The Analyst

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of The Chemical Society

A monthly international publication dealing
with all branches of analytical chemistry

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Summaries of Papers in this Issue

Sub-micrometre Particle Size Characterisation and Distribution by Mercury Penetration

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A determination of the particle size and distribution of three powders, in the one micrometre and sub-micrometre size range, has been obtained from mercury porosimetry breakthrough and intrusion pressures. The mercury intrusion particle diameters and distributions are compared with values obtained by gravitational and centrifugal sedimentation methods and electron microscopy counts for particle size measurement.

Keywords: *Particle size characterisation*; *mercury penetration*

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Analyst, 1979, **104**, 97–105.

Determination of Acrylonitrile Monomer in Plastic Packaging and Beverages by Headspace Gas Chromatography

A gas - liquid chromatographic method for determining trace amounts of acrylonitrile in plastic containers and carbonated beverages using a nitrogen-sensitive detector and headspace injection technique is described. The method is suitable for the determination of acrylonitrile at concentrations down to 0.1 mg kg^{-1} in plastics and 0.005 mg kg^{-1} in beverages.

Keywords: *Acrylonitrile determination*; *headspace gas chromatography*; *plastic packaging*; *foodstuffs*

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Analyst, 1979, **104**, 106–110.

Determination of Ethylenethiourea in Ethylenebisdithiocarbamate Fungicides: Comparison of High-performance Liquid Chromatography and Gas - Liquid Chromatography

A rapid, sensitive method is described for the determination of ethylenethiourea (imidazolidine-2-thione) in ethylenebisdithiocarbamate fungicides. High-performance liquid chromatography is used with an ultraviolet spectrophotometric detector. The results are compared with those obtained using gas - liquid chromatography. All fungicide samples assayed contained ethylenethiourea, and gas - liquid chromatography indicated higher concentrations than high-performance liquid chromatography.

Keywords: *Ethylenethiourea determination*; *ethylenebisdithiocarbamate fungicides*; *high-performance liquid chromatography*; *gas - liquid chromatography*

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Analyst, 1979, **104**, 111–116.

Fluorimetric Determination of Acetohexamide in Plasma and Tablet Formulations Using 1-Methylnicotinamide

A sensitive method is described for the fluorimetric determination of acetohexamide in plasma or in tablets by means of its reaction with 1-methylnicotinamide, which is shown to be a useful reagent for the determination of ketonic compounds. The limit of detection is approximately 0.2 $\mu g\ ml^{-1}$ and the relative standard deviation is 3.1% for 2 $\mu g\ ml^{-1}$ in plasma. Acetoacetic acid usually does not interfere, but can be separated, if necessary, from acetohexamide by means of a washing technique. No interference is caused by the presence of insulin, other (non-ketonic) oral hypoglycaemic drugs, acetone or pyruvic or α-ketoglutaric acid.

Keywords: *Acetohexamide determination*; *plasma*; *tablets*; *1-methylnicotinamide reagent*; *spectrofluorimetry*

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Analyst, 1979, **104**, 117–123.

Polarographic Determination of Trace Elements in Food from a Single Digest

The determination of 12 trace elements, namely copper, zinc, mercury, lead, cadmium, iron, tin, chromium, arsenic, antimony, selenium and tellurium, in admixture by means of a cathode-ray polarograph is described. The elements were investigated in concentrations ranging from 0.1 to 20 p.p.m. by means of normal, reverse-sweep or resistance - capacitance derivative techniques. The last technique could not be used for mercury, although it was used for all the other elements in concentrations less than 1 p.p.m. The complete determination took 4–7 h, and was applied to various kinds of food, such as bread, meat and vegetables.

Keywords: *Trace element determination*; *food analysis*; *polarography*; *single digest*

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Analyst, 1979, **104**, 124–135.

Apparatus for the Automatic Preparation of Soil Extracts for Mineral-nitrogen Determination

An apparatus is described that automatically prepares samples and feeds an AutoAnalyzer system. It consists of a reagent adder, which adds the correct volume of extractant for an approximately weighed amount of soil, and a sample preparation unit, which mixes, filters, dilutes and loads samples on to an AutoAnalyzer sampler. The results obtained using the apparatus were in good agreement with those obtained by manual sample preparation.

Keywords: *Mineral-nitrogen determination*; *soil analysis*; *automatic extraction*

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Analyst, 1979, **104**, 136–142.

