and by Crowther and Large[10] in the determination of ammonia by sodium phenoxide and sodium hydroxide. Similarly, by preparing the uranium^{IV} - thoronol complex in aqueous acetone media a stable and more sensitive coloured complex resulted. The sensitivity varied with the amount of acetone present, 80 per cent. of acetone being the optimum composition.

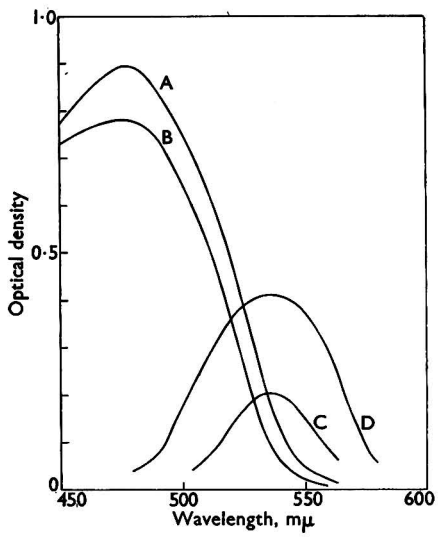

Fig. 1. Absorption spectra: curve A, thoronol in aqueous solution; curve B, thoronol in 80 per cent. acetone; curve C, uranium^{IV} - thoronol complex in aqueous solution; curve D, uranium^{IV} - thoronol complex in 80 per cent. acetone. Final volume of solution, 25 ml; cell length, 4 cm

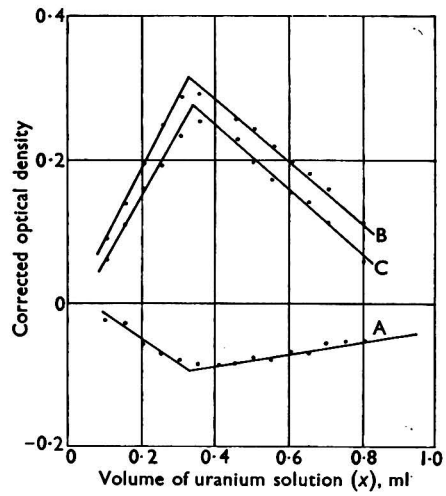

Fig. 2. Continuous-variation plots for the uranium^{IV} - thoronol complex: curve A, 500 mμ; curve B, 530 mμ; curve C, 560 mμ. Final volume of solution, 10 ml; cell length, 4 cm

Fig. 1 shows the spectra of the thoronol reagent and the uranium^{IV} - thoronol complex in both aqueous and aqueous acetone solution recorded with a Uvispek spectrophotometer. The introduction of acetone does not produce any spectral shifts, but merely decreases the optical density of the reagent while simultaneously increasing that of the complex. Other solvents, notably dioxan, ethyl methyl ketone and ethanol were less effective than acetone with respect to both sensitivity and stability of the complex.

Typical figures for the improved sensitivity when 80 per cent. acetone was used as solvent are given in Table I; this represents a calibration in which the quantities of reagents used were reduced in the appropriate proportions to give a final volume of 10 ml. Optical-density measurements were then made against a reagent blank, with use of a Spekker absorptiometer in conjunction with 4-cm Uvispek cells contained in a simple holder designed to fit the cell carriage of the Spekker instrument.

TABLE I

COMPARISON OF OPTICAL DENSITIES OF CERTAIN URANIUM^{IV} COMPLEXES

Uranium taken, μg	Optical density of uranium^{IV} - thoronol complex in 80 per cent. acetone	Optical density of uranium^{IV} - thoronol complex in aqueous solution	Optical density of uranium^{IV} - dibenzoylmethane complex
1·25	0·033	0·020	0·014
2·5	0·072	0·041	0·025
5·0	0·135	0·079	0·047
7·5	0·203	0·111	0·077
10·0	0·276	0·155	0·102
12·5	0·343	0·188	0·130

The sensitivity compares favourably with that of existing absorptiometric methods. Included in Table I are calibration results obtained by the dibenzoylmethane procedure for uranyl ion as given by Yoe, Will and Black,[2] which is amongst the most sensitive methods available. Again the final volume was 10 ml and Uvispek spectrophotometer cells were used with the Spekker absorptiometer, the filters being Ilford No. 601.

The coefficients of variation at three uranium concentrations, fifteen determinations being carried out at each level, were as follows—

Uranium concentration, μg per 25 ml ..	10	20	30
Coefficient of variation, %	3·3	3·0	2·5

The sensitivity of the thoronol method for thorium may be similarly enhanced by preparing the complex in aqueous acetone; this is shown by the results given in Table II.

TABLE II

COMPARISON OF OPTICAL DENSITIES OF THE THORIUM - THORONOL COMPLEX IN VARIOUS SOLVENTS

Thorium present, μg per 25 ml	Optical density in aqueous solution	Optical density in 80 per cent. acetone
0	0·044	0·023
10	0·122	0·154
20	0·197	0·285
30	0·266	0·415

MOLE RATIO OF THE URANIUM^{IV} - THORONOL COMPLEX—

Information about the empirical formula of the complex was obtained by applying the method of continuous variations.[11,12] Equimolar solutions (0·000726 *M*) of uranium^{IV} in 3 *N* hydrochloric acid and aqueous thoronol were mixed in the proportions x to $1-x$ ml, respectively, and made up to 25 ml to give solutions containing 80 per cent. of acetone. Their optical densities were measured at several wavelengths by means of the Uvispek spectrophotometer. Typical plots of corrected optical density of the complex against the parameter x are shown in Fig. 2, and it is evident that the complex contains two molecules of thoronol to one of uranium. For thorium Byrd and Banks[13] find the complex is predominantly 2 moles of thorium to 3 moles of thoronol.

In Fig. 3 is shown the extent of complex formation as the mole ratio of thoronol to uranium is increased for both aqueous and 80 per cent. acetone media. In addition to demonstrating the enhanced sensitivity resulting from the use of acetone, the curves show that the uranium^{IV} - thoronol complex is less dissociated in the mixed solvent.

By preparing solutions of the complex containing an excess of thoronol, corresponding to the plateau in Fig. 3, the molar optical density of the complex may be measured. Values of this property for the complex and pure reagent at any two wavelengths together with

optical-density measurements at these wavelengths for uranium^{IV} - thoronol mixtures of known composition permit the apparent stability constant, K, of the complex to be calculated, assuming the formation is in accordance with the following equation—

$$U^{IV} + 2 \text{ thoronol} \rightleftharpoons U^{IV}(\text{thoronol})_2,$$

whence

$$K = \frac{[U^{IV}(\text{thoronol})_2]}{[U^{IV}]\ [\text{thoronol}]^2}.$$

A number of determinations of this quantity gave a value of $K = 9{\cdot}2 \pm 0{\cdot}5 \times 10^9$.

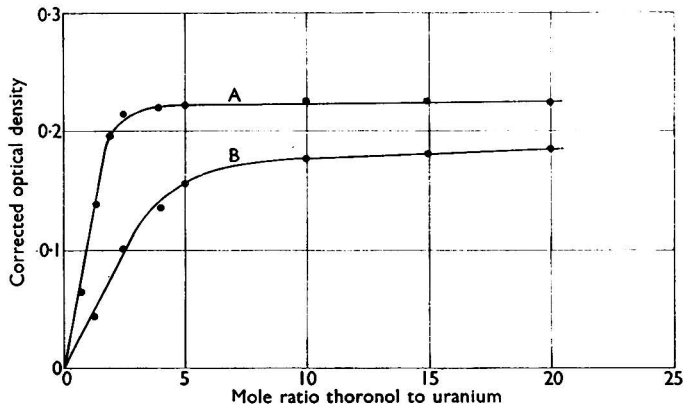

Fig. 3. Composition dependence of extent of complex formation: curve A, in 80 per cent. acetone; curve B, in aqueous solution. Final volume of solution, 10 ml; cell length, 4 cm

INTERFERENCES—

Foreign ions may interfere at the colour-development stage and also at the lead reductor, where anions capable of strongly complexing the uranyl ion will reduce the efficiency of the reduction, as also will the presence of reducible cations that are deposited on the surface of the lead. A number of cations and anions has been examined with respect to over-all interference by applying the method to fixed amounts of uranium in the presence of successively increasing quantities of the ion being studied. The results are depicted in Figs. 4, 5 and 6, the Spekker absorptiometer and 4-cm cells being used. It is evident that complexing anions represent the most serious interference, and that iron, chromium, vanadium, zirconium, copper, mercury, manganese, cerium and particularly molybdenum also interfere. Strontium, aluminium and nickel have no effect at the levels studied.

PRELIMINARY SEPARATION OF URANIUM—

It is evident from the previous paragraph that when uranium is a minor constituent of a sample its separation before being determined by the thoronol method is essential.

Uranyl ion forms only a weak complex with ethylenediaminetetra-acetic acid,[14] so permitting it to be separated from a large number of cations by its solvent extraction as a suitable complex. For efficient retention of cations by ethylenediaminetetra-acetic acid an extraction pH above about 4 is desirable. Hence sodium diethyldithiocarbamate was used, since its uranium complex is readily extracted into chloroform in the range pH 5 to 7·5.[15] This combination of reagents has been used successfully by Pohl[16] for the separation of copper from iron, cobalt and nickel, and by Cheng, Bray and Melsted[17] for the extraction of bismuth from impurities. The only elements that will accompany uranium through the separation are those that form a solvent-extractable sodium diethyldithiocarbamate complex that is more stable than the corresponding ethylenediaminetetra-acetate. Iron^{III}, bismuth and copper are in this category and their presence in the sample aliquot leads to low results for uranium. These elements may be removed by a prior extraction of their cupferrates into chloroform.

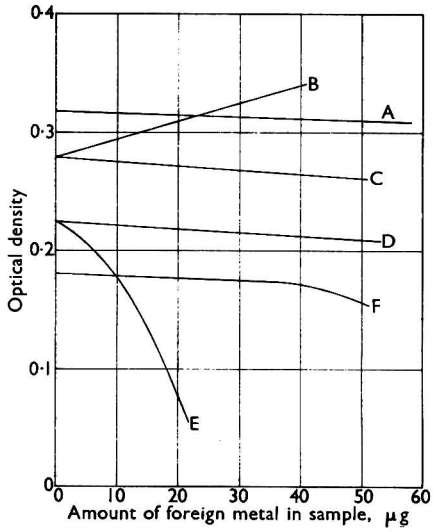

Fig. 4. Effect of cationic impurities on the uranium^{IV} - thoronol method: curve A, 30 μg of uranium + aluminium; curve B, 25 μg of uranium + zirconium; curve C, 25 μg of uranium + vanadium; curve D, 20 μg of uranium + iron^{III}; curve E, 20 μg of uranium + molybdenum; curve F, 15 μg of uranium + chromium^{III}. Final volume of solution, 25 ml; cell length, 4 cm

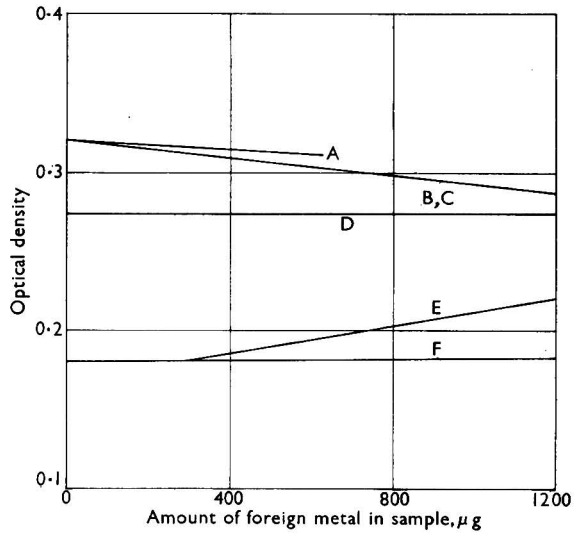

Fig. 5. Effect of cationic impurities on the uranium^{IV} - thoronol method: curve A, 30 μg of uranium + copper; curve B, 30 μg of uranium + manganese; curve C, 30 μg of uranium + mercury^{II}; curve D, 25 μg of uranium + strontium; curve E, 15 μg of uranium + cerium^{III}; curve F, 15 μg of uranium + nickel. Final volume of solution, 25 ml; cell length, 4 cm

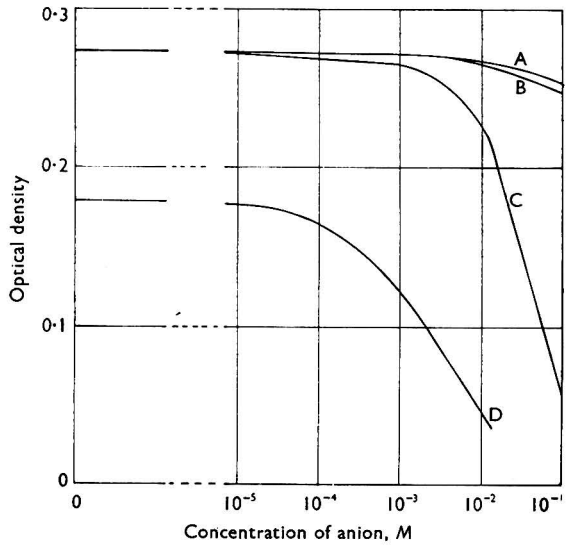

Fig. 6. Effect of anions on the uranium^{IV} - thoronol method: curve A, 25 μg of uranium + perchlorate; curve B, 25 μg of uranium + nitrate; curve C, 25 μg of uranium + sulphate; 15 μg of uranium + phosphate. Final volume of solution, 25 ml; cell length, 4 cm

The performance of the separation procedure (which is detailed in the next section) may be judged from Tables III and IV. Table III shows the reproducibility of a uranium

calibration, and Table IV lists the amounts of various ions that were shown not to affect the recovery of uranium. The concentrations quoted refer to the aqueous solution at the cupferron extraction stage. Whilst the figures quoted for the four anions represent safe working limits, this is not so for the cations, for which the concentrations quoted are merely levels at which the separations have worked satisfactorily. The uranium concentration was 4×10^{-6} *M*.

TABLE III

REPRODUCIBILITY OF A URANIUM CALIBRATION

Uranium taken, μg	Corrected optical density		
	Sample 1	Sample 2	Sample 3
5	0·051	0·050	0·049
10	0·098	0·101	0·097
20	0·197	0·205	0·198
30	0·300	0·302	0·303

TABLE IV

LEVEL OF CONCENTRATION OF VARIOUS INTERFERING IONS AT WHICH SEPARATION OF URANIUM^{IV} CAN BE SATISFACTORILY ACHIEVED

Ion	Concentration, *M*	Ion	Concentration, *M*	Ion	Concentration, *M*
SO_4^{2-}	2·5	Al^{3+}	0·3	Mn^{2+}	0·01
$C_2O_4^{2-}$	1·2	Hg^{2+}	0·003	Ni^{2+}	0·01
F^-	0·5	Cu^{2+}	0·05	Co^{2+}	0·01
PO_4^{3-}	0·2	Th^{4+}	0·003	Sr^{2+}	0·005
Fe^{3+}	0·01	Ce^{3+}	0·02	La^{3+}	0·003
				Sn^{2+}	0·005

METHOD

The sample should be in dilute nitric or hydrochloric acid solution.

Dilute 1 ml of the sample to 5 ml with 1 *M* hydrochloric acid (up to 1 *M* nitric acid can be tolerated at this stage; the concentration of complexing anions should not exceed the limits quoted in Table IV). Add 2 ml of 5 per cent. aqueous cupferron solution and extract with 15 ml of chloroform. Add a further 2 ml of cupferron solution and re-extract with chloroform. Separate the aqueous phase and add to it one drop of phenolphthalein solution and then 20 ml of saturated ethylenediaminetetra-acetic acid solution. Make just alkaline with sodium hydroxide and buffer to about pH 6 by adding 2 ml of 20 per cent. sodium acetate solution and 0·5 ml of 20 per cent. acetic acid. Add 5 ml of 5 per cent. aqueous sodium diethyldithiocarbamate solution and then 20 ml of 0·3 *M* calcium nitrate solution (if appreciable quantities of oxalate are present, magnesium nitrate must be used) to bind the excess of ethylenediaminetetra-acetate. Re-adjust to pH 6 as described above, if necessary.

Extract the aqueous phase with three 15-ml portions of chloroform, combine the chloroform solutions and wash once with water containing 1 ml of 5 per cent. sodium diethyldithiocarbamate buffered with acetate to about pH 6 as described above and made 0·05 *M* in calcium ethylenediaminetetra-acetate. Extract the chloroform phase with two 5-ml portions of 10 per cent. ammonium carbonate solution, combine the extracts and evaporate to dryness in a silica dish. Ignite the residue over a bunsen burner for half a minute, then dissolve it in 2 ml of concentrated hydrochloric acid and evaporate to dryness.

Dissolve the residue in 1 ml of 3 *N* hydrochloric acid and transfer this solution, together with a further 1 ml used for washing the dish, to a 10-ml flask containing 15 g of freshly cleaned lead shot. Shake for 1 minute, transfer the solution, together with about 1 ml of the distilled-water washings, to a 25-ml calibrated flask, add 20 ml of acetone, 1 ml of 0·5 per cent. thoronol solution and make up to volume with water. Measure the optical density of the solution against a blank, prepared by carrying out the method in the absence of uranium, using the Spekker absorptiometer in conjunction with 4-cm cells and Ilford No. 605 filters.

If it is desired to increase the sensitivity of the method by using 4-cm Uvispek spectrophotometer cells in the Spekker absorptiometer with a final volume of 10 ml, the quantity

of 3 *N* hydrochloric acid used at the lead-reduction stage must be reduced to give a final acidity not greater than 0·2 *M*.

We are indebted to Mr. E. F. Kemp for valuable discussions and to the Managing Director, United Kingdom Atomic Energy Authority (Industrial Group), for permission to publish this work.

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Chromatographic Separations in Phenol - Methanol - Hydrochloric Acid Solvents, with Special Reference to the Alkali Metals

BY ROBERT J. MAGEE* AND JAMES B. HEADRIDGE

A phenol - methanol - concentrated hydrochloric acid mixture (57·5: 22·5 : 20 per cent. w/v/v) has been used to separate the chlorides of lithium and sodium, and of potassium, rubidium, caesium and ammonium. Zinc uranyl acetate has been employed for the detection and determination of 0·25 to 10 μM (micromoles) of lithium and 0·1 to 10 μM of sodium, and sodium lead cobaltous hexanitrite for 0·1 to 10 μM of potassium, rubidium and caesium and 0·25 to 10 μM of ammonium. It was found that 10-μM amounts of any of the group IA metals, singly or combined, did not interfere with the detection and determination of the minimum amounts of any particular metal.

A phenol - methanol - concentrated hydrochloric acid mixture (50 : 20 : 30 per cent. w/v/v) has been used to separate small amounts of aluminium, gallium, indium, thallium and zinc, and titanium, zirconium and iron.

SEPARATION OF THE ALKALI METALS

THE separation of lithium, sodium and potassium is not difficult and references in the literature to this separation are numerous; usually the chlorides and nitrates have been used and these are generally separated with solvents consisting of one or more alcohols. For example, these three cations and the ammonium ion may be separated with a methanol - *n*-butanol mixture (70 : 30 per cent. v/v).[1] The separation of potassium, rubidium and caesium is not easy, and only two solvent mixtures have been found to accomplish this separation.[2,3] Miller and Magee[2] were able to separate 5 μg of any of these ions from a total weight of 1000 μg, but could not use the solvent mixture to separate lithium, sodium and

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ammonium from them. Steel[3] claimed a separation of 50-μg amounts of ammonium, potassium, rubidium and caesium ions with the phenol-rich layer from liquid phenol - dilute hydrochloric acid solution (1 + 2, by volume), but did not mention the effect of this solvent mixture on lithium and sodium chlorides.

A good separation of the alkali metals on one chromatographic strip was required, as it was later intended to determine the amount of each of the separated salts by micro-analytical methods. This separation was not found to be possible, but a solvent mixture giving a reliable separation of lithium and sodium salts, and of potassium, rubidium, caesium and ammonium salts is described.

Experimental

Most previous investigators have used the salts of inorganic acids, although the acetates[4] and citrates[5] of lithium, sodium and potassium have been separated. To investigate the influence of an organic anion on the solubility and distribution in organic solvents of the salts of group IA metals, in particular potassium, rubidium and caesium, *n*-butyrates were prepared. An ion-exchange resin, Amberlite IRA-400 (50 to 100 mesh), was used in their preparation. A column of the resin (10 cm long and diameter 1 cm) was regenerated with 2 *N* sodium hydroxide, washed free from alkali, converted to the butyrate form with 2 *N* butyric acid and finally washed with water to remove excess of acid. The alkali-metal chlorides (20 mg of cation) were separately changed to butyrates on this column. Of the solvents tried, the alkali-metal butyrates were found to be readily soluble only in water, methanol and anhydrous butyric acid.

Lithium, sodium and potassium butyrates were separated by using certain mixtures of simple alcohols, *e.g.*, methanol - *n*-butanol mixture (80 to 20 per cent. v/v), with which 200-μg amounts of the cations gave the following R_F values: lithium, 0·91 ± 0·06, *i.e.*, "0·85 to 0·97"; sodium, 0·52 ± 0·06; and potassium, 0·23 ± 0·07. With these and other solvent mixtures containing butyric acid, no separation of potassium, rubidium and caesium was achieved.

From the chromatograms produced it was apparent that butyrates were reacting in a similar manner to chlorides and other inorganic salts. Further investigations were made with chlorides.

Miller and Magee[2] used concentrated hydrochloric acid - methanol - *n*-butanol - *iso*butyl methyl ketone mixture (55 : 35 : 5 : 5 per cent., by volume) to separate potassium, rubidium and caesium; 100-μg amounts of these cations and of lithium, sodium and ammonium were chromatographed separately, with use of this solvent mixture. The R_F values for the mid-points of each spot are given in the first line of Table I. Sodium and potassium, ammonium and caesium were not separated. Chromatograms were prepared by using solvent mixtures similar to that described above, but these contained the organic base *symm.*-collidine. The R_F values found are shown in Table I.

Table I

R_F VALUES FOR THE ALKALI METALS WITH VARIOUS SOLVENTS

Composition of solvent mixture, per cent. by volume					R_F values for					
Concentrated hydrochloric acid	Methanol	*n*-Butanol	*symm.*-Collidine	*iso*Butyl methyl ketone	Na^+	K^+	Rb^+	Cs^+	NH_4^+	Li^+
55	35	5	–	5	0·45	0·47	0·51	0·60	0·63	0·68
35	55	–	10	–	0·38	0·28	0·25	0·33	–	0·75
40	55	–	5	–	0·36	0·25	0·28	0·34	0·51	0·68
70	25	–	5	–	0·58	0·58	0·60	0·69	0·69	0·76
95	—	–	5	–	0·75	0·79	0·80	0·83	0·83	0·80
85	—	10	5	–	0·65	0·70	0·72	0·78	0·77	0·76
65	20	10	5	–	0·52	0·51	0·55	0·63	0·66	0·73

It can be seen from Table I that no separation of all six cations is achieved. In fact, no solvent mixture gives a separation of potassium, rubidium, caesium and ammonium on one strip and so permit a mixture of these cations to be resolved and identified.

Steel[3] gives the order of the separated salts from the starting line as ammonium, potassium, rubidium and caesium. Although several chromatograms were made with chlorides

under similar conditions, these results were not reproducible. Potassium, rubidium and caesium were separated, but the ammonium and rubidium ions were always in the same position. However, it appears that the flow of the ammonium ion is at times erratic and is dependent on the anion present.[6] The phenol-rich layers obtained when other proportions of dilute hydrochloric acid and phenol were used gave almost identical chromatograms to those obtained with Steel's solvent mixture. Lithium coincided with and sodium was above the potassium position.

In attempts to get lithium below potassium, rubidium and caesium, methanol was introduced into the mixtures of phenol and concentrated hydrochloric acid. The relative positions of the cations with a number of these solvent mixtures and with others containing derivatives of phenol are given in Table II, Whatman No. 1 filter-paper being used. The R_F values refer to the mid-points of each band.

TABLE II

SEPARATION OF 1-μM AMOUNTS OF ALKALI-METAL AND AMMONIUM IONS

Solvent mixture No.	Solvent mixture			R_F values for Li+	Na+	K+	Rb+	Cs+	NH_4+
1	Phenol saturated with 20 per cent. hydrochloric acid			0·11	0·07	0·12	0·18	0·43	0·20
2	Of composition—								
	Phenol, g	Methanol, ml	Concentrated hydrochloric acid, ml						
(*a*)	33	33	33	0·55	0·24	0·21	0·28	0·42	0·42
(*b*)	42	16	42	0·42	0·27	0·31	0·43	0·55	0·31
(*c*)	20	30	50	0·70	0·44	0·43	0·50	0·64	0·64
(*d*)	50	30	20	0·37	0·14	0·10	0·16	0·30	0·30
(*e*)	25	50	25	0·57	0·19	0·11	0·14	0·23	—
(*f*)	40	50	10	0·51	0·11	0·05	0·08	0·15	—
(*g*)	50	40	10	0·37	0·06	0·04	0·06	0·13	—
(*h*)	40	40	20	0·45	0·12	0·08	0·12	0·21	—
(*i*)	60	20	20	0·27	0·11	0·12	0·19	0·38	0·24
3	Cresol-rich layer from *m*-cresol - concentrated hydrochloric acid (90:10 per cent. v/v)*			0·09	0·01	0·04	0·09	0·31	—
4	Catechol - methanol - concentrated hydrochloric acid (45:35:20 per cent. v/v)			Unsatisfactory chromatograms owing to the dark backgrounds produced with the spraying reagents					
5	Resorcinol - methanol - concentrated hydrochloric acid (50:30:20 per cent. v/v)			As with solvent mixture No. 4.					

* Whatman No. 4 filter-paper was used with this solvent mixture.

With a methanol content of 30 to 35 per cent. in solvent mixtures containing phenol and concentrated hydrochloric acid (mixtures No. 2, (*c*) and (*d*)), potassium, rubidium, caesium and lithium were separated in that order, but the sodium could not be removed from the vicinity of the potassium. The separation of potassium and rubidium could not be effected when the methanol content exceeded 35 per cent.

From these investigations it was evident that all the alkali metals and the ammonium ion could only be separated by using two-dimensional chromatography, with, for example, a methanol - *n*-butanol mixture (70 to 30 per cent. v/v) for the separation of potassium, sodium, ammonium and lithium, and a solvent mixture consisting of phenol, methanol and concentrated hydrochloric acid for potassium, rubidium and caesium. However, for the purpose of detection and rough estimation, a solvent mixture able to separate the groups potassium, rubidium, caesium and ammonium, and lithium and sodium would be sufficient, since selective spraying reagents could be used for the detection of the cations in each group.

The best spacing of potassium, rubidium, ammonium and caesium is achieved with a phenol - methanol - concentrated hydrochloric acid mixture (57·5:22·5:20 per cent. w/v/v), with which 1·7-μM amounts of the alkali-metal and ammonium ions (10 μM total) in a run of 50 cm on Whatman No. 1 filter-paper gave the following R_F values: potassium, 0·08 to 0·14;

sodium, 0·09 to 0·13; rubidium, 0·15 to 0·20; ammonium, 0·23 to 0·27; lithium, 0·27 to 0·32; and caesium, 0·33 to 0·40. The separations are better on the fast Whatman No. 4 filter-paper. This solvent mixture gives bands superior to those obtained with the solvent mixtures used by Miller and Magee, and by Steel.

Method

Apparatus and reagents—

Descending chromatography was employed. Chromatograms were produced in a Shandon 9-inch Universal-strip glass "Chromatank," having an over-all height of 22 inches and a solvent trough 7¾ inches long. This apparatus accommodates sheets of chromatographic paper 16 cm wide and permits the solvent-front to travel a distance of 50 cm. The temperature of the room was maintained at 18° to 20° C. Preliminary investigations with the alkali metals were carried out with use of Whatman No. 1 and No. 4 filter-papers, but in later work Whatman No. 41 filter-paper was used. For all experiments with other metals Whatman No. 1 filter-paper was used.

In each test a 0·01-ml portion of the solution was applied as a spot to the paper from capillary tubes calibrated by means of an Agla micrometer syringe. Four such spots could be accommodated by placing them 4 cm apart, across the paper. After their removal from the tank, the strips were dried by being heated in an oven at 110° C for 10 minutes. The strips were held taut and sprayed by means of a simple compressed-air atomiser.

Molar stock solutions of the group IA cations were prepared as follows—

Lithium—Prepared from anhydrous lithium chloride (obtained from The British Drug Houses Ltd.).

Sodium and potassium—Prepared from AnalaR sodium and potassium chlorides.

Rubidium and caesium—Prepared from ordinary grade rubidium and caesium carbonates (obtained from Johnson, Matthey & Co. Ltd.), dissolved in dilute hydrochloric acid.

While preparing standards, it was noticed that the rubidium solution contained some caesium (about 0·25 μM per 10 μM), and for further work a molar solution prepared from Specpure rubidium chloride was used.

The following reagents were used in the detection of the cations on the paper strip by spraying—

Lithium and sodium—Zinc uranyl acetate reagent prepared as described by Barber and Kolthoff.[7]

Potassium, rubidium, caesium and ammonium—Sodium lead cobaltous hexanitrite reagent prepared as follows—

> Dissolve 12·5 g of cobaltous acetate, $Co(CH_3COO)_2.4H_2O$, and 19·0 g of lead acetate, $Pb(CH_3COO)_2.3H_2O$, in about 100 ml of water. To this solution add a solution of 20·7 g of sodium nitrite in about 100 ml of water. Mix thoroughly and dilute to 250 ml. Set aside for 1 hour and then filter. The reagent corresponds to the formula $Na_2PbCo^{II}(NO_2)_6$.

This reagent is similar to that used by Sergienko[8] for the quantitative determination of potassium, but cobalt and lead acetates are substituted for cobalt and lead nitrates.

Procedure—

Place the solvent mixture in the solvent trough 1 hour or more before the experiment is started. On the appropriate Whatman filter-paper strip place 0·01 ml of the test solution and allow the strip to dry before inserting it in the chromatographic vessel. Allow the solvent to run down the paper for 16 to 18 hours (with Whatman No. 41 filter-paper the solvent-front reaches the foot of the paper within this time and is allowed to drip off evenly by notching the bottom edge before inserting the paper). Remove the paper from the apparatus and dry it.

Detection and determination of lithium and sodium—

Spray the paper with the zinc uranyl acetate reagent and hang it in the air for 1 hour to ensure complete drying. Examine it under ultra-violet light. Lithium and sodium fluoresce brilliantly (turquoise-green). For determination purposes compare the bands produced with a set of standards, prepared from 0·1, 0·25, 0·5, 1, 2·5, 5 and 10-μM amounts of the cations on Whatman No. 41 filter-paper.

DETECTION AND DETERMINATION OF POTASSIUM, RUBIDIUM, CAESIUM AND AMMONIUM—

Spray the paper with ethanol and then immediately after with sodium lead cobaltous hexanitrite reagent. Leave the paper in the air for 10 minutes to ensure the complete development of the ammonium precipitate and then wash off the excess of reagent, which itself forms a pale yellow background. Allow the paper to dry and compare it with a set of standards prepared as before. Potassium and ammonium appear as grey bands, rubidium as a brown band and caesium as a yellow-brown band. The best results are obtained when a freshly prepared reagent is used.

RESULTS

By using the above procedure, 0·1 μM of sodium, potassium, rubidium and caesium can be readily detected. The minimum amounts of lithium and ammonium detectable are 0·25 μM. The minimum amount of any of the metals can be detected in the presence of 10 μM of any other or total of the others. In an overnight experiment lasting 16 hours a mixture of 1 μM of each of the six cations travelled the following distances: sodium, 5·4 to 8·4 cm; potassium, 6·4 to 9·1 cm; rubidium, 10·2 to 13·3 cm; ammonium, 15·2 to 17·7 cm; lithium, 18·1 to 20·6 cm; and caesium, 21·3 to 25·3 cm. The first and second figures give the distances to the back and front of the spots, respectively.

As a test of the validity of the proposed scheme, 10 mixtures of unknown composition were submitted to one of us (J.B.H.) for analysis. The results are shown in Table III.

TABLE III

ANALYSIS OF UNKNOWN MIXTURES

Mixture No.	Lithium found, μM	Sodium found, μM	Potassium found, μM	Rubidium found, μM	Caesium found, μM	Ammonium found, μM
1	0	0·1(0·20)	9	0·25(0·2)	0·5	0·5
2	0	0·25(0·1)	0·5(0·1)	5(10)	0·25(0·2)	0·5(0·25)
3	9(10)	0·25(0·1)	0	0·25(0·1)	0·1	0·5(0·3)
4	2·5(3)	0·25(0)	0	0·25(0·1)	2(7)	0·5(0·3)
5	0·25	0·1	0·25(0·1)	0	8(10)	0
6	0	0	0·5(0·1)	0·1	0	9(10)
7	0·25	9	0·25(0·2)	0·25(0·2)	0·1(0·2)	1
8	0·25	0·1	5	0	0	3(5)
9	4(3)	0	0	0·4(0·1)	0·8(0·3)	5(7)
10	0·5	0·1(0)	0·25(0·2)	0·3(0·2)	10	0

NOTE—The figures in parentheses are the amounts present when they differ from the amounts found.

In mixtures No. 4 and No. 10, a small amount of sodium was found, although none had been added to these mixtures. It was observed that this sodium was present in mixtures containing large amounts of caesium and, by producing a chromatogram with 10 μM of caesium and spraying it with zinc uranyl acetate reagent, it was confirmed that the caesium stock solution contained a small percentage of sodium as an impurity. The results are, however, considered satisfactory.

APPLICATION TO THE ANALYSIS OF A SILICATE ROCK

The success of the method of separation described suggested its application to the analysis of rocks containing rubidium and caesium, such as lepidolite and pollucite. The method was therefore applied to the determination of the alkali metals present in a lepidolite that had been previously analysed by Miller and Traves[9] and contained 1·6 per cent. of lithium, 0·1 per cent. of sodium and 5·8 per cent. of potassium.

A 50-mg sample of the finely ground lepidolite was subjected to a Lawrence-Smith fusion.[10] The fused mass was digested with water for 30 minutes on a steam-bath and the insoluble material was filtered off. The calcium in the solution was precipitated as the carbonate, which was dissolved in dilute hydrochloric acid and re-precipitated. The final solution was evaporated to dryness in a platinum vessel and the residue was ignited to remove the ammonium salts. The residue was dissolved in 0·25 ml of dilute hydrochloric acid and 0·01-ml portions were spotted on to the paper strip. The separated salts were detected in the usual manner and it was estimated that 2 mg of the rock contained 5 μM of lithium, 0·1 μM of

sodium, 2 μM of potassium, 0·1 μM of rubidium and no caesium. These figures correspond to 1·7 per cent. of lithium, 0·1 per cent. of sodium, 3·9 per cent. of potassium and 0·4 per cent. of rubidium.

The use of the proposed method for separating the alkali metals with a view to their subsequent quantitative determination by micro-techniques[11] is at present under investigation.

SEPARATION OF CERTAIN OTHER METALS

The R_F values for 100-μg amounts of certain other metals with a phenol - methanol - concentrated hydrochloric acid mixture (57·5 : 22·5 : 20 per cent. w/v/v) on Whatman No. 1 filter-paper are given in Table IV.

TABLE IV

R_F VALUES OF SOME METALS WITH THE SOLVENT MIXTURE USED

Metal	R_F	Metal	R_F	Metal	R_F
Copper	0·22 ± 0·04	Chromium^{III}	0·12 ± 0·04	Nickel	0·14 ± 0·04
Mercury^{II}	0·34 ± 0·04	Aluminium	0·10 ± 0·05	Zinc	0·35 ± 0·05
Cadmium	0·30 ± 0·05	Gallium	0·66 ± 0·06	Beryllium*	0·23 ± 0·10
Bismuth	0·20 ± 0·04	Indium	0·20 ± 0·05	Magnesium†	0·17 ± 0·04
Antimony^{III}	0·44 ± 0·05	Thallium^{III}	0·53 ± 0·05	Calcium†	0·70 ± 0·04
Tin^{IV}	0·37 ± 0·04	Manganese^{II}	0·16 ± 0·04	Strontium†	0·03 ± 0·03
Iron^{III}	0·39 ± 0·05	Cobalt	0·16 ± 0·04	Barium†	0

* Sulphate taken. † Nitrate taken.

It can be seen that, in addition to the group IA metals of the Periodic Table, this solvent mixture separates many elements, including those of group IIIB, *viz.*, aluminium, indium, gallium and thallium. The separation of these elements with aqueous hydrochloric acid and aliphatic-alcohol solvent mixtures has been mentioned before.[12,13] Improvements in the separation of the group IIIB metals were made by varying the ratios of phenol, methanol and hydrochloric acid. They are separated with solvent mixtures containing 20 to 40 per cent. of concentrated hydrochloric acid and 15 to 20 per cent. of methanol. The best separation achieved was with a phenol - methanol - concentrated hydrochloric acid mixture (50 : 20 : 30 per cent. w/v/v).

METHOD

APPARATUS AND REAGENTS—

The apparatus was the same as that used for the alkali metals. Whatman No. 1 filter-paper was used throughout. Solutions of the metals were prepared by dissolving the chlorides in water or dilute acid. The concentration was 10 g of metal per litre.

The following reagents were used in the detection of the cations on the paper strips—

Aluminium, gallium, indium, zinc and iron—A 5 per cent. w/v solution of 8-hydroxyquinoline in a methanol - chloroform - water mixture (85 : 10 : 5 per cent. v/v). This reagent was used by Miller and Magee[2] for the detection of magnesium and calcium.

Thallium—A 5 per cent. w/v aqueous solution of potassium iodide.

Titanium—A 1 per cent. w/v aqueous solution of chromotropic acid.

Zirconium—Ethanol saturated with alizarin.

PROCEDURE—

The procedure was the same as that for the alkali metals (p. 98).

DETECTION OF ALUMINIUM, GALLIUM, INDIUM, ZINC AND IRON—

Spray the paper with 8-hydroxyquinoline reagent and hang it in a closed vessel containing a beaker of concentrated ammonia solution. Iron appears as a black band. Allow the paper to dry and examine it under ultra-violet light. Aluminium, gallium, indium and zinc fluoresce a brilliant yellow.

DETECTION OF THALLIUM, TITANIUM AND ZIRCONIUM—

Spray the paper with the appropriate reagent. Thallium shows as a yellow band, titanium as a red-brown band and zirconium as a purple band.

Results

The R_F values of 100-μg amounts of a few metals obtained with the phenol - methanol - concentrated hydrochloric acid mixture (50:20:30 per cent. w/v/v) in a 16-hour experiment are: zirconium, 0·09 ± 0·06; aluminium, 0·13 ± 0·05; indium, 0·28 ± 0·04; titanium, 0·34 ± 0·06; zinc, 0·40 ± 0·05; thallium^{III}, 0·58 ± 0·06; iron, 0·64 ± 0·06; and gallium, 0·73 ± 0·07.

It was found that 1-μg amounts of aluminium, gallium, indium and zinc were readily detected with 8-hydroxyquinoline and 1 μg of gallium could be separated from 1000 μg of aluminium with this solvent mixture.

These results suggest that the solvent mixture might be of use in the qualitative analysis of certain aluminium alloys.

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The Identification of Nylon and Related Polymers by Paper Chromatography

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A simple procedure has been developed for the identification of commercial samples of nylon and related polymers. It is based on earlier work carried out by Zahn and Wollemann and by Stühlen and Horn.

After hydrolysis of the sample with acid, the solution is evaporated to dryness and the hydrolysis products thus obtained are dissolved in ethanol and separated by paper chromatography, a mixture of *n*-propanol, ammonia and water being used as the developing solvent. The various products are identified by observation of the chromatogram under ultra-violet light and by using ninhydrin and methyl red - borate buffer solutions as spray reagents.