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The Analyst

Sub-micrometre Particle Size Characterisation and Distribution by Mercury Penetration

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The technique of mercury porosimetry is usually regarded as a method for the determination of surface areas and the evaluation of pore size distributions in porous solids. With fine non-porous or microporous materials the initial low-pressure region of a mercury penetration graph can be used to determine the inter-particle spaces or voids in an assembly of discrete particles.

A determination of the particle size and distribution of three powders, in the one micrometre and sub-micrometre size range, has been obtained from mercury porosimetry breakthrough and intrusion pressures. The mercury intrusion particle diameters and distributions are compared with values obtained by gravitational and centrifugal sedimentation methods and electron microscopy counts for particle size measurement.

Keywords: *Particle size characterisation; mercury penetration*

The high-pressure mercury intrusion technique is commonly used to characterise, in terms of the volume, number and distribution, the void or pore spaces in porous materials.

The evaluation and interpretation of the volume of mercury which penetrates, under pressure, into porous solids was initially proposed by Washburn,[1,2] who applied the Young - Laplace equation for capillary rise in cylindrical tubes to the measurement of the size of pores in solids:

$$P = (-2\gamma_{LG}\cos\theta)/r \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where P is the applied intrusion pressure, γ_{LG} the surface tension of mercury, θ the contact angle of mercury and r the radius of the cylindrical tube.

In reality, however, the results of pore size distribution analysis obtained by use of this equation give only an indistinct image of the real situation, as the model chosen is one of a collection of perfectly cylindrical tubes open at both ends.[3] The limitations of the Washburn model were recognised by de Boer,[4] Frevel and Kressley,[5] Kruyer[6] and Mayer and Stowe.[7,8] Frevel and Kressley proposed an alternative model to describe the penetration of mercury or fluid under pressure into void spaces within a solid sample composed of a collection of non-porous uniform spheres. The derived mathematical relationship allowed the determination and direct comparison, from the initial penetration or breakthrough pressure of mercury, P^*, into this assembly of non-porous spheres, of a surface-area equivalent spherical radius, r_s, with that of a mercury-porosimeter equivalent particle radius, r_m, over the porosity range 39.54–25.95%.

Later, Mayer and Stowe described, in more general terms, the breakthrough pressure required for the penetration of a fluid into a collection of non-porous, uniform, solid spheres over the extended porosity range of 47.64–25.95%. This model was subsequently modified to evaluate the toroidal void volume between touching spheres

$$P = -\gamma_{LG}(L'/A)/r_s \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where the function (L'/A) was regarded as equivalent to the breakthrough pressure P^* and was calculated for all degrees of packing between the two porosities of hexagonal close-packed and cubic-packed spheres (Tables II and III in reference 7).

Many attempts[3,9,10] have been made to measure the size of irregularly shaped non-porous particles by mercury intrusion. This technique has, however, been criticised because the Frevel and Kressley and Mayer and Stowe models of an assembly of regular monosized spheres are used to characterise a random polysized assembly of particles. Agreement of the mercury particle radius, r_m, calculated from the regular monosized sphere model of Mayer and Stowe, with the radii determined from other independent particle characterisation techniques is, according to Van Brakel,[11] fortuitous. Svata and Zabransky[10] have shown experimentally, however, that the regular sphere model of Mayer and Stowe can be used to evaluate the particle size and the size distribution of spherical and non-spherical particles. The particle size measurement by mercury porosimetry of various sized and shaped bodies of poly(methyl methacrylate), carbonyl iron and nickel and fritted glass, all non-porous solids, showed close agreement with the particle size determined by sedimentation and microscope-count techniques.

When the mercury intrusion technique is used with porous particles, there is a difficulty in separating the effect of the volume of mercury that penetrates the spaces between particles (voids) from the effect of the volume of mercury that penetrates the spaces within particles. The purpose of this investigation was to show whether mercury intrusion could be used to indicate the presence, and measure the shape of the size distribution, of micrometre and sub-micrometre particles in porous and non-porous powders.

Experimental

Powders

Steel shot

This is a plastic-coated steel shot with a mean particle size of 112 μm, as determined by sieve analysis with Endecott test sieves and shaker. The density, as determined with an air pycnometer (Beckman, Model 930) or by mercury displacement, is 7.952×10^3 or 7.615×10^3 kg m^{-3}, respectively. The theoretical density from the literature[12] is 7.750×10^3 kg m^{-3} (Fig. 1, Table I).

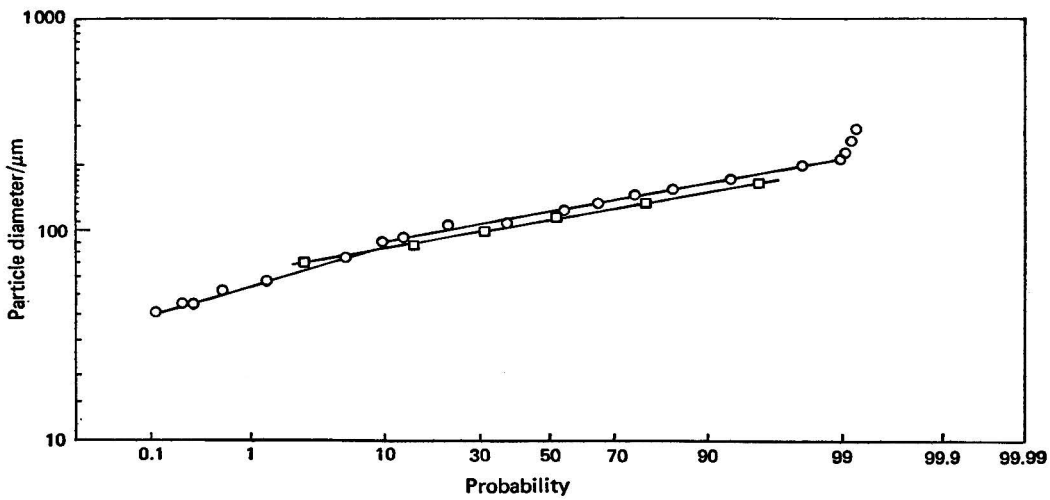

Fig. 1. Sieve and mercury intrusion size distributions for steel shot. ○, Mercury penetration 118 μm; □, sieve size 112 μm.

Magnesium trisilicate

This is a hydrated magnesium silicate. The mean particle size by wide-angle scanning photosedimentation (WASP) is 15.1 μm by mass, with a size range of 3.0–30.0 μm. The number - length particle size obtained from electron microscope photographs at 700× and 2600× magnification is 1.9 μm (Fig. 2, Table I), with a size range of 0.38–19.2 μm. The density, as determined by air pycnometry, is 2.17×10^3 kg m^{-3}.

Barium sulphate

This fine, white, insoluble powder has a mean particle size, by mass, with the wide-angle scanning photosedimentation technique, of 10.7 μm and a size range of 1.85–41.0 μm. The

TABLE I

PHYSICAL CHARACTERISTICS OF POWDERS

Characteristic	Steel shot	Barium sulphate	Magnesium trisilicate	Dicalcium phosphate
Density $\times 10^3$/kg m^{-3}	7.952	4.36	2.17	2.31
Specific volume of solid/cm^3 g^{-1}	0.1255	0.2294	0.460	0.4329
Mercury volume/cm^3 g^{-1} in voids	0.0829	0.845	1.596	0.777
Mercury volume/cm^3 g^{-1} in pores	—	0.132	0.066	
Structure	Non-porous	Microporous	Microporous	Mesoporous
Porosity (ϵ)	0.398	0.700	0.752	0.642
Packing angle/angular degrees	71–72	90	90	90
$(L'/A)_{min.} \equiv P^*$ p.s.i.a.	4.214	3.35	3.35	3.35
Mean particle diameter/μm	112 (sieve)			
WASP d_{st}	119.8	17.0	15.1	18.0
Centrifugal d_{st}	—	0.57	40% < 2.3	15% < 3.0
Microscope d_n	—	1.10	1.85	2.50
Mercury d_m	118.0	4.8	17.8	6.8

number - length particle size from electron microscope photographs at 650× and 2600× magnification is 1.1 μm, with a size range of 36.2–0.54 μm (Fig. 3). The density, as determined by air pycnometry, is 4.36 × 10^3 kg m^{-3}.

Dicalcium phosphate dihydrate

Dicalcium phosphate dihydrate is a white, crystalline, water-insoluble powder with a mean particle size, as determined by photosedimentation, of 18.0 μm and a size range of 5.3–27.5 μm. The number - length particle size from electron microscope photographs at 650× and 2600× magnification is 2.5 μm with a size range of 0.57–39.1 μm (Fig. 4, Table I).