In 1951, Zahn and Wolf,[1] stimulated, as they said, by work carried out by two of us,[2] described a method of differentiating between polyhexamethylenediamineadipamide (nylon 66), polyhexamethylenediaminesebacamide (nylon 610), polycaproamide (nylon 6) and polyurethane (Perlon U), as well as a mixed condensate of caprolactam with 5 per cent. of nylon 66. Their method involved the hydrolysis of the sample with hydrochloric acid, followed by the preparation of a solution of the hydrolysis product buffered to a pH of 7·5 and a paper-chromatographic test of this solution. Two chromatograms were prepared. The first chromatogram was used for the detection of amine components, a *sec.*-butanol - formic acid - water mixture being used as the developing solvent. The fluorescence of the dried paper was observed, as well as its behaviour after having been sprayed with the prepared ninhydrin reagent; Sanger's reagent and Pauly's diazo reagent were also tried. The second chromatogram was prepared with use of a mixture of *iso*butanol, ammonia and glycol as developing solvent. After the paper had been dried, it was sprayed with bromothymol blue

in order to obtain evidence of the presence of adipic acid and sebacic acid. It is obvious, however, from Zahn and Wolf's paper[1] that their separation of the two acids was unsatisfactory.

Realising this, Zahn and Wollemann[3] carried out further work and showed that, if such acids as adipic and sebacic were first extracted from the hydrolysis products of the nylon with ether, these mixed acids as their sodium salts could then be much more satisfactorily resolved by use of a developing solvent consisting of *n*-propanol, ammonia and water. Indeed, it was suggested in principle that it might be possible to separate all the hydrolysis products by using this solvent mixture.

Stühlen and Horn[4] examined the procedures adopted by Zahn and Wolf[1] and Zahn and Wollemann[3]; they used a mixture of *n*-propanol, ammonia and water as developing solvent in an effort to develop a simple method of differentiation of commercial polymers. They found no difficulty in detecting the amine components with Pauly's diazo reagent, but encountered considerable difficulties in detecting the acid components and in particular sebacic acid by the bromothymol blue spraying method of Zahn and Wollemann.[3] As a result of their investigation, Stühlen and Horn[4] put forward a procedure for the differentiation of commercial polyamides. This involved—

(*a*) the determination of the melting point of the original polyamide,
(*b*) the hydrolysis of 0·4 g of the polyamide with 6 *N* hydrochloric acid; observations were made of the appearance of the hydrolysis product before and after cooling, and
(*c*) the separation by filtration of any crystals formed from the hydrolysis product and the determination of their melting point. If necessary, mixed-melting-point determinations were carried out with adipic and sebacic acids.

This procedure permitted them to differentiate quite readily between nylon 66, nylon 610, nylon 6 and polyundecanoamide (nylon 11), but a satisfactory differentiation between the copolymers nylon 66/6 and nylon 66/6/PACM 6 was not achieved. They found it necessary to filter off any acid precipitated as a result of the hydrolysis, to evaporate the filtrate to dryness and then to chromatograph the residue on paper, with a *n*-propanol - ammonia - water mixture as developing solvent. Subsequent use of Pauly's diazo reagent permitted them to differentiate satisfactorily between the two copolymers.

Although we were aware of the work of Ayers[5] on the direct chromatographic separation of polyamides, it seemed to us that both of Zahn's papers[1,3] and Stühlen and Horn's paper[4] contained many useful points, some of which could be embodied in a simple procedure for the examination of commercial polyamides.

Such a simple method, in which only 25 mg of substance are used, has been worked out and is based on the following procedures—

(*a*) hydrolysis of the polyamide with 50 per cent. v/v hydrochloric acid: with observations of the behaviour of the polyamide in this hydrolysis,
(*b*) after evaporation of the hydrolysis product to dryness and removal of the excess of hydrochloric acid by further evaporation with water, the residue is dissolved in ethanol and observations are made on the solution process, and
(*c*) the ethanolic solution is chromatographed in duplicate, with *n*-propanol - ammonia - water mixture as the developing solvent: one of the chromatograms is dried and examined under ultra-violet light, after which it is sprayed with ninhydrin reagent; the other chromatogram is sprayed with a methyl red - borate buffer solution.

The methyl red - borate buffer reagent arises from work that was being carried out in this laboratory on the separation of adipic and sebacic acids. Kalbe[6] in an investigation of the paper chromatography of aliphatic dicarboxylic acids reached the conclusion that a reagent prepared so as to contain 0·03 per cent. of methyl red in 0·05 *M* borate buffer of pH 8·0 was an extraordinarily sensitive reagent for the detection of organic aliphatic acids on paper chromatograms. A reagent of slightly different concentration, which had proved to be particularly satisfactory in chromatographic work on adipic and sebacic acids, proved to be particularly valuable in this chromatographic examination of the hydrolysis products of polyamides. This particular spray reagent, on comparative testing, proved to be immeasurably superior to the bromothymol blue spray reagent of pH 10·0 used by Zahn and Wollemann.[3] From a reading of Zahn and Wollemann's paper it appeared that their spray reagent of pH 10·0 was adapted from the work of Brown,[7] who used a similar bromothymol

blue spray reagent of pH 7·5; the latter reagent was also found to be unsatisfactory. The methyl red - borate buffer spray gave trustworthy information about the acids present in the hydrolysis products and, moreover, about such amine components as hexamethylenediamine and *p*-diaminodi*cyclo*hexylmethane.

The complete procedure incorporating the principles enumerated above has permitted us to differentiate quite readily between commercial polyamides and copolymers. Full details of the tests are given below, followed by information about the behaviour of various polymers in the test.

METHOD

APPARATUS—

Aimer Universal chromatographic tank.

Filter-paper, Whatman No. 1, for chromatography, 10 inches × 10 inches, with corner holes.

Glass tubes, prepared from glass tubing having an internal diameter of about 6 mm; the length of each tube is approximately 100 mm.

REAGENTS—

Developing solvent—Mix 6 parts of *n*-propanol, 3 parts of ammonia solution, sp.gr. 0·880, and 1 part of water, by volume.

Methyl red - borate buffer reagent—Dissolve 12·368 g of analytical-reagent grade boric acid and 14·912 g of analytical-reagent grade potassium chloride in water in a 1-litre calibrated flask, then add 35·0 ml of *N* sodium hydroxide solution and dilute to the mark with water. Check the pH of this solution, which should be 8·0 ± 0·1. Dissolve 0·03 g of methyl red indicator in 100 ml of the buffer solution, shaking frequently to ensure complete solution.

Ninhydrin reagent—Dissolve 0·3 g of ninhydrin in a mixture of 95 parts of *n*-butanol and 5 parts of 2 *N* acetic acid, by volume.

Hydrochloric acid, diluted (1 + 1).

PROCEDURE—

Weigh 25 mg of the substance into a glass tube and add 0·5 ml of diluted hydrochloric acid (1 + 1). Seal the glass tube carefully in a gas flame, then place it in an upright position in an oven at 120° C and leave it overnight. After removing the tube from the oven, make observations on the appearance of the solution; allow it to cool and make further observations. On opening the tube, note any odour that may be evolved, and then transfer the contents of the tube to a 10-ml beaker, washing with 1 to 2 ml of water. Observe any change that may take place on the addition of water. Evaporate the contents of the beaker to dryness once or twice, with small additions of water to remove the hydrochloric acid completely, and finally dry in an oven at 105° C for 15 to 20 minutes. Dissolve the residue in 1 ml of ethanol and warm to effect solution. Note if there is any insoluble material. Now spot in duplicate 3 to 5 μl of the ethanolic solution on to a Whatman No. 1 10-inch × 10-inch filter-paper having corner holes on a line drawn 1½ inches from the edge of the paper. Six spots can be placed along this line, so that at least three samples can be dealt with at the same time. After spotting, fit the paper into the frame of the Aimer Universal chromatographic outfit. Put about 200 ml of the *n*-propanol - ammonia - water developing solvent into the trough in the chromatographic tank and, after allowing the solvent to equilibrate with the atmosphere in the tank for about 1 hour, place the frame in position in the tank and allow the chromatograms to develop for about 7 hours. At the end of this time remove the frame and allow the paper to dry in the air. When the paper is dry, place it in an oven at 105° C for 15 minutes and then examine it under ultra-violet light. Now separate the duplicate chromatograms and spray the first chromatogram with the ninhydrin reagent, and, after allowing the paper to dry in the air, place it in an oven at 105° C for 5 minutes. Observe the position and colour of the spots obtained.

Spray the duplicate chromatogram with the methyl red - borate buffer reagent and dry it by placing it between sheets of filter-paper. After 10 minutes make observations on the position and colour of the spots that develop, and repeat these observations after a further 20 minutes.

TABLE II: EXAMINATION OF COMMERCIAL

	Nylon 66	Nylon 610	Nylon 6	Nylon 11
1. Observation in hydrolysis tube—				
(*a*) while still hot	Complete solution	Complete solution	Complete solution	Small amount of insoluble matter
(*b*) after cooling	Tendency for crystal formation; white precipitate on adding water	Heavy white crystalline precipitate	Complete solution	Sets solid owing to mass of fine white crystals
2. Odour on opening hydrolysis tube	No characteristic odour	No characteristic odour	No characteristic odour	No characteristic odour
3. Solubility in ethanol	Completely soluble	Completely soluble	Completely soluble	Completely soluble
4. Examination under ultra-violet light	Spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). Also slight evidence of adipic acid spot (R_F 0·28 to 0·36)	Spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). Also slight evidence of sebacic acid spot (R_F 0·59 to 0·64)	Spot due to 5-aminocaproic acid hydrochloride (R_F 0·4 to 0·52).	Large spot in same place as for hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)
5. Examination after spraying with ninhydrin reagent	Purple spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). Also faint brown spot due to adipic acid (R_F 0·28 to 0·36)	Purple spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). Also faint brown spot due to sebacic acid (R_F 0·59 to 0·64)	Purple spot due to 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52)	Large purple spot in same place as hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)
6. Examination after spraying with methyl red - borate buffer reagent—				
(*a*) after initial 10 minutes; colour of paper: yellow	Pink spot due to adipic acid (R_F 0·28 to 0·36). Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spot due to sebacic acid (R_F 0·59 to 0·64). Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spot due to ammonium chloride (R_F 0·47 to 0·52)
(*b*) after 30 minutes; colour of paper: yellow turning to pink	Pink spots become more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Pink spots become more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Pink spot becomes more pronounced. No evidence of of any further spots	Pink spot becomes more pronounced. No evidence of of any further spots

NYLON POLYMERS AND COPOLYMERS

Igamide U	Nylon 66/610 (40:60)	Nylon 66/6 (60:40)	Nylon 66/610/6 (40:30:30)	Nylon 66/6/ PACM 6 (1:1:1)
Insoluble matter present	Complete solution	Complete solution	Complete solution	Complete solution
No change. Same insoluble matter present	Heavy white crystalline precipitate			
Sweet odour	No characteristic odour	No characteristic odour	No characteristic odour	No characteristic odour
Insoluble matter present	Completely soluble	Completely soluble	Completely soluble	Completely soluble
Spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). Also slight evidence of adipic acid spot (R_F 0·28 to 0·36) and sebacic acid spot (R_F 0·59 to 0·64)	Spots due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86) and 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52). Also slight evidence of adipic acid spot (R_F 0·28 to 0·36)	Spots due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86) and 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52). Also slight evidence of adipic acid spot (R_F 0·28 to 0·36)	Spots due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86) and 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52). Also evidence of a *p*-diaminodi*cyclo*hexylmethane dihydrochloride spot (R_F 0·98) and an adipic acid spot (R_F 0·28 to 0·36)
Purple spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Purple spot due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). Also faint brown spots due to adipic acid (R_F 0·28 to 0·36) and sebacic acid (R_F 0·59 to 0·64)	Purple spots due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86). and 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52). Also faint brown spot due to adipic acid (R_F 0·28 to 0·36)	Purple spots due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86) and 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52). Also faint brown spots due to adipic acid (R_F 0·28 to 0·36) and sebacic acid (R_F 0·59 to 0·64)	Purple spots due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86) and 5-aminocaproic acid hydrochloride (R_F 0·47 to 0·52) and *p*-diaminodi*cyclo*hexylmethane dihydrochloride (R_F 0·98). Also faint brown spot due to adipic acid (R_F 0·28 to 0·36)
Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spots due to adipic acid (R_F 0·28 to 0·36) and sebacic acid (R_F 0·59 to 0·64). Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spot due to adipic acid (R_F 0·28 to 0·36). Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spots due to adipic acid (R_F 0·28 to 0·36) and sebacic acid (R_F 0·59 to 0·64). Pink spot due to ammonium chloride (R_F 0·47 to 0·52)	Pink spot due to adipic acid (R_F 0·28 to 0·36). Pink spot due to ammonium chloride (R_F 0·47 to 0·52)
Pink spot becomes more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Pink spots become more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Pink spots become more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Pink spots become more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86)	Pink spots become more pronounced. Yellow spot develops due to hexamethylenediamine dihydrochloride (R_F 0·81 to 0·86) and *p*-diaminodi*cyclo*hexylmethane dihydrochloride (R_F 0·98)

Results

Chromatograms from typical commercial nylon samples after being sprayed with ninhydrin and methyl red - borate buffer are shown in Figs. 1 and 2, respectively.

Observations made on the behaviour of the various substances obtained on hydrolysis of nylon-type materials when they are subjected to the conditions of the test described above are shown in Table I. Examination of the Table shows that a spot of R_F value 0·48 to 0·55 is common to all the hydrochloride materials. This spot does not fluoresce under ultra-violet light and gives no colour with the ninhydrin reagent; it does, however, give a pink colour with the methyl red - borate buffer reagent. The R_F values of this spot and the spot given by 5-aminocaproic acid hydrochloride are similar, but these spots can easily be differentiated, as the latter gives a fluorescence under ultra-violet light and a purple colour with ninhydrin reagent. It has been proved that this spot is due to the presence of ammonium chloride, formed by the reaction of the ammonia in the developing solvent with the chloride ion in the base hydrochlorides.

Table I

Examination of the substances obtained on hydrolysis of nylon-type polymers

Substance	R_F value	Examination under ultra-violet light	Effect of ninhydrin reagent	Effect of methyl red - borate buffer reagent added	
				after 10 minutes	after 30 minutes
Adipic acid	0·28 to 0·36	Faint fluorescence	Faint brown spot	Pink spot	Pink spot
Sebacic acid	0·59 to 0·64	Faint fluorescence	Faint brown spot	Pink spot	Pink spot
Hexamethylenediamine dihydrochloride	0·47 to 0·52 0·81 to 0·86	No fluorescence Strong fluorescence	No spot Purple spot	Pink spot Faint yellow spot	Pink spot Definite yellow spot
p-Diamino*dicyclo*hexyl-methane dihydrochloride	0·47 to 0·52 0·98	No fluorescence Strong fluorescence	No spot Purple spot	Pink spot Faint yellow spot	Pink spot Definite yellow spot
5-Aminocaproic acid hydrochloride	0·47 to 0·52	Strong fluorescence	Purple spot	Pink spot	Pink spot
10-Aminoundecanoic acid hydrochloride	0·47 to 0·52 0·81 to 0·86	No fluorescence Strong fluorescence	No spot Purple spot	Pink spot No spot	Pink spot No spot

Various commercial nylon polymers and copolymers have been examined by the test described above and details of the results obtained are shown in Table II.

We have found that the small purple spots that are sometimes observed after use of the ninhydrin reagent are not due to the normal base hydrochlorides. These spots are particularly noticeable with copolymers. We have no satisfactory explanation as yet for this behaviour, although it may be due to small proportions of impurities in the commercial samples. For the time being such spots are disregarded and have not been described in Table II.

The test for the identification of nylons described in this paper has been found to be useful when applied to various types of nylons that are not strictly speaking commercial samples, for example—

(*i*) Examination of a sample of polyheptoamide (nylon 7) showed that a spot due to 6-aminoheptoic acid hydrochloride was obtained; it had an R_F value of approximately 0·72. This spot behaved in the same manner in the test as that due to 5-aminocaproic acid hydrochloride. It was also found by applying the test that the presence of nylon 7 in nylon 6 could quite readily be detected.

(*ii*) Examination of a sample of nylon 66/610/6 containing 14 per cent. of Santiciser 8 (a mixture of N-ethyltoluene-*o*-sulphonamide and -*p*-sulphonamide) as plasticiser gave results that were exactly similar to those of unplasticised nylon 66/610/6 (see Table II); there was no evidence of any interference by the plasticiser.

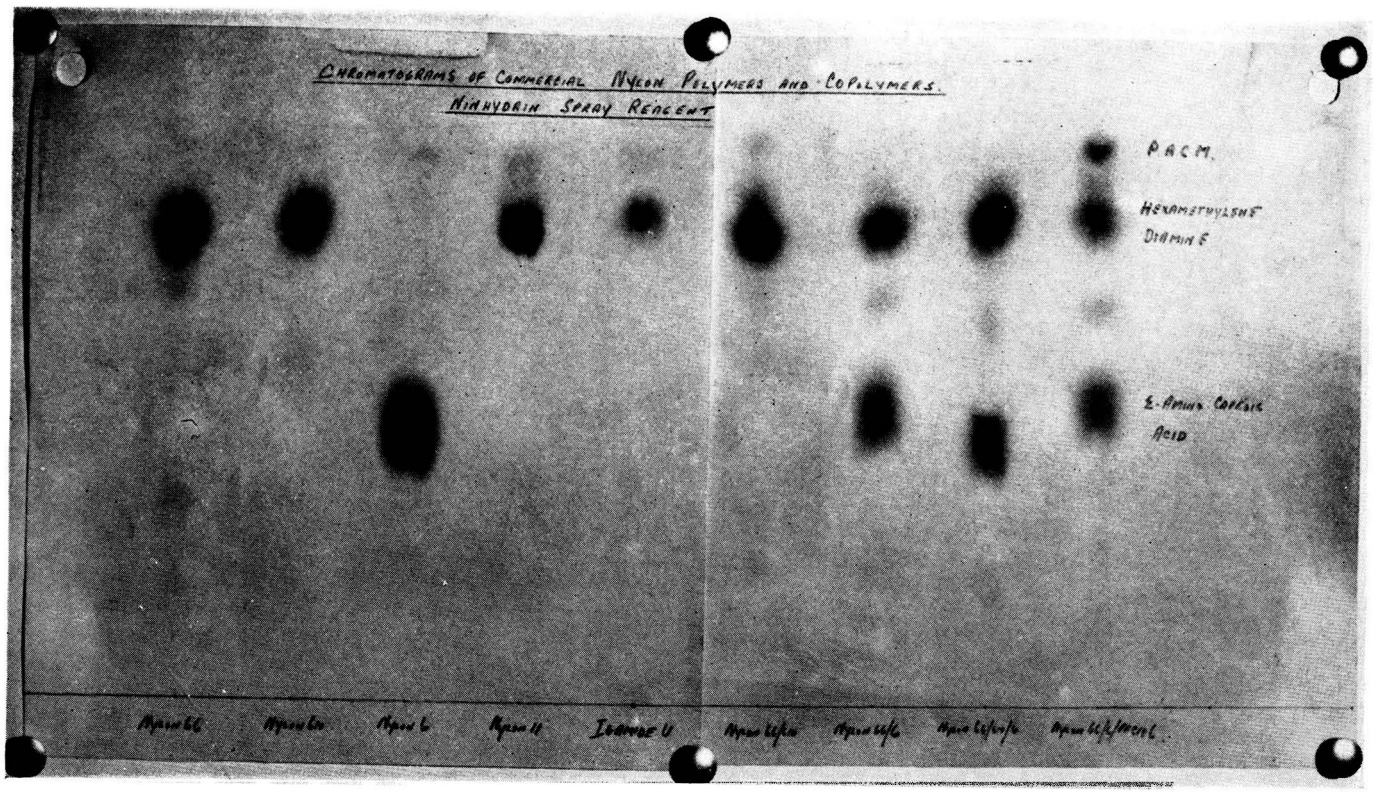

Fig. 1. Chromatograms of commercial nylon polymers and copolymers: ninhydrin spray reagent

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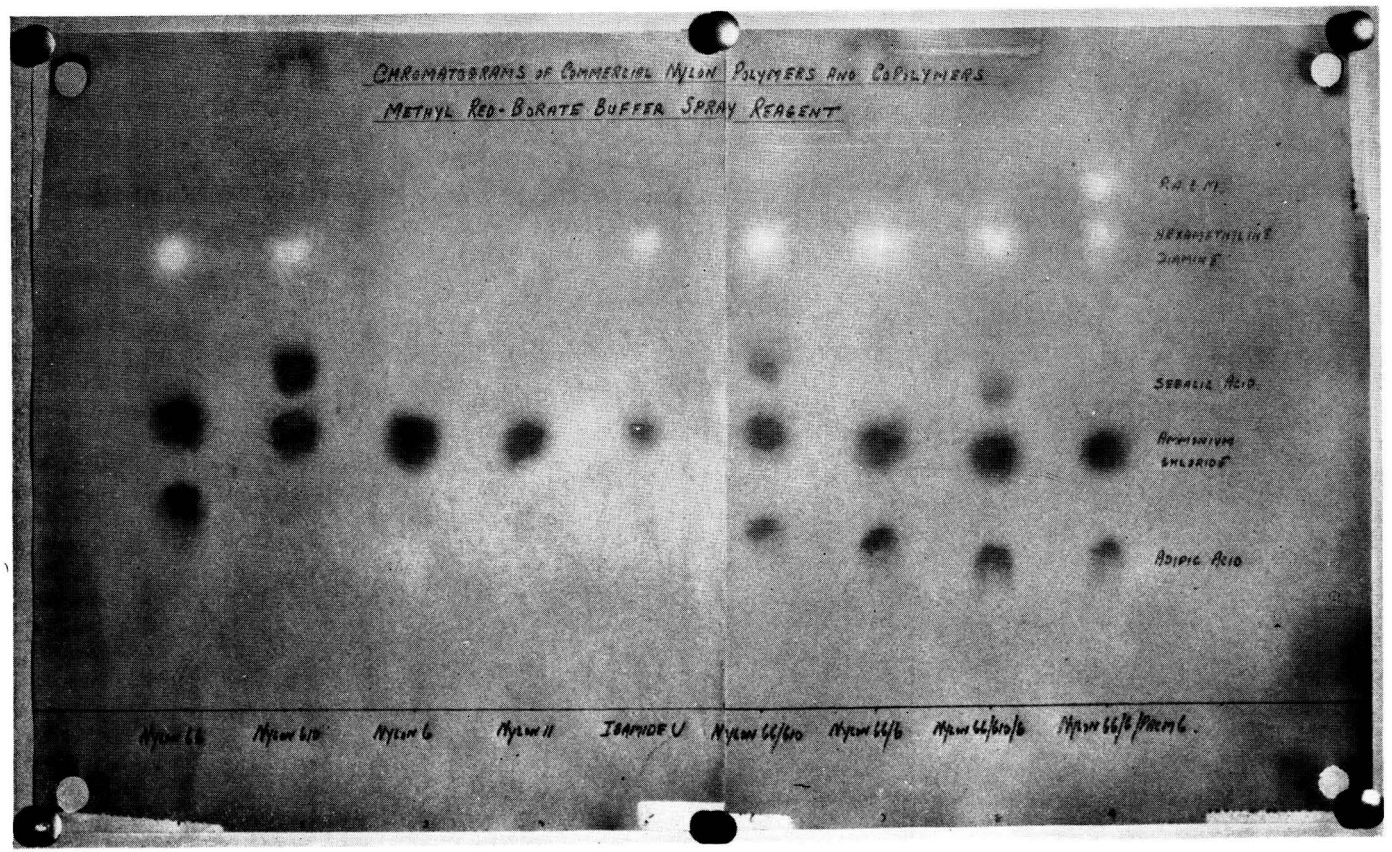

Fig. 2. Chromatograms of commercial nylon polymers and copolymers: methyl red - borate buffer spray reagent

(*iii*) Examination of an interpolymer of nylon 66/6 containing 5 per cent. of nylon 6 showed that this amount could readily be detected; the spot due to the 5-amino-caproic acid hydrochloride in the hydrolysis products from the nylon 6 was quite apparent under ultra-violet light and with the ninhydrin spray reagent.

NOTE—If a derivative of nylon 66, such as methoxymethylnylon, is suspected, it is obviously desirable to test whether formaldehyde is liberated on treatment with acid. If this derivative is present, a distinct odour of formaldehyde will be detected on evaporation of the hydrolysis product.

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Determination of Glycollic Acid in Used Antifreeze Solutions

BY H. GREEN

A method is described for the determination of the glycollic acid content of used antifreeze solutions of the inhibited ethylene glycol type. Insoluble matter is removed by treatment with zinc oxide and soluble cations are removed by means of an ion-exchange resin. Separation of the glycollic acid from ethylene glycol is achieved by the use of an anion-exchange resin. The glycollic acid is then eluted with sulphuric acid and treated with 2:7-dihydroxynaphthalene in concentrated sulphuric acid to give a coloured complex suitable for photometric measurement.

IN connection with the analysis of used antifreeze solutions of the inhibited ethylene glycol type (D.T.D. 779) taken from water-cooled automobile engines, it became necessary to determine glycollic acid (or its salts) occurring as a decomposition product of the glycol. The available literature indicated that the most sensitive test for glycollic acid was based on its reaction with 2:7-dihydroxynaphthalene in concentrated sulphuric acid at 100° C. At this temperature the initial product of the reaction is readily oxidised by air to form a pronounced red-violet colour.[1]

Used antifreeze solutions of the type referred to above contain various amounts of soluble colloidal and precipitated iron compounds, and Squires[2] has found that dissolved iron compounds can catalyse the air-oxidation of aqueous ethylene glycol at moderately elevated temperatures. It seemed possible, therefore, that aqueous glycol containing iron salts would give rise to glycollic acid when treated with 2:7-dihydroxynaphthalene reagent as described above, and exploratory tests proved this to be so. In these circumstances, it was decided to try to separate the glycol from glycollic acid before determining the latter.

As the pH of all the antifreeze solutions encountered lay between 4 and 8, it seemed probable that a separation of glycollic acid and ethylene glycol could be effected by the use of an anion-exchange resin, and, in practice, this proved to be so. A series of experiments was carried out in which aqueous solutions of ethylene glycol and glycollic acid were passed through the resin, which was then washed with water. The pH of the resulting solution was always the same as that of a similar volume of distilled water and ethylene glycol passed through the same resin. Tests with 2:7-dihydroxynaphthalene showed that the acid had not passed through the resin.

With the anion-exchange resin, the separation of ethylene glycol from glycollic acid was satisfactorily achieved with synthetic solutions, but when used antifreeze solutions were passed through the resin some of the precipitated iron compounds were adsorbed and, since they might interfere with subsequent manipulation of the sample, complete separation of the iron compounds from the glycollic acid was desirable.

Although the addition of a salt, such as sodium sulphate, followed by filtration was found to clear the solution of colloidal iron compounds, this procedure involved the use of a large quantity of resin to remove the added ions. This difficulty was overcome by treating the used antifreeze solution with a large excess of zinc oxide, followed by centrifuging. The colloidal iron compounds were collected in the insoluble residue of zinc oxide and massive ferric hydroxide. Any iron and zinc then in solution could be removed by using a cation-exchange resin.

Experimental conditions that gave concentrations of glycollic acid capable of being measured colorimetrically after reaction with the 2:7-dihydroxynaphthalene reagent were then established and an absorption graph was plotted. This showed a maximum absorption at 535 mμ (see Fig. 1). A series of standard glycollic acid solutions in aqueous glycol was

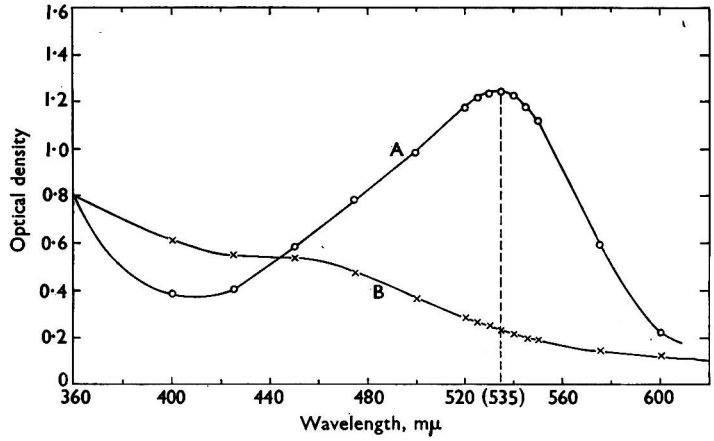

Fig. 1. Absorption curves: curve A, glycollic acid complex; curve B, reagent

then prepared, and the glycollic acid was separated from the glycol by using an anion-exchange resin as described above. The glycollic acid in the eluate was treated with the 2:7-dihydroxynaphthalene reagent to convert it into the red-violet coloured complex and

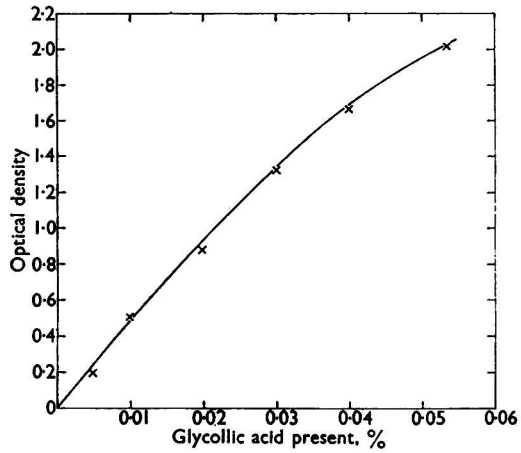

Fig. 2. Typical calibration curve for glycollic acid complex

the optical density of the resulting solutions was measured at 535 mμ. The calibration graph obtained (Fig. 2) showed a slight curve, but the deviation from Beer's law was small. The absorption of the solutions was measured at intervals up to 4 hours after they had been prepared without significant changes in optical density taking place. This procedure was repeated with the same standard solutions of glycollic acid in aqueous glycol and the 2:7-dihydroxynaphthalene reagent, and within experimental error the same calibration graph was obtained. A second solution of 2:7-dihydroxynaphthalene was prepared and the calibration graph was re-determined. The results obtained with the first and second 2:7-dihydroxynaphthalene solutions are shown in Table I.

TABLE I

REPRODUCIBILITY OF REAGENT SOLUTIONS

Concentration of glycollic acid, g per 100 ml of antifreeze	Optical density	
	Reagent 1	Reagent 2
0·005	0·313	0·320
0·010	0·480	0·486
0·020	0·845	0·840
0·030	1·153	1·150
0·040	1·356	1·358

A solution of the 2:7-dihydroxynaphthalene reagent was taken and the calibration graph was re-determined over a period of 3 weeks to investigate the effect of ageing of the reagent. The results obtained are shown in Table II, and indicate that up to 4 days after preparation of the reagent the calibration graph is reproducible. However, after 5 or 6 days the reagent solution gradually acquires a violet tint and the higher concentrations of glycollic acid fail to produce maximum colour intensity.

The method described below is designed for contents of glycollic acid in antifreeze solutions from 0·002 to 0·040 per cent. The method determines total glycollate-ion content of the solution and is not selective for "free" glycollic acid. The range of the determination can be extended by amending the original aliquot for zinc oxide treatment, since concentration of the glycollic acid into the required volume of 80 per cent. sulphuric acid is effected by the ion-exchange resin.

TABLE II

RELATIONSHIP BETWEEN OPTICAL DENSITY AND AGE OF REAGENT

Concentration of glycollic acid, g per 100 ml of antifreeze	Optical density on—				
	2nd day	3rd day	4th day	7th day	21st day
0·0025	0·182	0·196	0·190	0·185	0·185
0·005	0·313	0·321	0·319	0·308	0·302
0·010	0·480	0·475	0·476	0·472	0·475
0·020	0·845	0·856	0·854	0·855	0·860
0·030	1·153	1·156	1·150	1·152	1·140
0·040	1·356	1·341	1·340	1·275	1·258

METHOD

REAGENT—

2:7-*Dihydroxynaphthalene reagent*—Dissolve 0·01 g of 2:7-dihydroxynaphthalene in concentrated sulphuric acid to 100 ml. The solution changes colour on standing from yellow to blue, and this change, which takes about 24 hours, must be allowed to occur before the reagent is used.

PROCEDURE—

Put a 10-ml sample of the antifreeze solution by pipette into a centrifuge tube and add 40 ml of water, measured accurately, followed by 5 to 10 g of solid zinc oxide. Mix the solution thoroughly and revolve it in a centrifuge at 2000 r.p.m. and 10 cm radius for 5 minutes. Transfer a 25-ml aliquot of the supernatant liquid to a 100-ml beaker and add 1 to 2 g of activated cation-exchange resin Zeo-Karb 225. Stir the solution well and set it aside for a few minutes before filtering through a small pulp pad, which is subsequently washed well with cold water. The filtrate, containing the ethylene glycol, glycollic acid and possibly small amounts of phosphoric acid derived from the inhibitor, should be quite clear at this stage. To this filtrate add 1 g of dry activated anion-exchange resin De-Acidite FF. Stir

the solution and set it aside for a few minutes, and then filter it through a sintered crucible of porosity 2, washing the filter well with water. Reject the filtrate, which contains the ethylene glycol. Dry the crucible containing the resin as far as possible, both inside and out, by means of a filter-paper to remove extraneous moisture. Elute the glycollic acid from the resin in the crucible, adding to it by pipette two 5-ml portions of 80 per cent. sulphuric acid and, after addition of each aliquot, gently suck the crucible dry, using a vacuum-desiccator and collecting the eluate in a small dry glass-stoppered bottle. Add 20 ml of concentrated sulphuric acid to the bottle by pipette and mix the solution thoroughly. Transfer a 5-ml aliquot to a dry test-tube and add exactly 10 ml of the 2:7-dihydroxynaphthalene reagent. After mixing, place the test-tube in a beaker of boiling water for a timed period of 10 minutes and then cool it to room temperature. After cooling, transfer the solution to a 25-ml calibrated flask, using 50 per cent. sulphuric acid for the transfer. Cool the solution and dilute it to 25 ml with the 50 per cent. sulphuric acid. Measure the absorption of the solution, using a 2-cm cell and a wavelength of 535 mμ.

Set the spectrophotometer on a blank value, by placing 10 ml of 80 per cent. sulphuric acid in a dry glass-stoppered bottle, adding 20 ml of concentrated sulphuric acid and proceeding as described above. If a 20 per cent. solution of ethylene glycol in water is subjected to the entire procedure, then both relative and absolute values can be found for the glycollic acid. (NOTE—Ethylene glycol appears to contain a small quantity of glycollic acid, probably as a by-product during preparation. The accuracy of the determination is improved if the glycollic acid content of the ethylene glycol, used in the preparation of the antifreeze, can be determined. Otherwise, all results are a measure of this *plus* the increase in glycollic acid content of the antifreeze mixture after use.)

I express my thanks to the Director and Council of the British Cast Iron Research Association for permission to publish this paper.

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BRITISH CAST IRON RESEARCH ASSOCIATION
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The Determination of Uric Acid, Particularly in Avian Excreta

BY JOSEPH TINSLEY AND T. Z. NOWAKOWSKI

Methods for the determination of uric acid in blood, milk, urine and avian excreta have been reviewed. As more than half the nitrogen in poultry excreta is present as uric acid, the accurate determination of this component is important both for nutritional studies with poultry based on a nitrogen balance and for manurial studies involving the transformation of nitrogen compounds.

A simple procedure is described for the quantitative titration of uric acid in acid lithium chloride solution with 0·01 *N* ceric sulphate. The accuracy is within $\pm$ 2 per cent. for quantities of the order of 1 mg.

In accordance with previous methods, the uric acid is extracted from poultry excreta with lithium carbonate solution and separated by precipitation with an ammoniacal silver - magnesium reagent. Lithium carbonate is shown to give rapid solution, especially on heating, but the uric acid decomposes at high pH values so that solutions in lithium carbonate, especially standards, must be freshly prepared and analysed directly.

BEFORE 1912 the methods employed for the determination of uric acid in urine were either gravimetric or volumetric. Then Folin and Denis[1] introduced their colorimetric method based on the reduction of phosphotungstic acid, and subsequently many colorimetric procedures have been described for use on whole blood and plasma and on urine. Details of

the earlier methods, until 1932, are given by Peters and Van Slyke[2] and, until 1947, by Hawk, Oser and Summerson.[3] Mostly, these methods involve use of Benedict's highly sensitive and specific arsenophosphotungstic acid reagent[4] in the presence of cyanide, and recently a detailed spectrophotometric study of the reaction was reported by Norton, Plunkett and Richards.[5]

For blood, it is usual to determine uric acid in the filtrate after precipitation of the proteins with the tungstic acid reagent of Folin and Wu,[6] and the same procedure was followed by Shahani and Sommer[7] for milk. However, there have been conflicting reports on possible errors arising from loss of uric acid in the protein precipitate or from the reducing action of sugars and ascorbic acid. To cancel such errors, Blauch and Koch[8] made duplicate colorimetric determinations on blood samples before and after treatment with the highly specific enzyme uricase, and the same principle of differential measurements was applied to urine by Schaffer[9] and by Buchanan, Block and Christman.[10] In place of the traditional colorimetric measurements Kalckar[11] made differential measurements of the ultra-violet absorption in the 290-mμ band without deproteinising the blood plasma. Tilden[12,13] also used ultra-violet absorption for the determination of traces of uric acid in fruit juices, the uric acid being first isolated chromatographically on paper or an absorption column.

In the case of avian excreta, the uric acid is mostly present in the solid phase, some as the highly insoluble ammonium salt, and its accurate determination depends on efficient extraction and separation from other faecal compounds. Many determinations have been made in droppings from hens by using the procedure developed by Woodman[14] on the basis of Hopkins's method.[15] This method is, however, tedious to operate and does not give accurate results, because uric acid decomposes in the highly alkaline lithium hydroxide solution used for extraction and also because the precipitated uric acid is contaminated with variable amounts of extraneous materials and the end-point of the titration with permanganate is often obscure.

Hutchinson[16] greatly simplified the extraction and isolation of uric acid from dried droppings by heating with lithium carbonate solution and then precipitating the uric acid from an aliquot of filtrate with the ammoniacal silver - magnesium reagent of Benedict and Hitchcock.[17] The centrifuged precipitate was dissolved in acidified lithium chloride solution and the determination completed colorimetrically. Reasonably good results were obtained by adding the arsenotungstate reagent of Newton[18] directly to the lithium carbonate extracts, but Hutchinson recommended the precipitation procedure as being more reliable.

Bose[19] maintained that hot extraction with lithium carbonate caused some destruction of uric acid and preferred shaking at room temperature. He chose to titrate the uric acid at pH 9 with 0·01 *N* iodine solution, which has a low oxidation potential, $E_0 + 0{\cdot}58$. Direct titration of the extracts without separating the uric acid gave results about 15 per cent. too high. Bose and Ghosh[20] also developed a procedure in which the iodine titration is used in conjunction with uricase treatment of the lithium carbonate extracts. Baker[21] re-examined the extraction procedure and obtained higher yields of uric acid with hot 0·25 per cent. lithium carbonate than with a cold 0·5 per cent. solution. Using Bose's iodine titration, he found little destruction of uric acid during a 15-minute period of heating. In this laboratory reproducible results could not be secured with Baker's method, the end-point of the titration being indistinct and liable to fade. Because the rate of oxidation of uric acid is so greatly affected by pH in alkaline solutions, it was considered that ceric sulphate in acid solution might prove simpler and more reliable. Ceric sulphate appeared to offer the advantage over hot permanganate in that it is very stable in dilute solution and, since it was found to oxidise uric acid rapidly at room temperature, it should be less likely to react with extraneous organic matter. Tests with standard amounts of uric acid dissolved in lithium carbonate solution showed that, after precipitation with Benedict and Hitchcock's reagent and re-dissolving in acid lithium chloride solution, it could be titrated with 0·01 *N* ceric ammonium sulphate solution, the *o*-phenanthroline - ferrous complex being used as indicator. This gave a sharp end-point from red to blue, which changed back to red on standing only slowly, as farther slight oxidation of the allantoin occurred.