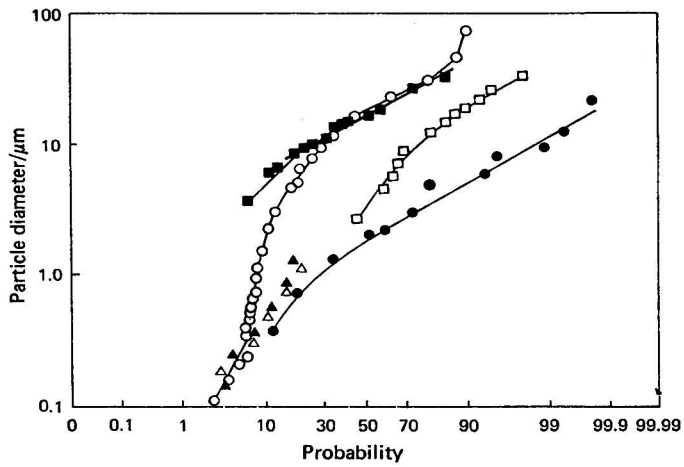

Fig. 2. Size distributions for magnesium trisilicate. ■, WASP by mass distribution; □, WASP by surface distribution; △, centrifuge by mass; ▲, centrifuge by mass (different sample mass); ○, mercury penetration (ϵ = 0.75); and ●, electron microscope number count.

Adsorption Isotherms

Adsorption isotherms of all powders were obtained by low-temperature nitrogen adsorption. The apparatus used was similar to that described in British Standard 4359, Part I.[13] All samples were de-gassed at room temperature (24 ± 1 °C) for 16 h at a vacuum of less than

10^{-3} Torr prior to adsorption measurements being made. The temperature of adsorption was 77 K and the nitrogen gas used was research grade XX from the British Oxygen Company, Wembley, Middlesex.

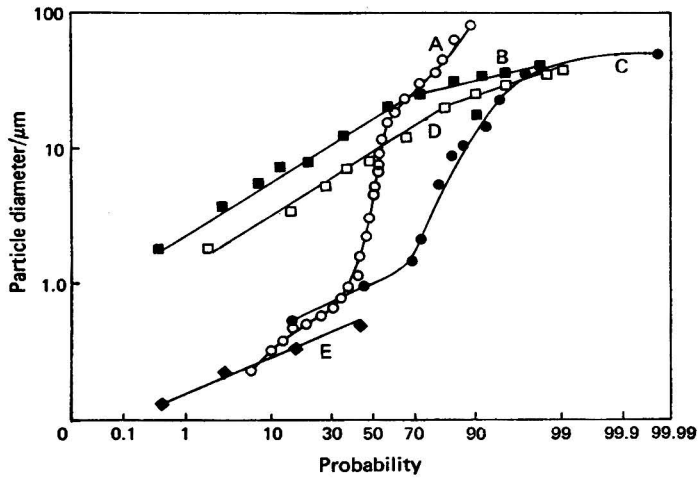

Fig. 3. Size distributions for barium sulphate. A, Mercury penetration (ϵ = 0.70); B, WASP by mass distribution; C, electron microscope number count; D, WASP by surface distribution; and E, centrifuge by mass.

Brunauer, Emmett and Teller surface area

The specific surface area of the powders was calculated from the monomolecular volume of nitrogen adsorbed between the relative pressure range of 0.05–0.35 and the Brunauer, Emmett and Teller equation.[14]

Mesopore size range (2.0–100 nm)

The nitrogen adsorption isotherms of magnesium trisilicate, barium sulphate and dicalcium phosphate dihydrate, measured over the relative pressure range 0.08–0.98, were used to

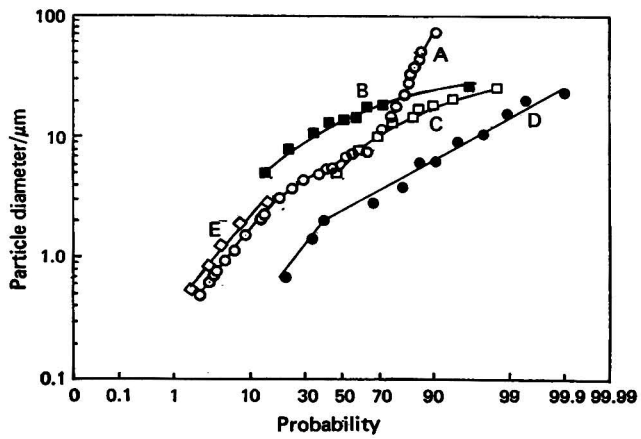

Fig. 4. Size distributions for dicalcium phosphate. A, Mercury penetration (ϵ = 0.64); B, WASP by mass distribution; C, WASP by surface distribution; D, electron microscope number count; and E, centrifuge by mass.