Method for determining uric acid in poultry excreta

Reagents—

Ammoniacal silver - magnesium reagent—Three grams of silver lactate were dissolved by heating with water containing about 1 ml of lactic acid, and the solution was cooled, diluted to

100 ml and filtered. To 70 ml of this solution were added 30 ml of magnesia mixture and then 100 ml of concentrated ammonia solution, sp.gr. 0·880. The magnesia mixture contained 8·75 g of hydrated magnesium sulphate, 17·5 g of ammonium chloride and 30 ml of concentrated ammonia per 100 ml. The final solution of ammoniacal silver - magnesium reagent was filtered and stored in a dark glass bottle.

Acid lithium chloride solution—A 3·5 per cent. w/v solution of lithium chloride in 0·1 *N* hydrochloric acid.

Ceric ammonium sulphate, 0·01 N—A solution in *N* sulphuric acid.

o-*Phenanthroline - ferrous complex indicator*—A commercial solution.

PROCEDURE—

By pipette put 5 ml of standard uric acid solution or filtered extract of droppings, in duplicate, into a 15-ml conical centrifuge tube and then add from a pipette 2 ml of ammoniacal silver - magnesium reagent. Mix the contents by stirring with a glass rod drawn out to a fine stem and set aside for ½ hour. Spin in a centrifuge for 5 minutes at about 2000 r.p.m. and 17 cm radius and pour or siphon off the supernatant liquid. Invert the tube in a stand to drain for 10 minutes before wiping any liquid from the rim with filter-paper.

Add 10 ml of acid lithium chloride solution from a pipette and stir the precipitate well with the fine glass rod for 2 to 3 minutes to ensure that the uric acid is dissolved. Spin in the centrifuge again to collect the silver chloride and other insoluble material, and then pour the solution carefully into a 50-ml conical flask. Wash the inside walls and rim of the tube with a fine jet of water without disturbing the precipitate and collect the washings in the flask.

Add 1 drop of indicator solution and titrate with 0·01 *N* ceric ammonium sulphate solution from a 5-ml burette graduated in 0·01-ml divisions and having a fine tip. Correct for a blank titration performed on 10 ml of acid lithium chloride solution.

1 ml of 0·01 *N* ceric ammonium sulphate solution ≡ 0·8403 mg of uric acid.
≡ 0·2801 mg of nitrogen.

ACCURACY OF METHOD—

Duplicate determinations, whether on standard solutions or extracts of droppings, almost invariably agree to within ± 2 per cent. of the mean for titration values ranging from 0·5 to 1·3 ml, equivalent to 0·4 to 1·1 mg of uric acid. If a higher titre is required, a 10-ml aliquot can be taken initially.

STANDARD URIC ACID SOLUTIONS—

Solutions in lithium carbonate with pH values exceeding 9 decompose rapidly, as the results given later show. Such solutions should be freshly prepared as required. Solutions in the acid lithium chloride reagent may be stored for several days without loss.

Benedict[4] employed a stock solution dissolved in a near neutral phosphate buffer and preserved with chloroform, but this was not recommended by Hutchinson. Folin and Wu[22] added sodium sulphite to lithium carbonate solutions to stabilise the uric acid, but later, Folin[23] preferred to add formaldehyde and acidify the solution with sulphuric acid. Such a solution was shown to be stable for 5 years when stored out of the light and its use was recommended by Hutchinson.

EXTRACTION AND RECOVERY OF URIC ACID

Having settled the method of determination, it was decided to investigate the conditions of extraction and related factors in order to establish a reliable procedure for routine use on poultry excreta.

RECOVERY OF URIC ACID FROM COLD AND HOT LITHIUM CARBONATE SOLUTIONS—

Cold solutions—Weighed quantities of pure uric acid were dissolved in 50 ml of 0·5 per cent. w/v lithium carbonate solution in 250-ml calibrated flasks. After standing for 1½ hours, the solutions were diluted to volume, mixed, and 5-ml aliquots were taken for analysis.

Hot solutions—Weighed quantities of uric acid were dissolved in 50 ml of 0·25 per cent. w/v lithium carbonate solution in 250-ml calibrated flasks mounted on a boiling-water bath for 5, 15 and 30 minutes. After being cooled under the tap, the solutions were diluted to volume before analysis.

The results given in Table I show that the mean recovery from four separate cold solutions was 98·8 per cent., but recovery from the heated solutions varied from 71·4 per cent. for 30 mg to 89·8 per cent. for 80 mg. Shortening the time of heating to 5 minutes or extending it to 30 minutes had no appreciable effect on the recovery and there appeared to be a fairly constant loss of 8 to 9 mg irrespective of the initial quantity taken.

TABLE I

RECOVERY OF URIC ACID FROM LITHIUM CARBONATE SOLUTIONS

Cold solutions (after standing for 90 minutes)—								
Uric acid taken, mg ..	40	47	77	80				
Recovery, %	99·5	100·1	100·4	95·1	Mean = 98·8			
Heated solutions—								
Time of heating, minutes ..	15	5	15	30	15	5	15	30
Uric acid taken, mg ..	30	40	40	40	77	80	80	80
Recovery, %	71·4	77·7	78·8	78·8	88·9	89·3	89·3	89·8

EXTRACTION OF URIC ACID FROM POULTRY DROPPINGS WITH LITHIUM CARBONATE—

Five samples of dried and ground droppings were used to compare cold and hot-extraction procedures. Duplicate 1-g samples were weighed into 250-ml calibrated flasks and one of each pair was extracted for 90 minutes at room temperature with 50 ml of 0·5 per cent. lithium carbonate solution, while the other was extracted for 15 minutes on a boiling-water bath with 50 ml of 0·25 per cent. lithium carbonate solution. Both extracts were diluted to the mark and filtered before analysis.

The results given in Table II show close agreement between the extraction procedures for four of the five samples and do not support the findings of Baker, that much less uric acid is dissolved on cold extraction than on hot extraction.

TABLE II

EXTRACTION OF URIC ACID FROM 1-g SAMPLES OF POULTRY DROPPINGS WITH COLD AND HOT LITHIUM CARBONATE SOLUTIONS

Sample No.	1	2	3	4	5
Uric acid extracted { with cold 0·5% lithium carbonate solution, mg ..	57·1	27·3	56·3	51·3	63·4
Uric acid extracted { with hot 0·5% lithium carbonate solution, mg ..	56·7	27·7	61·3	51·3	63·9

RECOVERY OF URIC ACID ADDED TO POULTRY DROPPINGS—

The close agreement observed in the previous experiment may have been due to cancellation of errors, in that more uric acid may have been dissolved on hot extraction, but some decomposition may have occurred during extraction. Accordingly, a series of determinations was made with pure uric acid added to poultry droppings, before the cold and hot extractions were done. It was found that the total uric acid present in 250 ml of final solution should not exceed 100 mg.

The results given in Table III show that the mean recovery of 30 mg of uric acid added to 1 g of dried droppings was 100·1 per cent. for hot extraction and 99·8 per cent. for cold extraction. Hence there does not appear to be any significant destruction of uric acid during 15 minutes' hot extraction of droppings.

TABLE III

RECOVERY OF URIC ACID ADDED TO POULTRY DROPPINGS, BY COLD AND HOT EXTRACTION

Sample No.	Uric acid in 1 g of droppings, mg	Uric acid added, mg	Uric acid found, mg	Recovery of added uric acid, %
Cold extraction—				
1	57·1	30	88·2	101·3
2	41·6	20	60·1	97·6
3	29·0	30	60·5	102·5
4	56·3	30	84·5	97·9
				Mean 99·8
Hot extraction—				
1	56·7	30	87·4	100·8
2	27·7	30	57·6	99·7
3	61·3	30	91·2	99·8
				Mean 100·1

The full recovery of uric acid from heated lithium carbonate solution in the presence of poultry droppings conflicts with the destruction that was found in the absence of droppings in the first series of experiments. It was thought this may be due to the droppings having some "protective action" on the uric acid or to their exerting a buffering action and reducing the pH of the lithium carbonate solution to a lower level at which destruction was prevented.

EFFECT OF pH ON THE DECOMPOSITION OF URIC ACID IN SOLUTION—

Two series of lithium solutions were used with the pH of the lithium carbonate solution reduced either by dilution with an equivalent lithium acetate solution or by addition of boric acid. Uric acid itself reduced the pH and so this was measured before and after solution of the uric acid. It is well known that uric acid decomposes in alkaline solution, and therefore the solutions of different pH values were kept and the amount of uric acid present was determined after storage at room temperature for 3 and 6 days.

The results given in Table IV show that, as the pH was reduced from above 10 in the lithium carbonate solution to 7, the rate of decomposition of uric acid decreased, so that after 6 days only 30 per cent. of the 40 mg of uric acid dissolved in 0·1 per cent. lithium carbonate solution remained at pH 10·4, whereas over 90 per cent. remained in the equivalent lithium carbonate - acetate mixture at pH 7. However, it was noticed that the uric acid dissolved more slowly as the pH was reduced, so that it became necessary to heat the mixtures on the water bath to effect solution. Whereas the lithium carbonate - acetate solutions are not well buffered and the pH varies with the amount of dissolved uric acid, the boric acid mixtures are highly buffered.

TABLE IV

RECOVERY OF URIC ACID FROM LITHIUM SOLUTIONS OF DIFFERENT pH VALUES

Uric acid dissolved in 50 ml of cold solution and diluted to 250 ml—

No.	Solution used: Volume of 0·5 % lithium carbonate solution, ml	Solution used: Volume of 1·11 % lithium acetate solution, ml	Initial pH	Uric acid added, mg	pH of solution	Recovery of uric acid after—: 1½ hours, %	3 days, %	6 days, %
1	50	—	11·2	40	10·4	99·5	59·2	30·5
2	25	25	10·9	40	9·5	96·6	83·0	68·3
3	5	45	10·3	40	7·0	97·7	92·5	91·4
4	—	50	7·5	40	(remained dissolved)			

Uric acid dissolved by heating for 15 minutes and diluting to 250 ml—

	Solution used: Volume of 0·5 % lithium carbonate solution, ml	Solution used: Volume of 1·11 % lithium acetate solution, ml						
1	50	—	—	40	—	83·0	—	—
2	25	25	—	40	—	85·1	—	—
3	5	45	—	40	—	91·4	—	—
4	not tested		—	--	—	--	—	—

	Solution used: Volume of 0·25% lithium carbonate solution, ml	Solution used: Concentration of boric acid added to solution, %						
5	50	0·42	9·36	40	9·33	87·2	71·4	68·3
				80	—	93·0	—	—
6	50	0·84	8·84	40	8·96	89·3	79·8	77·7
				80	—	94·0	—	—
7	50	1·68	8·21	40	8·63	90·3	86·1	81·9
				80	—	95·6	—	—

EFFECT OF pH ON EXTRACTION AND RECOVERY OF URIC ACID FROM DROPPINGS—

A single sample of dried ground droppings was used to test the initial extraction of uric acid and also the recovery of added uric acid in lithium salt solutions of different pH

values by cold and hot treatments. It was previously shown that cold 0·5 per cent. lithium carbonate solution extracted nearly the same amount of uric acid as hot treatment with a 0·25 per cent. solution. In the present series of tests comparison was made between hot and cold extraction with the same 0·25 per cent. solution. Table V gives the results, each value being the mean of duplicate determinations on each of duplicate extracts from 1-g portions of droppings.

TABLE V

EFFECT OF pH ON EXTRACTION AND RECOVERY OF URIC ACID FROM DROPPINGS

Solution used								
Volume of 0·25% lithium carbonate solution, ml	Volume of 0·56% lithium acetate solution, ml	Concentration of boric acid added to solution, %		Uric acid extracted, mg	pH of solvent	Uric acid added, mg	pH of extract	Recovery of uric acid, %
50	—	—	cold	26·7	10·6	20	9·87	84·1
			hot	29·0	9·86	20	9·66	91·4
5	45	—	hot	28·2	—	20	7·31	95·6
50	—	0·5	cold	24·0	8·85	20	8·84	79·9
			hot	29·4	8·80	20	8·78	95·6
50	—	0·42	hot	*	—	20	8·96	102·9
			hot	*	—	40	—	99·8
50	—	0·84	hot	*	—	20	8·68	92·5
			hot	*	—	40	—	98·7
50	—	1·68	hot	*	—	20	8·44	88·3
			hot	*	—	40	—	96·6

* 29·4 mg assumed for calculating recovery.

From these results it seems clear that the pH of the solution is the main factor in the decomposition of uric acid, and the reason that recovery from standards prepared by heating in pure lithium carbonate solution proved to be less than in the presence of droppings was because droppings lower the pH to a greater extent than pure uric acid.

Satisfactory extraction of uric acid from droppings is secured with a 0·5 per cent. solution of lithium carbonate at room temperature, but not with a 0·25 per cent. solution, although the latter solution is preferable for hot extraction, since less destruction of uric acid is likely to occur than with the stronger solution. If hot extraction is preferred, the 0·25 per cent. solution of lithium carbonate buffered at pH about 9 with 0·5 per cent. boric acid is very safe, but this is not satisfactory for cold extraction, at least under the conditions of these tests.

EFFECT OF DRYING ON TOTAL NITROGEN AND URIC ACID CONTENT OF DROPPINGS—

It is well known that some nitrogen is lost when fresh droppings are dried, and that these losses are considerably increased if the droppings are set aside for more than a day at room temperature before being dried, because of microbiological decomposition of the uric acid to volatile ammonia. When an accurate knowledge is required of the contents of total nitrogen and of the various forms of nitrogen, the handling of the material is very important. Analysis of the fresh moist material is not always convenient and therefore it is desirable to compare the determination of uric acid in fresh material with dried material.

Samples of droppings were collected from a feeding experiment each evening and morning. The evening collection was stored overnight in a refrigerator and then mixed with the morning collection to make a composite sample for a period of 24 hours. Three such samples were taken and the contents of total nitrogen and uric acid were determined in duplicate subsamples, one fresh and the other dried overnight in an oven at 105° C. The mean loss of total nitrogen from drying was 6·2 per cent. and the mean yields of uric acid per gram of dry matter were, respectively, 42·8 mg from the fresh and 41·6 mg from the dried material. The apparent loss of uric acid from drying was thus 2·8 per cent., which is relatively small.

METHOD FOR EXTRACTING URIC ACID FROM POULTRY EXCRETA

The following method is adapted from that of Bose.[19]

FROM FRESH DROPPINGS—

Mix the sample well with a spatula and disperse a weighed quantity of 4 to 5 g in about 100 ml of water by vigorous shaking or stirring, preferably in a blender or macerator. Transfer the suspension to a 250-ml calibrated flask, add 50 ml of 0·5 per cent. lithium carbonate solution and shake intermittently by hand or continuously by machine for 1½ hours. Dilute to the mark, mix well, filter through a dry 18-cm Whatman No. 1 filter-paper and discard the first runnings before collecting the clear filtrate.

FROM DRIED GROUND MATERIALS—

Weigh about 1 g into a 250-ml calibrated flask, add 50 ml of 0·5 per cent. lithium carbonate solution and continue the extraction as described above.

When the equipment or routine favours hot extraction, this could be done by following the practice of Hutchinson[16] and Baker[21] of heating on a boiling-water bath for 15 minutes with a 0·25 per cent. solution of lithium carbonate or the 0·5 per cent. solution buffered with 0·5 per cent. boric acid.

CONCLUSIONS

A wide choice of methods is available for the determination of uric acid in very varied materials and concentrations. The most sensitive is the ulta-violet absorption method, which can be used directly on solutions containing from 1 to 5 μg per ml if there is little or no interference from other compounds having similar absorption characteristics, or differentially in conjunction with uricase when necessary.

The conventional colorimetric procedures are also well suited to the routine examination of blood and urine samples, although perhaps more subject to error through loss of uric acid or interference by other substances with reducing properties.

When, as in avian excreta, the uric acid is present in high concentration and largely undissolved, the method of extraction assumes primary importance. It is likely that a direct or a differential ultra-violet-absorption procedure could easily be applied to extracts of droppings prepared by heating with the lithium carbonate - boric acid buffer solution, since the pH of 9 is immediately right for incubation with uricase, although considerable dilution would be needed. Nevertheless, isolation of the uric acid with ammoniacal silver - magnesium reagent and titration with ceric sulphate solution, as described in this paper, is a simple and convenient procedure that can readily be used in almost any laboratory without specialised equipment other than a centrifuge.

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DEPARTMENT OF AGRICULTURAL CHEMISTRY
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The Titration of Bivalent Metals with the Disodium Salt of Ethylenediaminetetra-acetic Acid

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A method first developed by Schwarzenbach in which account is taken of the acid liberated as a result of the interaction of certain bivalent metals, such as copper and zinc, with the disodium salt of ethylenediaminetetra-acetic acid has been shown to be unsatisfactory. An alternative form of titration is proposed in which the bivalent metal is titrated with a standardised solution of the disodium salt of ethylenediaminetetra-acetic acid until further addition of titrant no longer produces a change in the pH of the solution.

In Schwarzenbach's book[1] attention is drawn to the general alkalimetric titration of bivalent metals such as manganese, cobalt, nickel, copper, zinc, cadmium and mercury, as well as total rare-earth cations, with the disodium salt of ethylenediaminetetra-acetic acid (EDTA). The solution of the bivalent metal should not contain a weak acid or the salt of a weak acid and 10 ml of *M* ammonium chloride solution should be present for each 100 ml of solution being titrated. This solution is first neutralised very carefully to methyl red - bromocresol green indicator with sodium hydroxide solution to pH 5, *i.e.*, the grey tint of the indicator. Solutions of EDTA (0·1 *M*) and sodium hydroxide (0·1 *N*) are placed in two separate burettes. A few millilitres of EDTA solution are added to the solution being titrated. This releases acid, which is then neutralised by adding standard sodium hydroxide solution from the other burette. A further small quantity of EDTA solution is added, and then the liberated acid is neutralised with the standard sodium hydroxide solution. This procedure is repeated until, finally, on the addition of a small volume of EDTA solution, the pH of the solution no longer falls below 5·0. The volume of sodium hydroxide is noted and hence the amount of bivalent metal present is calculated. The assumption is made that the reaction is stoicheiometric and that the sodium hydroxide required in the titration is a direct measure of the bivalent metal, *e.g.*, that 1 ml of 0·1 *N* sodium hydroxide solution is equivalent to 3·177 mg of copper. It is pointed out that the procedure may be applied to suspensions of lead sulphate. Moreover, it is indicated that difficulty is experienced in the detection of the end-point when solutions of copper, cobalt and nickel are being titrated, as the complex solution is highly coloured.

In trying out the method, we confirmed this difficulty with the end-point and, in view of the general applicability of such a simple method of complexometric titration, decided to test the principle of the method, using a pH meter throughout and omitting indicators altogether. We confined our attention to the titration of zinc, which forms a colourless complex with EDTA, and copper, which forms a highly coloured blue complex. Accordingly, samples of AnalaR copper sulphate and zinc sulphate were first standardised by conventional procedures. For copper sulphate, $CuSO_4.5H_2O$, 25·33 per cent. of copper was found by the potassium iodide - thiosulphate method and 25·35 per cent. by electro-deposition, giving a mean of 25·34 per cent. For zinc sulphate, $ZnSO_4.7H_2O$, 23·09 and 23·11 per cent. of zinc were found by the 8-hydroxyquinoline gravimetric method, giving a mean of 23·10 per cent.

In a particular experiment a known amount of copper sulphate was dissolved in 100 ml of water, 10 ml of *M* ammonium chloride solution were added and the solution was neutralised to pH 5·0 by adding 0·1 *N* sodium hydroxide solution. A small volume of 0·1 *M* EDTA solution was added and, with use of a pH meter, the solution was re-adjusted to pH 5·0 by adding 0·1 *N* sodium hydroxide solution. This procedure was repeated until, on completion of the titration, the addition of a small volume of EDTA solution no longer caused an alteration in the pH of the solution. Details of a typical titration are as follows—

The 0·1 *M* EDTA solution was prepared by dissolving 37·25 g of the disodium salt of sequestric acid (obtained from Hopkin & Williams Ltd.) in distilled water and diluting to

1 litre; it had a pH value of 4·43. In the titration 0·1275 g of copper and 10 ml of *M* ammonium chloride were present. The results were as follows—

Volume of 0·1 *M* EDTA solution added, ml	pH value after the addition	Volume of 0·1 *N* sodium hydroxide required to make the solution pH 5, ml
0	4·3	adjusted to pH 5·0 with NaOH
0	5·0	0
5·0	< 3	9·90
10·0	< 3	19·35
15·0	3·18	29·1
18·0	3·55	34·88
19·0	4·08	36·81
19·25	4·58	37·31
19·50	4·58	37·81
19·75	4·62	38·30
20·0	4·58	38·82
20·28	4·82	39·10 (end-point)
20·50	4·99	39·10
20·75	5·0	39·10
21·0	4·99	39·10

The end-point was found when 39·10 ml of 0·1 *N* sodium hydroxide had been added, the theoretical end-point requiring 40·13 ml of 0·1 *N* sodium hydroxide.

In similar titrations, the results were as follows—

Copper present, g	Volume of 0·1 *N* sodium hydroxide to end-point, ml	Volume of 0·1 *N* sodium hydroxide to theoretical end-point, ml
0·1273	39·09	40·07
0·1268	38·80	39·91
0·1268	38·73	39·91
With ammonium chloride omitted—		
0·1284	39·89	40·41
0·1272	39·50	40·04
0·1266	39·20	39·85
0·1266	39·25	39·85
With the volume of M *ammonium chloride increased from* 10 *to* 30 *ml—*		
0·1286	38·80	40·48
0·1268	38·30	39·91

In corresponding tests with zinc solutions containing 10 ml of *M* ammonium chloride, the results were as follows—

Zinc present, g	Volume of 0·1 *N* sodium hydroxide to end-point, ml	Volume of 0·1 *N* sodium hydroxide to theoretical end-point, ml
0·1319	39·81	40·34
0·1319	39·95	40·34
0·1332	40·18	40·75
0·1335	40·29	40·83

Although the results for zinc were well below the theoretical values, they were more accurate than those for copper.

We reached the conclusion at this stage that the method in its present form was unsound and decided on a modified form of approach, *i.e.*, although maintaining the general procedure of the titration, to determine the volume of EDTA solution that would complex the metal ions so that further addition would not produce a change of pH. Moreover, working at pH 5·0 with the bivalent metals we were considering, liberation of a small amount of acid in the course of the test would be expected to produce a pronounced change in the pH of the test solution. By using the older methods of pH titration, this form of titration probably takes quite a long time, but the introduction of automatic titrimeters into analytical work has permitted this type of titration to be performed with rapidity and certainty.

The procedure that we have employed in the titration, for example, of a known weight of copper as copper sulphate is described in detail below. We have used the automatic titrimeter manufactured by Messrs. Electronic Instruments Ltd., Richmond, Surrey, although we see no reason why other automatic instruments should not be used for the same purpose.

Method

The instrument is switched on and adjusted with a standard buffer solution, in the normal manner for the standardisation of a pH meter. The automatic burette is filled with 0·1 *N* sodium hydroxide and the instrument is set for titration to a rising pH. The copper solution to be titrated, containing 10 ml of *M* ammonium chloride per 110 ml of solution, is placed in position and the stirrer is started. The pH of the solution is measured by turning the appropriate pH indicator dial, which is then set to the desired pH of 5·0. By moving the automatic switch to the "on" position, automatic addition of 0·1 *N* sodium hydroxide is made until the pH is raised to 5·0. The instrument then switches off, and the solution is ready for the main titration.

A 0·1 *M* solution of EDTA is placed in an external burette and small additions (5 ml decreasing to 1 ml) are made to the titration beaker. Between each addition the automatic apparatus is switched on and the pH is automatically brought back to 5·0 by adding 0·1 *N* sodium hydroxide, the instrument being so set that the alkali is added dropwise from pH 4·8. When within about 1 ml of the end-point, the automatic instrument is left switched on while dropwise addition of the EDTA solution is made. Each drop of EDTA solution added causes further automatic delivery of alkali to return the pH to 5·0 until, of course, all the metal in solution has been complexed. At this point the pH remains at 5·0 and there is no automatic "switch on" of the alkali burette tap, and, with our instrument, the yellow end-point indicator light remains on. The volume of EDTA solution at this point is noted, and then one more drop is added. The end-point of the titration is taken as the point at which the addition of 2 or 3 further drops of EDTA solution causes no automatic addition of sodium hydroxide solution.

Application of the method

If sufficient sample is available, it is often convenient and time-saving to get an approximate idea of the end-point in a preliminary test. This can be readily achieved by adding directly to the solution of the samples an excess of EDTA solution, and automatically returning the pH to 5·0 by means of the automatic titrimeter. The approximate end-point in terms of EDTA solution is then given by $\frac{\text{volume of 0·1 } N \text{ sodium hydroxide}}{2}$, and in the actual titration dropwise addition of the EDTA solution is made 1 ml before this approximate end-point. The pH of the EDTA solution used was 4·43, and no adjustment of this solution to pH 5·0 was made. At the end-point, however, a considerable volume of the solution could be added without causing any significant departure from pH 5·0 of the solution being titrated.

With this method of titration it will be seen from the following results that the relationship between, for example, the weight of copper or zinc titrated and the amount of 0·1 *M* EDTA solution used in the titration is nearly stoicheiometric, on the assumption that the reagent purchased has the formula $C_{10}H_{14}O_8N_2Na_2.2H_2O$—

Amount of metal present, g	Theoretical volume of 0·1 *M* EDTA solution required, ml	Actual volume of 0·1 *M* EDTA solution required, ml
Copper—		
0·1267	19·93	19·90 19·95 19·98
0·0576	9·07	9·17
0·1056	16·62	16·68
0·0680	10·70	10·70
0·1091	17·17	17·20
Zinc—		
0·1316	20·13	20·18 20·18 20·18
0·1249	19·10	19·15
0·06721	10·28	10·35
0·0888	13·58	13·68
0·1103	16·87	16·95

We believe, however, as is our practice in other potentiometric titrations, that the best method of carrying out this test is to standardise the EDTA solution against the particular metal under consideration, the apparatus and method described above being used.

When this procedure was applied to amounts of copper unknown to the operator at the time of test, the results were as follows—

Operator 1—Standardisation: 1 ml of EDTA solution	≡	0·006321 g	of copper.
Copper present, g		0·0576	0·1056
Copper found, ...		0·0579	0·1054
Operator 2—Standardisation: 1 ml of EDTA solution	≡	0·006346 g	of copper.
Copper present, g		0·0680	0·1091
Copper found, g		0·0679	0·1092

When a similar procedure was applied to the titration of zinc, with the initial adjustment of pH made with 0·1 *N* hydrochloric acid, the results were as follows—

Operator 1—Standardisation: 1 ml of EDTA solution	≡	0·006521 g	of zinc.
Zinc present, g		0·1249	0·06721
Zinc found, g		0·1249	0·06715
Operator 2—Standardisation: 1 ml of EDTA solution	≡	0·006521 g	of zinc.
Zinc present, g		0·0888	0·1103
Zinc found, g		0·0892	0·1105

We feel that this new principle, *i.e.*, titration of a bivalent metal solution with a standardised 0·1 *M* solution of EDTA until further addition of titrant produces no change in the pH of the solution being titrated may have applications in the titration of other bivalent metals at different pH values. The important thing to realise is that the advent of automatic titrimeters has made this form of titration very simple.

The suggested method of titration is likely to be of greatest value with highly coloured solutions, or for metals forming highly coloured complexes with EDTA.

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The Determination of Sodium Laurate in Sodium N-Lauroylsarcosinate

By D. C. CULLUM

A method is described for the determination of sodium laurate in sodium N-lauroylsarcosinate by partition chromatography of the corresponding acids on kieselguhr impregnated with an alkaline buffer, ether being used as the moving phase. The lauric acid is finally titrated with standard alkali.

Sodium N-lauroylsarcosinate is manufactured by means of a Schotten - Baumann condensation of lauroyl chloride with sodium sarcosinate—

$$C_{11}H_{23}\cdot COCl + CH_3\cdot NH\cdot CH_2\cdot COONa \rightarrow C_{11}H_{23}\cdot CO\cdot N(CH_3)\cdot CH_2\cdot COONa + NaCl + H_2O.$$

During this process a little of the lauroyl chloride reacts with some of the excess of alkali to give sodium laurate, which appears in the final product. The content of sodium laurate is usually less than 3 per cent., so that its determination by calculation from the apparent molecular weight of the product is not practicable. The determination can, however, be made easily and with good accuracy if the lauric acid is first separated by partition chromatography.

METHOD

APPARATUS—

Chromatographic column, 400 mm × 18 mm, fitted with a jet and stopcock at the lower end and a detachable reservoir at the upper end. The apparatus in use in this laboratory was made with ordinary glass tubing, rubber bungs and a 250-ml separating funnel, but apparatus with standard joints would serve equally well.

Microburette, of capacity 10 ml, graduated in 0·01 ml.

REAGENTS—

Kieselguhr—Any chromatographic grade; Celite filter aid was used.

Buffer solution—Dissolve 25 g of sodium phosphate crystals, $Na_2HPO_4.12H_2O$, in 250 ml of water and add *N* hydrochloric acid dropwise until the pH is 8·50.

Sodium hydroxide solution, 0·02 N, *accurately standardised.*

Phenol red indicator solution.

PROCEDURE FOR EXTRACTION OF ACIDS—

Accurately weigh out a sample containing about 5 g of crude sodium N-lauroylsarcosinate in a small beaker. Dissolve it in water and transfer the solution quantitatively to a 250-ml separating funnel. Acidify with 10 ml of concentrated hydrochloric acid and extract with three 100-ml portions of ether. Wash the ether extracts in turn with 50 ml of water. Combine the extracts and evaporate on a steam-bath to a small volume. Transfer quantitatively to a 100-ml calibrated flask and dilute to volume with ether. If the solution is turbid owing to the separation of water droplets, a maximum of 10 ml of ethanol may be added.

PROCEDURE FOR PREPARATION OF COLUMN AND SEPARATION OF ACIDS—

In a 400-ml beaker mix 25 g of kieselguhr and 12·5 ml of buffer solution. An almost dry powder should be produced. Pack this into the column a few centimetres at a time, tamping lightly with a suitable rod. The complete column should be about 30 cm deep, and will have a banded appearance but no air pockets. Fill the top of the column with ether and apply suction at the lower end until the whole column is wet with ether. Do not draw any air into the column.

Suck the ether down to the top of the kieselguhr bed and by pipette put into the column 10 ml of the ethereal solution of the extracted acids. Suck this into the bed. Rinse the walls of the column with ether and suck this into the bed. Fill the top of the column with ether and attach the reservoir, also full of ether. Allow the ether to flow at 3 ml per minute (usually with the stopcock wide open) and collect 10-ml fractions. Evaporate each fraction to dryness in a small titration flask, add 10 ml of ethanol previously neutralised to phenol red and titrate with 0·02 *N* sodium hydroxide from a 10-ml microburette until one drop produces a distinct pink colour persisting for 10 seconds. Record the fraction number and the volume of alkali absorbed. The evaporation step may be omitted with a slight loss of precision.

The first three or four fractions will require one drop of alkali (about 0·04 ml). Then the lauric acid appears in the eluate and the next few fractions will require appreciable volumes of alkali. Finally the titration drops again to the blank value.

CALCULATION OF RESULTS—

Subtract from each titration the mean blank figure, and add together the net titration. The following is an example—

Fraction No.	1	2	3	4	5	6	7	8	9	10
Volume of 0·02 *N* sodium hydroxide, ml ..	0·08	0·04	0·04	0·04	0·82	2·10	1·40	0·32	0·04	0·04

Mean blank = 0·04 ml,

Net titration = 0·78 + 2·06 + 1·36 + 0·28 = 4·50 ml.

1 ml of 0·02*N* NaOH ≡ 0·004 g of lauric acid or 0·00444 g of sodium laurate.

Results

The method was tested on mixtures of recrystallised sodium laurate, prepared from pure lauric acid, and recrystallised sodium N-lauroylsarcosinate. The results are shown in Table I.

Table I

Recovery of sodium laurate

Nominal soap content, %	Total soap recovered, %	Net soap recovered,* %	Soap added, %	Recovery, %
0	0·229	—	0	—
0	0·245	—	0	—
1	1·255	1·018	1·035	98·4
1	1·279	1·042	1·035	100·7
3	3·331	3·094	3·158	98·0
3	3·379	3·142	3·158	99·5
5	5·488	5·251	5·291	99·3
5	5·418	5·181	5·291	97·9

* The total percentage of soap minus the mean of the two soap figures found for the "pure" sodium N-lauroylsarcosinate.

The mean recovery was 99·0 per cent., and the average error was 1·3 per cent. In view of the small titration volumes—the largest was 7 ml—this was considered satisfactory.

I thank Mr. J. M. Blakeway of the Research Department, Colgate - Palmolive Ltd., for preparing the pure sodium N-lauroylsarcosinate used in this work, and the Management of the company for permission to publish this paper.

Colgate - Palmolive Ltd.
371 Ordsall Lane
Manchester, 5

September 26th, 1956

Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Methods for the Determination of Non-volatile Matter Extractable by Light Petroleum and the Determination of Volatile Immiscible Liquids

Non-volatile Matter Extractable by Light Petroleum

THE commonly used terms "oil," "fat," "grease" and so on do not form a sufficiently precise basis on which to found a comprehensive method of analysis. No solvent is known that will selectively extract only fatty or hydrocarbon oils; the term "non-volatile matter extracted by light petroleum" is recommended, since this will normally include fatty and non-volatile hydrocarbon oils and greases, but the analyst must bear in mind that the extract may contain a variety of chemical substances that cannot properly be classified as either oil or grease.

Light petroleum is not a good solvent for bituminous substances, nor can it be used directly to determine volatile organic liquids such as petrol or benzene. If a determination of bituminous substances or of substances that are appreciably volatile at 100° C is required, special methods appropriate to the nature of the problem must be applied.

REAGENTS—

Magnesium sulphate solution—Dissolve 1 g of magnesium sulphate, $MgSO_4.7H_2O$, in 100 ml of distilled water.

Light petroleum, boiling range below 40° C.

Lime cream—Prepare a thin cream by mixing 2 g of calcium oxide with distilled water into a paste and dilute the suspension to 100 ml.

Hydrochloric acid, diluted (1 + 3).

Sodium sulphate, anhydrous.

PROCEDURE—

Make a mark on the outside of the sample bottle at the level of the top of the contained liquid. If the sample includes a measurable layer of floating matter, carefully decant as much of it as possible into a 500-ml separating funnel (see Note 1) and return to the sampling bottle any water that separates. To the residue of the sample in the bottle add 5 ml of magnesium sulphate solution. Stir the liquid slowly in a rotary direction with a glass rod and cautiously add small volumes of lime cream (see Note 2), until flocculation occurs. Continue the stirring for about 2 minutes, then withdraw the glass rod, rinsing it with light petroleum, and collect the washings in the separating funnel containing the decanted liquid. Allow the precipitate in the sample bottle to settle. (This usually takes place fairly rapidly, but if a clear top layer does not begin to form within 5 minutes, see Note 2.) When the slurry has completely settled, siphon off the clear top layer to within about half an inch of the top of the precipitate. If any floating oil still remains in the top layer, leave this in the bottle. Dissolve the precipitate in diluted hydrochloric acid and pour the acid liquid into the separating funnel, taking care not to transfer any large adventitious solids (*e.g.*, twigs and leaves) that may be included with the sample. Wash the bottle with 50 ml of light petroleum and add this to the liquid in the funnel. Shake the funnel continuously but not vigorously for 1 minute. Allow the layers to separate, draw off the aqueous layer into a second separating funnel and extract it again with a further 50 ml of light petroleum. Withdraw and reject the aqueous layer and combine the two light-petroleum extracts in the first

funnel. Draw off and reject any further water layer that may separate, add 2 g of powdered anhydrous sodium sulphate to the non-aqueous layer and shake at intervals over a period of about half an hour. Filter this layer through a 9-cm Whatman No. 30 filter-paper (see Note 3) into a previously weighed wide-necked flask having a capacity of 250 ml, covering the filter-paper with a clock-glass to reduce evaporation. Wash the paper with two successive 20-ml quantities of light petroleum, allowing the first washing to pass completely through the paper before adding the second. Distil off most of the light petroleum, finally evaporating the last traces in a current of warm air; then heat the flask on a boiling-water bath for exactly 10 minutes, dry the outside, cool and weigh. (If, after the solvent has evaporated, the residue contains visible water, add 2 ml of acetone, evaporate on a water bath, and repeat the addition and evaporation until all visible water has been removed.)

Determine the volume of the original sample in the bottle and report the amount of non-volatile matter extractable by light petroleum as milligrams per litre.

NOTES—1. The taps of separating funnels should not be lubricated with matter that is soluble in light petroleum.

2. Some effluents do not readily flocculate with lime. For such effluents preliminary tests should be made with a fresh sample in order to find a suitable flocculating agent. The following are suggested—

Aluminium sulphate, 1 per cent. solution, with suitable adjustment of pH.
Ferric chloride, 1 per cent. solution, and ammonium hydroxide.
Zinc acetate, 10 per cent. solution, and potassium ferrocyanide, 5 per cent. solution.
Zinc acetate, 10 per cent. solution, and sodium carbonate, 5 per cent. solution.

3. This size of filter-paper is recommended, but it may be necessary to use a slightly larger paper if the light-petroleum extract contains much suspended matter.

Volatile Immiscible Liquids

PRINCIPLE OF METHOD—

This method[1] depends on the miscibility of volatile oils with acetone and their relative insolubility in water. The oils are removed from the sample in a current of air, adsorbed on activated carbon and then eluted with acetone. Dilution of the acetone solution with acid Teepol produces a turbidity varying in intensity with the amount of oil present: this turbidity is measured against standards.

RANGE—

For volatile oil contents of between 3 and 500 mg per litre of sample.

APPARATUS—

Absorption tube—A small cylindrical or thistle funnel with a stem about 18 to 20 cm long and 0·5 cm in diameter. The stem is fitted with a rubber stopper that will fit tightly into the upper end of a reflux condenser.

A 2-necked flask having a capacity of about 1·5 litres, fitted with a reflux condenser in one neck and with an air-delivery tube, reaching to the bottom of the flask, in the other.

Matched test-tubes (6 inch × $\frac{5}{8}$ inch), graduated at 2 ml and 10 ml.

REAGENTS—

Acid Teepol solution—A solution containing 1 ml of sulphuric acid, sp.gr. 1·84, and 1 ml of Teepol per litre of distilled water. Cool to between 5° and 10° C before use.

*Activated carbon.**

Sodium hydroxide solution—A 10 per cent. w/v solution of the analytical-quality reagent in distilled water.

Acetone—Analytical-quality reagent.

* Obtainable from Messrs. Sutcliffe, Speakman & Co. Ltd., Leigh, Lancashire, quality No. 207, type B, B.S. mesh 20 to 40.

PROCEDURE FOR DETERMINING VOLATILE OIL IN THE RANGE 3 TO 15 mg PER LITRE—

Weigh 0·2 g of activated carbon and pack it lightly between tight plugs of glass-wool in the narrow stem of the absorption tube. Arrange the flask and condenser for reflux distillation in a current of air and fit the absorption tube into the outlet end of the apparatus (Fig. 1).