characterise the porous or non-porous structure of the powders in the pore size range 2.0–100 nm (0.1 μm). From the adsorption isotherm 40 values of the amount of nitrogen adsorbed into or on to the solid surface at specific relative pressures were taken. The specific relative pressure values were taken in known, positive incremental steps and these, together with the appropriate nitrogen volumes adsorbed per gram of powder, formed the data input to a computer. The pore size and number distribution were calculated from a computer program of the modified mathematical porous model of Barrett, Joyner and Halenda.[15] The computer program was written in FORTRAN for use on an ICL 1904 computer. The values of the Kelvin radius were calculated from the Kelvin equation and the statistical thickness of the adsorbed layer was calculated from the data of Schull.[16]

Micropore surface area (*pore radii* 1.6–2.0 nm *or less*)

The volume of nitrogen adsorbed at specific relative pressures obtained from the experimental adsorption isotherms was compared with the thickness of the unimpeded adsorbed nitrogen layer at the same specific relative pressure as obtained from the Lippens and de Boer t curve.[17] The resultant V_a - t graph was used to measure the amount of microporous area within the powders.

When a straight line passes through the origin of the V_a - t graph the slope of the line is a measure of the non-microporous surface area:

$$S_t = 1.547 \frac{V_a}{t} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where S_t is the non-microporous area in $m^2\ g^{-1}$, V_a is the volume of nitrogen adsorbed at specific relative pressures and a specific thickness measured in $cm^3\ g^{-1}$ and t is the statistical thickness of an unimpeded adsorbed nitrogen layer in nanometres. A convex deviation of the line with the statistical thickness axis indicates a microporous powder. A curve concave to the statistical thickness axis, at large values of layer thickness, indicates a solid with mesoporous structure (pore radii of 2.0–100 nm).

Mercury Intrusion

Measurement of the volume of mercury penetrating the voids and pores within the four samples were made by use of a high-pressure Micromeritics Mercury Porosimeter, Model 905–1. All of the powder samples were de-gassed at room temperature (24 $\pm$ 1 °C) for at least 16 h at a vacuum of less than 10^{-2} Torr. The steel shot was de-gassed until a vacuum of less than 10^{-2} Torr was maintained for 1 h. The pressure on the mercury was increased incrementally from below atmospheric, 1.11 p.s.i.a. (7.5 kPa), up to 47 800 p.s.i.a. (328 MPa). The particle sizes of the collection of particles were calculated from the Mayer and Stowe equation, assuming a surface tension of 0.474 N m^{-1} and a contact angle of 130° for mercury. The particle diameter from mercury intrusion (d_m) in μm was calculated from

$$d_m = \frac{137.5 \times P^*}{P} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where P is the experimental pressure in p.s.i.a. and P^* the reduced breakthrough pressure obtained from Table II of reference 7 at various powder sample porosities and mercury contact angles.

Electron Microscopy Number - Length Counts

Scanning electron photographs of the powders were obtained from a Cambridge Stereoscan S4-10 after coating the particles with pure gold.

A total of more than 300 particles for each powder were individually measured by the Feret diameter and the particle sizes classified into different size classes in order to obtain a number - length distribution similar to that specified in British Standard 3406.[18]

Pipette Centrifuge for Sub-micron Powders

The size analysis of particles below approximately 5 μm is carried out in a centrifugal field by using a modified pipette centrifuge.[19] The centrifugal head is a 16 cm diameter, horizontally mounted bowl in which six narrow-bore tubes, 7 cm in length, are radially attached to a hollow central shaft. The centrifugal bowl is driven by a constant-speed motor at either 750 or 1500 rev s^{-1} and it contains an initial volume of 150 ml of a dilute suspension of powder (less than 0.1% V/V) in 0.1% m/V Calgon dispersant. Aliquots of 10 ml are extracted from the spinning bowl through the tubes via the hollow central shaft at various time intervals.

The amount of powder extracted at these known time intervals is determined gravimetrically after being dried in a hot oven. The particle size is determined from the Stokes equation, modified for centrifugal force, and the percentage undersize of the powder evaluated for the Kamak equation.[20]

Results and Discussion

Spherical Steel Shot

The sieve size distribution of these spherical, non-porous solids is shown in Fig. 1. The mean sieve size was 112 μm.