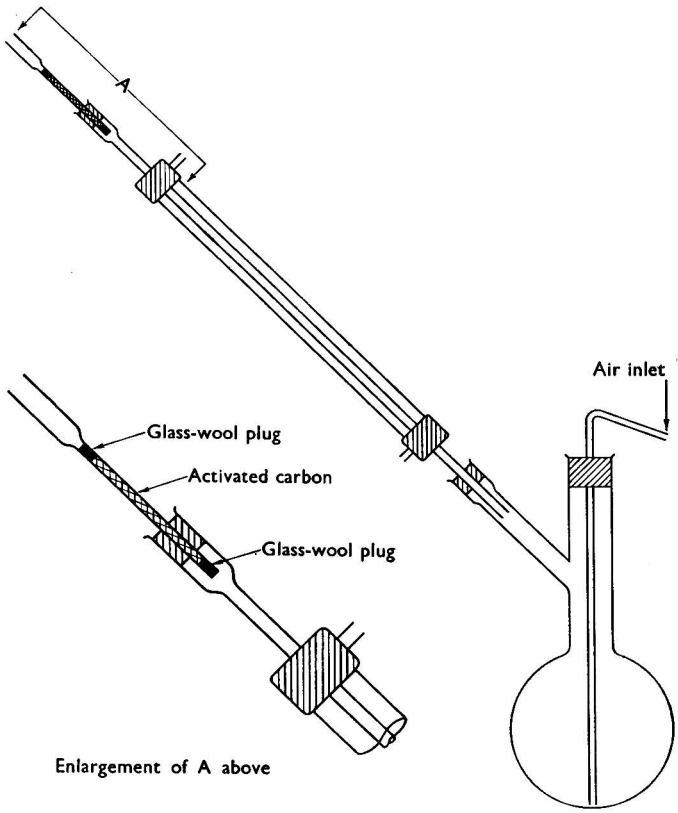

Fig. 1. Adsorption apparatus

Samples of effluent or river water in which oils are to be determined should be collected with precautions to ensure that they are representative and should be contained in wide-necked bottles of clear glass, holding approximately 1 litre. (See "Sampling."*) Mark the level of the liquid on the outside of the bottle for subsequent determination of the volume, and pour all the sample into the distillation flask. Rinse out the bottle with two 2·5-ml portions of acetone, taking care that the acetone dissolves any traces of oil left adhering to the inside of the sample bottle; pour the acetone into the distillation flask. Add sodium hydroxide solution to raise the pH of the sample to approximately 10. Connect the gas-delivery tube to the flask and pass a current of air through the sample at such a rate that the bubbles can just be counted; gently boil the liquid. About 10 minutes after drops of acetone can no longer be observed forming in the condenser, discontinue heating the flask and remove the absorption tube containing the carbon. Wash the carbon with acetone, added drop by drop, and collect the filtrate in one of the graduated test-tubes. When exactly 2 ml of acetone have been collected, dilute the filtrate to the 10-ml mark with acid Teepol solution.

* See *Analyst*, 1956, **81**, 492, or *Reprint No.* 3.

Preparation of standards—Prepare a solution containing 1·00 ml of petrol, sp.gr. 0·73 (or other volatile oil—see Note), dissolved in 100 ml of acetone. From a microburette measure into a series of the graduated test-tubes 0·4, 0·8, 1·2, 1·6 and 2·0 ml of this solution and dilute to the 2-ml mark with acetone. Finally dilute each of the standards to the 10-ml mark with acid Teepol solution and match the turbidity of sample visually against these standards. Calculate the amounts of petrol (or other reference oil) in the standards as milligrams per litre.

NOTE—Equal weights (or volumes) of different classes of commercial fuel oils do not give the same degrees of turbidity when subjected to the above procedure, but variations between different oils of roughly the same category, *e.g.*, kerosene and white spirit, or two different brands of petrol, are insignificant. For the most accurate work, therefore, it is necessary to prepare comparison standards with oil of the type that is present in the sample. The classes of oil likely to be present in effluents can usually be closely identified by their odour. An alternative procedure, if identification is impossible, is to refer the sample to some arbitrary standard, such as petrol or white spirit, on the same principle that "phenols" in water are usually referred to hydroxybenzene. This may lead to some sacrifice of accuracy, which, however, is not likely to be of practical importance.

PROCEDURE FOR DETERMINING OILS IN THE RANGE 12 TO 40 mg PER LITRE—

The apparatus and reagents are as described above, but 50-ml graduated cylinders should be used instead of test-tubes.

Pack 1 g of carbon into the stem of the small funnel and transfer the oil to the carbon by volatilising it in the manner already prescribed. At the end of this operation extract the oil from the carbon with successive 1-ml quantities of acetone, collecting the filtrate in a 50-ml graduated cylinder. When exactly 5 ml of filtrate have passed through the carbon, dilute the acetone to 50 ml with acid Teepol solution.

Preparation of standards—Prepare, as before, a 1 per cent. v/v solution of the reference oil in acetone. From a burette measure into a series of 50-ml graduated cylinders 1·0, 2·0, 3·0, 4·0 and 5·0 ml of this solution and dilute to the 5-ml mark with acetone. Finally dilute each of the standards to 50 ml with acid Teepol solution and match the sample against these standards, either instrumentally or visually.

PROCEDURE FOR DETERMINING QUANTITIES OF OILS IN EXCESS OF 40 mg PER LITRE—

If quantities of volatile oil in excess of 40 mg per litre are present, the final turbidity will be too dense to measure. This difficulty cannot be overcome by making the determination on only a portion of the sample, as it is impossible to distribute floating oil uniformly. The following method is suitable—

After all the volatile oil from a fresh sample has been absorbed on the carbon, as already described, extract the oil from the carbon with successive small volumes of acetone. Dilute the acetone solution to a measured volume and, on a suitable aliquot of this solution, determine the turbidity, using the standards as described for the range 12 to 40 mg per litre.

REFERENCE

1. Sherratt, J. G., *Analyst*, 1956, **81**, 518.

Mercury (addendum)

The method for mercury printed at p. 177 (March, 1956, *Analyst*, and *Reprint No.* 2) is based on a paper by A. C. Rolfe, F. R. Russell and N. T. Wilkinson (*Analyst*, 1955, **80**, 523), and in the final brochure a reference to this paper will be inserted in the "Principle of the Method" after the word "treatment" in line 2.

Notes

THE DETERMINATION OF GRADE STRENGTH OF PECTINS BY THE TEEPOL - GEL PROCEDURE

(Presented at a Joint Meeting of the Society with the Food Group of the Society of Chemical Industry on Wednesday, May 23rd, 1956)

A PROVISIONAL standard method for the determination of grade strength of pectins, described by a Sub-Committee of the British Food Manufacturing Industries Research Association,[1] has recently been critically examined, and inconsistencies have been found; these can be overcome by suitable modifications.[2] In this modified procedure the pectin solution is mixed with a determined quantity of Teepol, or other suitable surface-active agent, before being boiled with sugar and acid to produce a gel of soluble-solids content of 70·5 ± 0·5 per cent. (by refractometer) and a pH of 3·10 ± 0·05 (50 per cent. w/w solution of gel). The gel strength is measured by the Ridgelimeter.[3] Details of this method follow.

METHOD

REAGENTS—

Teepol—A 10 per cent. v/v solution of Teepol (530 or 410) in distilled water.

Buffer solution (*pH* 2·90 ± 0·02)—Dissolve 84 g of crystalline citric acid in about 600 ml of distilled water and add 185 ml of *N* potassium carbonate solution. After boiling for 5 minutes to remove carbon dioxide, cool and dilute with distilled water to 1 litre.

Commercial refined white sugar.

Hydrochloric acid, approximately N.

Sodium hydroxide, approximately 0·1 N.

CALCULATION OF WEIGHT OF SAMPLE TAKEN—

Under the conditions of test 0·705 per cent. of 100-grade pectin gives a gel of 23·5 per cent. sag. The weight of sample taken per kg of gel should therefore be adjusted according to the grade assumed for the pectin, *i.e.*, $\frac{705}{\text{assumed grade}}$ g.

PREPARATION OF SAMPLE—

Powder pectins are dispersed in accordance with the following procedure. Thoroughly mix the required weight of powder pectin with 30 g of sugar and add boiling distilled water with constant stirring until the total weight of the mixture is 225 g. Continue stirring until the solution is homogeneous, cover it and set it aside for 1 hour. If, at the end of this time, microscopical examination shows that the powder has not fully dispersed, a fresh preparation of the sample should be made with the addition of 0·2 g of trisodium citrate.

Liquid pectins are used without preliminary preparation.

PREPARATION AND TESTING OF GELS—

Transfer the pectin solution to a pan having a capacity of approximately 8 litres and dilute with distilled water to 500 g. Using either *N* hydrochloric acid or 0·1 *N* sodium hydroxide, adjust the solution to a pH of 3·05 to 3·10, measured electrometrically. This usually results in the specified pH being attained in the resultant gel, but samples of liquid pectins containing sulphite or sulphur dioxide may present some difficulty. For such samples, adjustment to about pH 2·80 to 2·90 with 0·1 *N* sodium hydroxide is recommended.

Now add 5 ml of Teepol solution and 50 ml of buffer solution, and adjust the weight of the pan contents to 850 g with distilled water. Add sugar equivalent to 695 g *minus* the soluble-solids content (by refractometer) of the pectin solution and, with stirring, bring to the boil within 2½ to 5 minutes. Boil, with continued stirring, at a rate rapid enough to yield a final product weighing 1 kg at the end of 10 minutes, ± 1 minute, after the boiling point is reached. Immediately pour into three Ridgelimeter glasses, prepared as described by Cox and Higby,[3] cover the gel surface with waxed paper discs and leave undisturbed at room temperature for 2 hours before ageing for a further 22 hours in an incubator at 30° C. Immediately after removing from the incubator, determine the strength of each gel, using the Ridgelimeter. The percentage sags of three gels from the same mixing should not differ by more than 0·4. The total soluble-solids content is

determined, by refractometer at 20° C, on a thin slice taken from the centre of one gel, and should be 70·5 ± 0·5 per cent. The pH is measured electrometrically, on a 50 per cent. w/w aqueous solution of the blended gel, and should be 3·10 ± 0·05.

Calculation of results

If the average sag of the gels is 23·5 per cent., then the assumed grade of the pectin is the true grade. If, however, the sag differs from 23·5 per cent., the true grade of the pectin may be calculated by multiplying the grade assumed for the pectin by a factor derived from experimental data as described below.

A number of pectins of different setting temperatures and from different sources is taken, and for each pectin a series of gels is prepared containing various weights of the pectin. Curves of percentage sag against weight of pectin are then plotted and the true grade of each pectin is obtained from the weight of pectin that gives a sag of 23·5 per cent. The value of the ratio of $\frac{\text{true grade}}{\text{assumed grade}}$ is calculated for each experimental point on the curves and a composite graph is prepared by plotting the values of the logarithms of these ratios against the percentage sags. The best straight line containing the point (23·5, 0) is drawn by inspection.

The equation of such a line has been found to be—

$$\log_{10} \frac{\text{true grade}}{\text{assumed grade}} = 0{\cdot}0188\ (23{\cdot}5 - \text{average percentage sag of gels}).$$

This equation is only valid between the limits of sag 20·0 to 27·0 per cent.

We thank the Directors of Chivers & Sons Limited, for permission to publish this Note.

References

1. Pectin Sub-Committee of the Jam Panel, British Food Manufacturing Industries Research Association, *Analyst*, 1951, **76**, 536.
2. Olliver, M., Wade, P., and Dent, K. P., *J. Sci. Fd Agric.*, 1957, **8**, in the press.
3. Cox, R. E., and Higby, R. H., *Food Industr.*, 1944, **16**, 441; **16**, 505.

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September 6th, 1956

SOME INVESTIGATIONS OF THE SENSITIVITY AND RESOLVING POWER OF A SIMPLE D.C. AMPLIFIER POLAROGRAPH

A paper giving details of comparative tests carried out by using the following three polarographs has been published[1]—

(*i*) the Univector unit, manufactured by Cambridge Instrument Co. Ltd.,
(*ii*) the Mervyn - Harwell square-wave polarograph,[2] manufactured by Mervyn Instruments, and
(*iii*) the single-sweep cathode-ray polarograph,[3] manufactured by Southern Instruments Ltd.

Comparisons are made of sensitivity and resolving power under a variety of conditions.

It has been considered worth while to compare some of these results with the performance of the Tinsley recording polarograph, mark 15/6, in use in this laboratory.

Experimental

It must be pointed out that the three polarographs previously studied[1] were designed with improved sensitivity and resolving power particularly in mind. The polarograph in use in this laboratory was obtained for its general suitability and utility for a variety of purposes. Whereas the new instruments are more complex electronically, the Tinsley instrument has only a simple d.c. amplifier, with a mirror galvanometer and photocell feeding a single electrometer valve.

For most of the comparisons made, particularly with regard to resolving power, a derivative circuit was required. With the three precision instruments, the effect of the condenser current has been virtually eliminated by using an a.c. derivative or the rapid scanning of potential on a single drop. This important refinement in technique is responsible for the increased complexity of the circuit. The derivative polarograms obtained with the Tinsley instrument are produced by a modified Leveque - Roth circuit,[4] a differentiating condenser being placed in series with

the galvanometer. With this arrangement the condenser current due to the mercury-drop variation is manifested as a zero correction on the recorder, but the normal oscillations from the drop are still present in the input to the galvanometer, although considerably damped in the recorder signal.

INSTRUMENT MODIFICATIONS—

The Tinsley instrument used had the following circuit variations from the standard mark 15/6—

(*i*) damping ranges: 0, 500 μF, 1500 μF, 2500 μF and 3500 μF.
(*ii*) two values of derivative time constant, allowing increased resolution at the cost of sensitivity in the approximate ratio of 1 to 2.

All measurements were made at 25° C against a mercury-pool anode.

SENSITIVITY—

Three solutions were used to measure the sensitivity, being examples of the three normal classes of polarographic waves—

(*a*) reversible reduction of Cd^{2+},
(*b*) partly irreversible reduction of Ni^{2+}, and
(*c*) catalytic reduction of an organic dye (the Pontachrome violet complex of Al^{3+}).

The waves obtained are shown in Figs. 1, 2, 3 (*a*) and 3 (*b*); full-scale deflection on the recorder is given by an input of 10 mA.

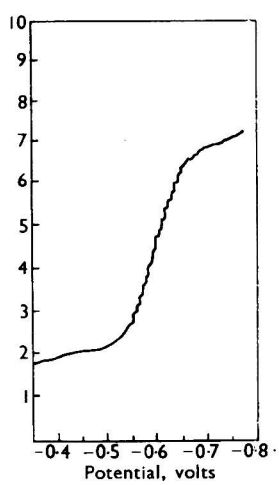

Fig. 1

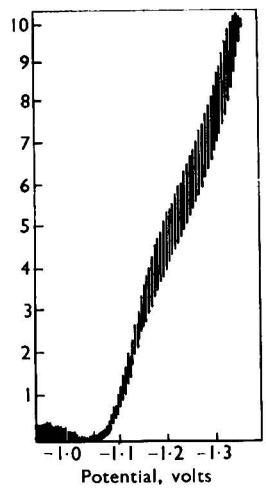

Fig. 2

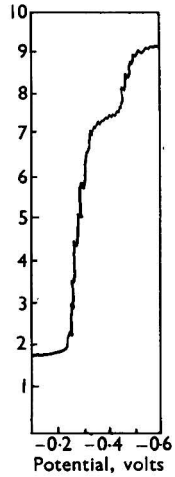

Fig. 3(*a*)

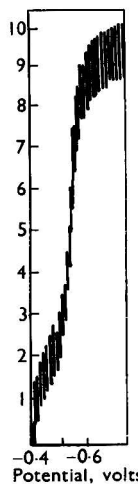

Fig. 3(*b*)

Fig. 1. Polarogram of a 0·18 μg per ml solution of Cd^{2+} in *M* potassium chloride: sensitivity, $\frac{1}{4}$ maximum

Fig. 2. Polarogram of a 5 μg per ml solution of Ni^{2+} in *M* potassium chloride: sensitivity, $\frac{1}{8}$ maximum

Fig. 3(*a*). Polarogram of a 2 μg per ml solution of Al^{3+} in Pontachrome violet SW; the waves from complexed and uncomplexed dye: sensitivity, $\frac{1}{40}$ maximum

Fig. 3(*b*). Polarogram of Al^{3+} - Pontachrome violet SW complex at an increased sensitivity of $\frac{1}{10}$ maximum

The polarograms were all obtained with use of a normal drop time of approximately 3 seconds. The first two required considerable application of counter current, but with cadmium at a concentration of 0·18 μg per ml there is no possible doubt as to the position and angle of the diffusion-current plateau.

RESOLUTION—

For the examination of resolution the derivative circuit was employed, a capillary with a drop rate of 1·2 seconds being used. Two particular examples of adjacent waves were studied, with *M* potassium chloride as base electrolyte—

Cd^{2+} and In^{3+}: $E_{\frac{1}{2}}$, − 0·642, − 0·597 volt.
Pb^{2+} and Tl^{+}: $E_{\frac{1}{2}}$, − 0·435, − 0·475 volt.

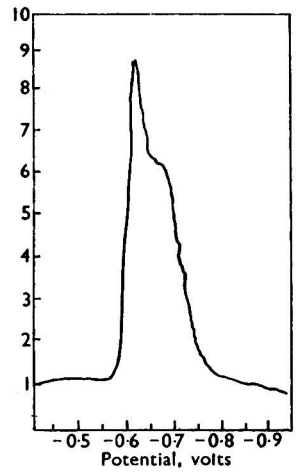

Fig. 4

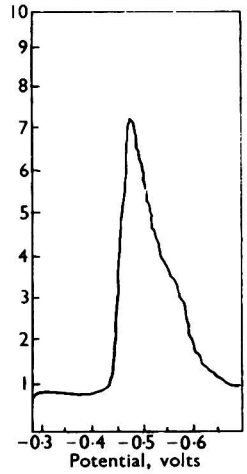

Fig. 5

Fig. 4. Polarogram of a solution containing 20 μg of Cd^{2+} and of In^{3+} per ml in *M* potassium chloride: sensitivity, $\frac{1}{16}$ maximum

Fig. 5. Polarogram of a solution containing 10 μg of Pb^{2+} and of Tl^{+} per ml in *M* potassium chloride: sensitivity, $\frac{1}{5}$ maximum

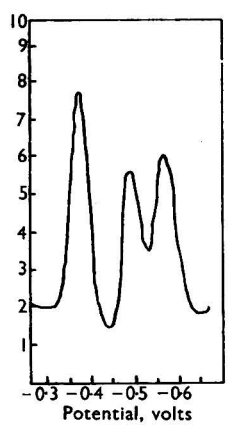

Fig. 6

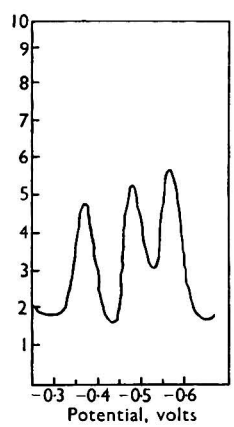

Fig. 7

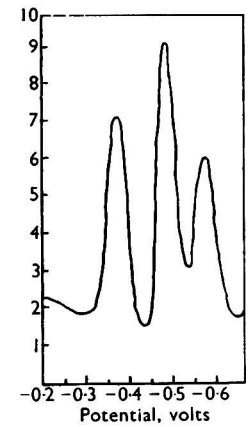

Fig. 8

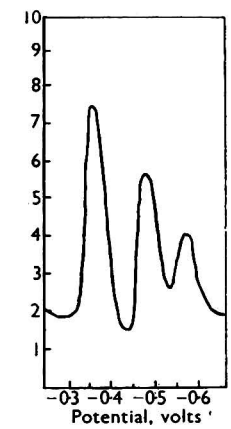

Fig. 9

Fig. 6. Polarogram of 25 ml of solution containing 1 ml of hydrochloric acid, 5 ml of saturated potassium bromide solution, 50 μg of Pb^{2+}, 25 μg of In^{3+} and 50μg of Cd^{2+}: sensitivity, $\frac{1}{2}$ maximum

Fig. 7. Polarogram of 25 ml of solution containing 1 ml of hydrochloric acid, 5 ml of saturated potassium bromide solution, 25 μg of Pb^{2+}, 25 μg of In^{3+} and 50 μg of Cd^{2+}: sensitivity, $\frac{1}{2}$ maximum

Fig. 8. Polarogram of 25 ml of solution containing 1 ml of hydrochloric acid, 5 ml of saturated potassium bromide solution, 50 μg of Pb^{2+}, 50 μg of In^{3+} and 50 μg of Cd^{2+}: sensitivity, $\frac{1}{2}$ maximum

Fig. 9. Polarogram of 25 ml of solution containing 1 ml of hydrochloric acid, 5 ml of saturated potassium bromide solution, 50 μg of Pb^{2+}, 25 μg of In^{3+} and 25 μg of Cd^{2+}: sensitivity, $\frac{1}{2}$ maximum

These mixtures were partly resolved when the ratio of concentrations was 1 to 1 (Figs. 4 and 5). Attempts to record polarograms at greater concentration ratios, showing the existence of the dilute species, were, however, unsuccessful. Nevertheless, on departing from the very exacting conditions mentioned above, polarograms demonstrating the resolution of the instrument were obtained. In particular, Figs. 6, 7, 8 and 9, which show Pb^{2+}, In^{3+} and Cd^{2+} in an acidified base electrolyte of *M* potassium bromide ($E_{\frac{1}{2}}$ — 0·38, — 0·49, — 0·57 volt) at various concentrations.