From the mercury intrusion data the total volume of mercury that penetrated the assembly of steel shot when in the high-pressure mercury sample tube was 0.0829 cm^3 g^{-1} (Fig. 5 and Table I). The porosity of this collection of spheres, or any assembly of particles, can be calculated from the relationship

$$\epsilon = \frac{\text{Total volume of mercury penetrating the assembly of particles per gram}}{\text{Total volume of mercury per gram} + 1/\text{density of solid}}$$

Thus, for steel shot, the porosity of the sample in the mercury sample tube was 0.0829/(0.0829 + 0.1255) = 0.398. The single acute angle for this packing arrangement and porosity, from Table I in reference 7, is between 71° and 72°. Interpolation of the Mayer and Stowe general function $(L'/A)_{min.}$ or reduced breakthrough pressure P^* for "square" access openings for the above acute packing angle gives a value of 4.214 p.s.i.a. for breakthrough when mercury has a contact angle of 130°. The particle size and distribution of non-porous spherical steel shot determined from equation (4) and the percentage of mercury penetrating the packed spherical particles is shown in Figs. 1 and 5. The over-all shape of the distribution graph determined by mercury intrusion is similar to that determined by sieve analysis.

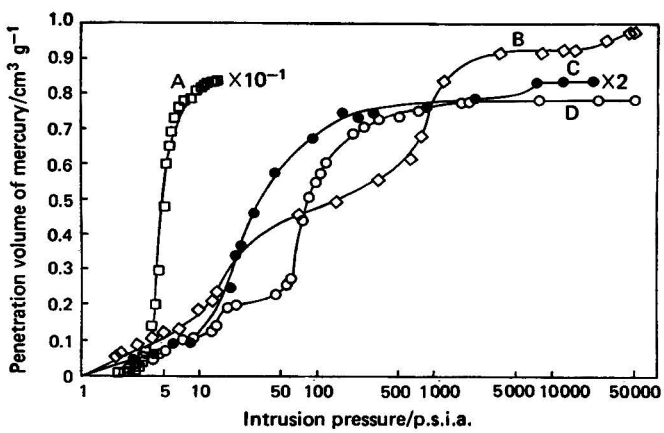

Fig. 5. Mercury intrusion graphs: A, Steel shot; B, barium sulphate; C, magnesium trisilicate; and D, dicalcium phosphate.

Barium Sulphate

The mass and surface distributions obtained by photo- and centrifugal sedimentation are shown in Fig. 3, together with the distribution obtained by a number - length electron-microscope count and the mercury intrusion technique.

Nitrogen adsorption isotherm analysis by the Barrett, Joyner and Halenda method and the Lippens and de Boer V_a - t method shows that barium sulphate contains a large number of pores in the size range up to 2.0 nm radius. The number of pores then decreases rapidly so that barium sulphate can be regarded as having no meso- or macropores (Fig. 6). This characterisation of the non-mesoporous structure of barium sulphate is substantiated by the linearity of the V_a *versus* t graph (Fig. 7) at nitrogen layer thicknesses greater than 1.4 nm.

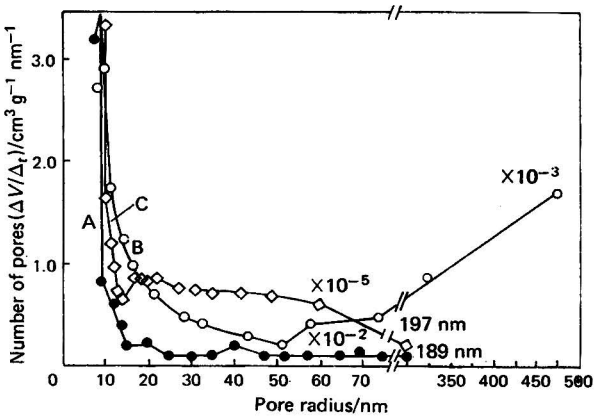

Fig. 6. Nitrogen adsorption pore size distributions: A, magnesium trisilicate; B, dicalcium phosphate; and C, barium sulphate.

The mercury intrusion graph (Fig. 5) indicates, however, the presence of pores, using the Washburn model, in the diameter range 160–0.0036 μm (160000–3.6 nm). The barium sulphate mercury intrusion graph can be readily divided into regions, one region in which mercury is being forced between particles and has a volume of 0.845 cm^3 g^{-1}, and another in which mercury is being forced into particles and has a volume of 0.132 cm^3 g^{-1} (Table I, Fig. 5).