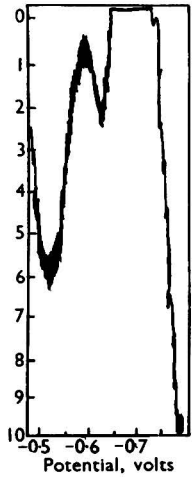

Fig. 10

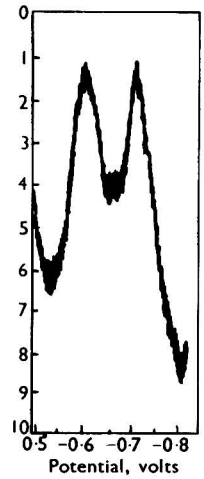

Fig. 11

Fig. 10. Polarogram of 25 ml of solution containing 1 ml of hydrochloric acid (neutralised with ammonium hydroxide to the end-point with methyl violet), 4 mg of Pb^{2+} and 40 μg of Cd^{2+}: sensitivity, maximum

Fig. 11. Polarogram of 25 ml of solution containing 1 ml of hydrochloric acid (neutralised with ammonium hydroxide to the end-point with methyl violet), 4 mg of Pb^{2+} and 80 μg of Cd^{2+}: sensitivity, $\frac{1}{2}$ maximum

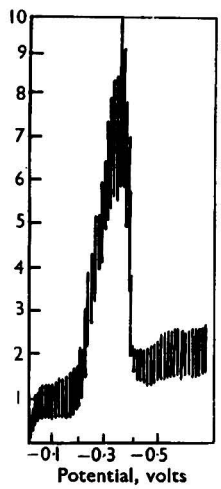

Fig. 12

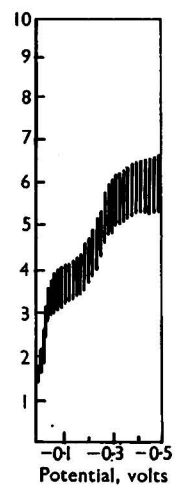

Fig. 13

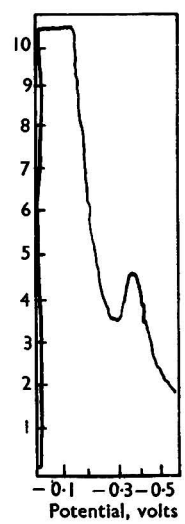

Fig. 14

Fig. 12. Polarogram of a solution containing 1 mg of Fe^{3+} per ml and 50 μg of Cu^{2+} per ml in *M* hydrochloric acid: sensitivity, $\frac{1}{600}$ maximum

Fig. 13. Polarogram of a solution containing 1 mg of Fe^{3+} per ml and 50 μg of Cu^{2+} per ml in *M* hydrochloric acid, but with 0·005 per cent. of gelatin added: sensitivity, $\frac{1}{200}$ maximum

Fig. 14. Polarogram of a solution containing 1 mg of Fe^{3+} per ml and 50 μg of Cu^{2+} per ml in *M* hydrochloric acid, but with 0·005 per cent. of gelatin added: sensitivity, $\frac{1}{100}$ maximum

The above are examples of resolution of waves with close $E_{\frac{1}{4}}$ values, but similar concentrations. The second advantage of the derivative polarograph, particularly exemplified in the square-wave polarograph, is the determination of small amounts of an ion in the presence of a considerable excess (greater than 10 to 1) of an ion reduced at a less negative potential. This is clearly shown in Figs. 10 and 11, which show derivative peaks for Cd^{2+} in the presence of 50 to 1 and 100 to 1 concentration ratios of Pb^{2+}. In this case the potential sweep was in the reverse direction, giving a concave derivative peak as opposed to convex. (For ease of reading the polarograms have been reversed in the Figures, but note the reverse deflection axis.)

Ferrett, Milner, Shalgosky and Slee[1] examined the system Fe^{3+} – Cu^{2+} in *M* hydrochloric acid with a Fe^{3+} to Cu^{2+} concentration ratio of 20 to 1. As a preliminary examination of this solution, a direct polarogram was recorded. This is shown at Fig. 12 and has a very large maximum, which was suppressed by the addition of gelatin to give the polarogram shown in Fig. 13. A derivative of the second "normal" solution was obtained (Fig. 14), but all attempts at producing a recognisable trace for the solution with the maximum failed. This point raised the question, in regard to the precision polarographs without an undamped direct circuit, as to the validity of their "derivative" wave on solutions having maxima.

Conclusion

The investigation has provided a datum line for comparison of precision polarographs with simple polarographs, in which use is made of d.c. amplification of the signal. It is considered that in view of the complexity and cost of the new instruments the performance of the Tinsley instrument is of a high order. The general utility of the instrument both for large batches of routine samples and special investigations has been fully confirmed in a year's practical use.

In this Note attention has been drawn to the potentialities of the instrument, which had not been fully investigated before.

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DETERMINATION OF IMPURITIES IN FILTER-PAPER

During the course of studying various reactions on filter-paper by the ring-oven technique,[1,2,3] it was found that certain filter-papers contained soluble inorganic substances, which interfered with these reactions. Hence the ring-oven method has been used to determine the impurity in the filter-paper. This method should be of general interest to many workers who utilise techniques such as spot analysis, paper chromatography and paper electrophoresis.

The principle of the method is that soluble materials can be washed out of a circular area of the paper and concentrated in a sharply defined ring zone. This effects concentration of the impurities as follows—

> The ring-oven has a bore-hole of diameter 22 mm. This means that the impurities are extracted from an area of $11^2 \times 3{\cdot}14$, *i.e.*, approximately 380 sq. mm. At these low concentrations, the width of the ring zone is of the order of 0·1 mm. Consequently, the area in which the soluble materials have been concentrated is given by the expression $2\pi r \times 0{\cdot}1$, *i.e.*, approximately 7 sq. mm. The concentration factor is therefore $\frac{380}{7}$, *i.e.*, 54.

Selective extraction can be achieved by judicious choice of solvent, *e.g.*, acid, ammonia, water, etc. In this way it is possible to classify the various types of impurity.

For example, if 0·02 *M* hydrochloric acid purified by the isothermal-distillation technique of Abrahamczik[4] is used, heavy metals are concentrated in the ring zone. The paper is then dried and cut in half. Iron, which is one of the commonest contaminants, can be revealed in one half

of the ring by spraying with potassium ferrocyanide solution.[1] Heavy metals in general can be revealed in the other half of the ring by bathing it in ammonium sulphide solution. This fixes the heavy metals as insoluble sulphides. The excess of ammonium sulphide is washed away. Finally, the paper is treated with silver nitrate solution, which produces an equivalent ring of the more visible silver sulphide. (Zinc sulphide, manganese sulphide, etc., are not readily visible on paper.) Specific reagents can be applied by spraying in order to detect the presence of any particular ion, *e.g.*, dimethylglyoxime for nickel.

Chloride, which is another common impurity, can be detected in the following way. The impurities are washed out into the ring zone by means of distilled water. The filter-paper is dried and then bathed in silver nitrate solution, which precipitates chloride (and, of course, other anions that form insoluble silver salts). The excess of silver nitrate is washed away. The ring is then made visible by bathing the paper in ammonium sulphide solution.

By visual comparison of the intensities of the various rings obtained from a selection of papers, it is possible to select the one that is best suited for any particular purpose. The method described here may be used to follow the purification of paper for special methods such as chromatography and electrophoresis.

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THE MICRO-DETERMINATION OF ARSENATE IN THE PRESENCE OF ARSENITE

IN the course of work on the radiation chemistry of the arsenite - arsenate system, a method was required for the determination of micro amounts of arsenate in the presence of excess of arsenite. Volumetric procedures involving either the oxidation of arsenic^{III} by tri-iodide ion at a neutral pH or the reduction of arsenic^{V} by iodine at an acid pH (see Kolthoff and Sandell[1]) were rejected on the grounds of (*a*) interference by hydrogen peroxide, which was known to be formed in amounts comparable to arsenic^{V}, and (*b*) dependence of the method on a small difference and consequent insensitivity, especially when initial oxidation rates were to be measured at high (approximately 0·1 *M*) concentrations of arsenic^{III}. Similar considerations eliminated the method of Gleu,[2] involving catalysed titration of arsenic^{III} with a ceric salt, although this can be, and was, used as a check at large percentage oxidation and relatively low initial concentrations (approximately 10^{-3} *M*) of arsenite, the reduction of the ceric salt due to hydrogen peroxide in solution being concurrently determined.

Colorimetric or spectrophotometric procedures seemed to be indicated, and a sensitive method has been developed and applied in this laboratory based on the formation of 12-molybdoarsenic acid, its selective extraction into an organic phase and subsequent reduction to the intensely coloured molybdenum blue. Since the work of Denigès,[3] several similar procedures have been described (see Snell and Snell[4]); many of these have suffered from the drawback that the poly acid (12-molybdoarsenic acid) is reduced in the presence of the excess of molybdic acid used in the formation of the complex. Slow concurrent reduction of the uncomplexed molybdic acid, together with possible reduction by other substances present (especially in the presence of organic solutes) and interference by hydrogen peroxide (reactions of which are known to be catalysed by molybdates[5]), decrease the reliability of such methods. Wadelin and Mellon[6] separated the 12-molybdoarsenic acid from excess of reagent by extraction with *n*-butanol and proposed a method based on ultraviolet absorption of the extract, although this did not show any absorption maximum. As well as having a low sensitivity ($\epsilon = 5100$ litres per mole per cm at 370 mμ), interference from the yellow peroxymolybdic acid complex might be expected. The present method combines the procedure involving the formation and extraction of the 12-molybdoarsenic acid with its subsequent reduction to molybdenum blue.

TABLE I

EFFECT OF TIME OF SHAKING ON EXTRACTION OF 12-MOLYBDOARSENIC ACID

Concentration of arsenic^{V}, μM per 25 ml	Time of shaking, minutes	Absorptiometer reading
0·33	½	0·085
	1	0·125
	2	0·150
	4	0·152
0·82	½	0·308
	1	0·377
	2	0·385
	3	0·387
	4	0·387
1·37	½	0·540
	1	0·615, 0·630
	2	0·650
	4	0·650

EXPERIMENTAL

12-Molybdoarsenic acid is formed by addition of the test solution to excess of molybdic acid in 2 *N* sulphuric acid and extracted by shaking with *iso*butanol. After being washed with sulphuric acid to remove excess of molybdate, the organic layer is reduced by the addition of stannous chloride in sulphuric acid, and the colour intensity of the organic layer is measured in a Spekker photo-electric absorptiometer, a Kodak No. 570 red filter being used.

With the reagents and conditions to be described (see "Method"), the critical feature of this method was found to be the time of shaking for the extraction of the 12-molybdoarsenic acid. Typical results obtained with standard arsenate solutions are shown in Table I.

The method is not sensitive to small changes in final acid concentration, *i.e.*, up to 10 per cent.; with a solution containing 1·0 μM of arsenic^{V} per 25 ml, at a pH of 1·2 the absorptiometer reading was 0·480, while at a pH of 6·0 it was 0·478. Only strongly alkaline solutions (0·1 *N* sodium hydroxide) are neutralised before determination. The method may be safely employed between pH 1 and 13.

Reaction between the arsenate and molybdate is too rapid to be measured by this method and the reduction of the 12-molybdoarsenic acid occurs within a few seconds; the final colour is stable for at least 3 hours away from bright sunlight.

METHOD

By pipette place a 10-ml sample in a 50-ml shaking flask containing 5 ml of 2 *N* sulphuric acid, and add 5 ml of 5 per cent. ammonium molybdate solution and then 20 ml of *iso*butanol. Shake the mixture for 3 minutes, allow it to separate into layers and discard the aqueous layer. Wash the organic layer twice by shaking it with 10-ml portions of *N* sulphuric acid for 30 seconds. Reduce the solution by adding 30 ml of a 0·4 per cent. solution of stannous chloride in *N* sulphuric acid and shaking for 30 seconds (the stannous chloride solution should be prepared daily by diluting a stock solution of 25 g of the solid, $SnCl_2.2H_2O$, in 100 ml of concentrated hydrochloric acid). Run off the acid layer, and wash the blue organic layer into a 25-ml calibrated flask and make up to the mark with ethanol (the ethanol serves to make miscible any droplets of acid carried over from the reduction). Measure the colour by means of a Spekker absorptiometer, using a 1-cm cell and a red filter.

RESULTS

With standard arsenate solutions, it was found that Beer's law is obeyed for absorptiometer readings up to 1·00, the relationship with arsenate concentration being—

$$[\text{arsenic}^{V}] = \frac{\text{absorptiometer reading}}{0{\cdot}475},$$

where [arsenic^{V}] is expressed as μM per 25 ml of final solution. Measurements with a Unicam spectrophotometer indicate an absorption maximum at a wavelength of 740 mμ (ϵ = 17,700 litres per mole per cm). The usual lower limit of determination is 5×10^{-6} *M*.

Arsenic^{III} does not interfere at concentrations up to 0·1 M (the limit of solubility in acid solution) and hydrogen peroxide only at concentrations greater than 10^{-3} M, as can be seen by the following results for a solution containing 1·0 μM of arsenic^{V} per 25 ml—

Concentration of arsenic^{III}, μM ..	0	10^{-3}	10^{-1}	0	10^{-3}	10^{-3}	10^{-3}
Concentration of hydrogen peroxide, M	0	0	0	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
Absorptiometer reading	0·480	0·480	0·482	0·478	0·480	0·475	0·488, 0·405

Anions forming extractable *iso*poly acids with molybdic acid, *e.g.*, phosphate, silicate and germanate, naturally interfere, but as this method has only been applied to work on very pure arsenite solutions, no attention has been paid to this problem; it could probably be solved by a selective extraction procedure (see Alexeev[7] and DeSesa and Rogers[8]).

Thanks are due to Professor J. Weiss for his encouragement in the course of this work and to Miss J. Elstob for technical assistance. Thanks are also due to the Director of the Atomic Energy Research Establishment, U.K.A.E.A., for support of this investigation and for permission to publish this Note.

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A METHOD FOR THE DETECTION AND DETERMINATION OF *iso*QUINOLINE AND QUINOLINE IN THE PRESENCE OF ONE ANOTHER

While investigating the nitration of quinoline and *iso*quinoline,[1] it was observed that, if the nitrations were carried out with an excess of nitric acid, both compounds could be nitrated virtually to completion (96 per cent. of the theoretical value for quinoline and 99 per cent. for *iso*quinoline) under comparatively mild conditions. Since a mixture of nitroquinolines and nitro-*iso*quinolines can easily be separated by chromatography on alumina, this provides a simple method for the determination or detection of one base in the presence of the other.

Experimental

The *iso*quinoline used was obtained from The British Drug Houses Ltd.; after one recrystallisation it contained no impurity detectable by the analytical method described. Peter Spence type-H alumina (100 to 200 mesh) was used for chromatography.

A mixture of 1·125 g of synthetic quinoline and 0·065 g of *iso*quinoline was dissolved in 9 ml of concentrated sulphuric acid at 0° C, and 0·8 ml of fuming nitric acid, sp.gr. 1·5, was added with stirring. The nitration was allowed to continue for 15 minutes at 0° C. The mixture was then poured on to ice, neutralised with aqueous ammonia and the resulting solution, having a volume of about 200 ml, was extracted with four 70-ml portions of chloroform. The chloroform extract was dried and distilled to leave a residue of mixed nitroquinolines and nitro*iso*quinolines. This was dissolved in ether and the solution was chromatographed on a column of alumina (approximately 1·5 cm × 25 cm). When viewed under ultra-violet light in the dark (Note—It is necessary to place the column very close to the lamp), two distinct bands soon appeared on the column, a thin dark-coloured band at the top that hardly moved (nitro*iso*quinolines) and a larger lighter band (nitroquinolines) that was eluted rapidly. The upper band was eluted separately with chloroform. The separation was repeated on both fractions until they were homogeneous (weight of pure nitroquinolines, 1·453 g; weight of nitro*iso*quinolines, 0·080 g: proportion of *iso*quinoline in the original mixture, 5·4 per cent.; proportion of nitro*iso*quinolines in the mixture of nitro compounds, 5·2 per cent.).

Results

The method described above was applied to several artificial mixtures of various compositions. The results were as follows—

*iso*Quinoline in original mixture, % ..	98·1	90·1	51·7	48·2	5·4	1·2
Nitro*iso*quinolines found, %	97·4	90·5	52·0	48·9	5·2	1·2

In practice the procedure is easier to apply for the detection of small amounts of *iso*quinolines in quinoline than *vice versa*, since the nitro*iso*quinoline band is more clearly visible under ultraviolet light than the nitroquinoline band. When the amounts of the two bases are roughly equal, best results are obtained by using a smaller amount of sample (about 0·2 to 0·3 g) than quoted under "Experimental." The accuracy of the determinations is estimated at ± 1 per cent.; the limits of determination are about 1 per cent. of *iso*quinoline in quinoline and 2 per cent. of quinoline in *iso*quinoline. However, it is possible to detect much smaller amounts of *iso*quinoline in quinoline.

Three determinations were carried out on a sample of commercial "Puriss." quinoline and *iso*quinoline contents of 6·0, 5·9 and 5·9 per cent. were found.

Two experiments were also carried out with mixtures (A and B) containing known amounts of quinaldine. It was found that, if the *iso*quinoline was in excess and only small amounts of quinoline and quinaldine were present, the analytical procedure was still applicable. The nitration mixture could be resolved into three components by chromatography—nitroquinolines, nitro*iso*quinolines and nitroquinaldines, the last-named compounds appearing as a dark band between the two preceding bands. When, however, the mixture contained a large excess of quinoline and only small amounts of quinaldine and *iso*quinoline, it was not possible to separate the nitroquinaldines from the nitroquinolines. Separation from the nitro*iso*quinolines could still be achieved.

Details of the mixture are as follows—

Mixture A—Composition of original mixture: quinoline, 5·0 per cent.; *iso*quinoline, 90·9 per cent.; quinaldine, 4·1 per cent. Composition of nitro compounds found: nitroquinolines, 5·6 per cent.; nitro*iso*quinolines, 90·4 per cent.; nitroquinaldines, 4·1 per cent. (all values are quoted as molar percentage).

Mixture B—Composition of original mixture: quinoline, 92·3 per cent.; *iso*quinoline, 3·4 per cent.; quinaldine, 4·3 per cent. Composition of nitro compounds found: nitroquinolines + nitroquinaldines, 96·8 per cent.; nitro*iso*quinolines, 3·2 per cent. (proportions by weight).

I thank the Department of Scientific and Industrial Research for the award of a maintenance grant and Kodak Ltd. for a gift of synthetic quinoline.

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June 20th, 1956

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Erratum

January (1957) issue, p. 60, 16th line from foot of page. *For* "2·34 mm of mercury" *read* "234 mm of mercury."