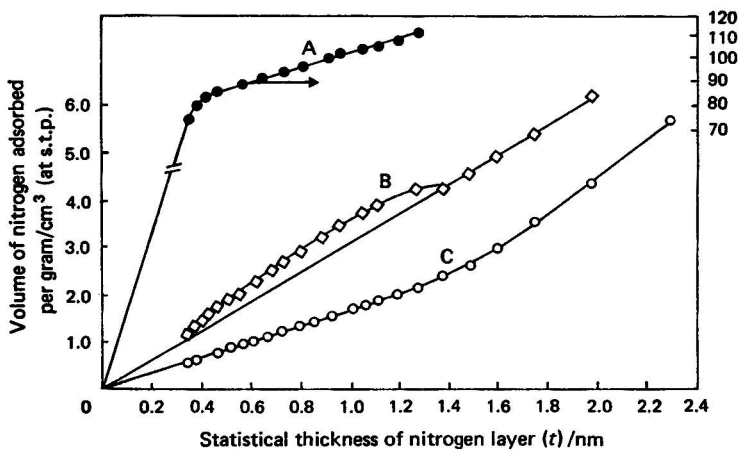

Fig. 7. Micropore V_a *versus* t graphs. A, Magnesium trisilicate; B, barium sulphate; and C, dicalcium phosphate.

The porosity of the collection of particles assembled in the mercury sample tube can be evaluated in a similar manner to that shown under Spherical Steel Shot. The porosity of barium sulphate is thus $0.845/(0.845 + 0.132 + 0.2294) = 0.700$. This porosity value is beyond the range of porosity *versus* acute angle of packing studied by Mayer and Stowe. Their relationship of breakthrough pressure *versus* packing angle (Fig. 9 in reference 7) does show, however, a smooth function over the higher packing angles, which tends to a constant value. A measure of the distribution of sizes, although not the surface diameter size predicted by theory, can be obtained when a breakthrough pressure value of 3.35 p.s.i.a. at a 90° packing angle is taken and substituted into equation (4). The size distribution determined from the percentage of mercury penetrating between these irregularly shaped poly-sized particles follows a similar distribution to that determined by a number - length count. The sub-micrometre tail of the barium sulphate is coincidental with the sub-micrometre particle size distribution determined by centrifugal sedimentation, but is not detected by the gravitational photosedimentation technique.

Magnesium Trisilicate

The mass and surface size distributions obtained by photosedimentation and the mass distribution from centrifugal sedimentation are shown together with the number - length and mercury intrusion size distributions in Fig. 2. The nitrogen adsorption isotherm analyses by the Barrett, Joyner and Halenda and V_a - t methods (Figs. 6 and 7) show that magnesium trisilicate is solely a microporous solid. The mercury volume intrusion graph (Fig. 5) falsely indicates that, like barium sulphate, magnesium trisilicate has pores in the diameter size range 160000–3.6 nm. The intrusion graph can be readily divided into two regions, one in which the volume of mercury can be attributed to the filling of voids between particles (a volume of 1.596 $cm^3 g^{-1}$), and the second attributable to the filling of pores in particles (a volume of 0.066 $cm^3 g^{-1}$). The porosity of the collection of magnesium trisilicate particles in the mercury sample tube is 0.752 and the breakthrough pressure can be taken as being 3.35 p.s.i.a. The size distribution calculated from equation (4) shows a similarly shaped distribution to that obtained by a combination of the micrometre photosedimentation and the sub-micrometre centrifugal sedimentation techniques, as well as that of the number - length microscope count. The particle radius or diameter predicted by the mercury intrusion theory is a surface diameter but the diameter measured by intrusion,with both magnesium trisilicate and barium sulphate, shows a closer correlation with a mass Stokes diameter than with a surface diameter. Adjustment of the experimental mercury diameter distribution to that of a surface diameter distribution would necessitate a breakthrough pressure value, at a packing range of 90° and mercury contact angle of 130°, in the region of 1.52 p.s.i.a. This breakthrough pressure value could only be achieved at a 90° pack if the contact angle between mercury and solid was in the range 110–100°.

Dicalcium Phosphate Dihydrate

The particle size distributions obtained by photosedimentation and centrifugal sedimentation techniques are shown in Fig. 4. The nitrogen adsorption isotherm analysis (Figs. 6 and 7) shows that dicalcium phosphate is non-microporous but has a mesoporous structure. The mercury volume intrusion graph (Fig. 5) cannot readily be divided into two regions; the volume of mercury filling both the voids between particles and the pores in particles is 0.777 $cm^3 g^{-1}$. The porosity of the collection of dicalcium phosphate particles in the mercury sample tube has been calculated to be 0.642, with a breakthrough pressure of 3.35 p.s.i.a. The shape of the calculated mercury intrusion size distribution graph bears little resemblance, at large diameters, to the sedimentation or number - length distributions. The presence of mesopores in dicalcium phosphate has destroyed the physical model upon which the mercury intrusion diameter calculations are based. After mercury has filled the mesopores within the solid, with the result that the solid can be regarded as non-porous at higher mercury pressures, the mercury size distribution obtained from the Mayer and Stowe physical model is similar to that of the centrifugal and number - length distributions.

Conclusions

With non-porous spheres the mercury intrusion technique evaluates a similarly shaped distribution of particle sizes to that determined by other, more conventional, particle sizing techniques. The diameter (or radius) measured is not, however, the surface diameter (or radius) predicted by the Mayer and Stowe model. The particle sizes measured by the mercury technique are greater than the sieve sizes of spherical shot.

With microporous, irregularly shaped particles, the mercury intrusion particle size technique detects both micrometre and sub-micrometre particles, which can usually only be sized by two separate characterisation methods. The mercury diameter evaluated from the microporous magnesium trisilicate powder correlates with the mass Stokes diameter rather than a surface diameter. The correlation between the mercury diameter and the Stokes diameter is, however, dubious for barium sulphate, except for sub-micrometre particles.

With meso- or macroporous material little correlation exists between the mercury diameter and the Stokes diameter. This result supports the observations of Van Brakel and Mayer and Stowe that the spherical model does not perfectly represent a real solid. The mercury particle size technique can, however, be used, with additional adsorption information, in order to obtain an over-all size distribution characterisation in the micrometre and sub-micrometre particle size ranges.

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Determination of Acrylonitrile Monomer in Plastic Packaging and Beverages by Headspace Gas Chromatography

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A gas - liquid chromatographic method for determining trace amounts of acrylonitrile in plastic containers and carbonated beverages using a nitrogen-sensitive detector and headspace injection technique is described. The method is suitable for the determination of acrylonitrile at concentrations down to 0.1 mg kg^{-1} in plastics and 0.005 mg kg^{-1} in beverages.

Keywords: *Acrylonitrile determination*; *headspace gas chromatography*; *plastic packaging*; *foodstuffs*

Polymers with acrylonitrile monomer as a component are used for packages and household articles designed for foodstuffs. During the manufacture of acrylonitrile copolymers, a small fraction of unreacted acrylonitrile monomer becomes physically entrapped in the polymer and can migrate slowly during storage and when in contact with food or other materials. Recently, experiments with rats in the USA[1] have indicated that acrylonitrile might be carcinogenic. This has led to a proposed ban in the USA on the use of acrylonitrile copolymer materials for beverage packaging.

These packages are also used in Sweden, and this work was initiated to develop a sensitive gas-chromatographic method for the determination of residual acrylonitrile in carbonated beverages and food packages. The methods for the determination of acrylonitrile in food and beverages published so far are often time consuming, require special laboratory equipment and are not very sensitive.

Jones and Smith[2] described a headspace gas-chromatographic method for acrylonitrile in fat with a sensitivity of 0.2 mg kg^{-1}. The Nederlandse Vereniging-federatie voor Kunststoffen[3] has recommended a method that involves distillation into xylene followed by gas chromatography. This method gives a sensitivity of 0.4 mg kg^{-1} in aqueous simulants and about 1 mg kg^{-1} in fat. The Food and Drug Administration[4] has recommended a method for the determination of acrylonitrile in aqueous extracts, which involves an azeotropic distillation with methanol, followed by differential-pulse polarography. The sensitivity of this method is reported to be 0.01 mg kg^{-1} in water and 0.03 mg kg^{-1} in beer. The determination of acrylonitrile in food processing plants using spectrophotometry involving photochemical bromination and the formation of a red pyridine - benzidine complex was described by Kröller.[5] The detection limit was reported to be 0.01 mg kg^{-1}.

Several methods have been published on the determination of acrylonitrile in plastics,[6–8] involving various solvents, gas-chromatographic columns and techniques. The method proposed here is applicable to plastics, carbonated beverages and simulating solvents. The headspace technique and the nitrogen-sensitive detector make the determination of acrylonitrile monomer rapid, accurate and sensitive (Fig. 1).

Experimental

Apparatus

Normal laboratory equipment, 20-ml glass vials equipped with natural rubber seals and aluminium caps, a Fermpress H 207 for sealing the bottles and a 1-ml gas-tight syringe were used.

Reagents

All reagents were of analytical-reagent grade.

Acrylonitrile.

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Propionitrile.

Propylene carbonate. The propylene carbonate was purified prior to use by the following procedure. The propylene carbonate was heated to 120 °C with magnetic stirring and nitrogen bubbling for 12 h,[8] then stored under nitrogen.

Gas Chromatography

The gas chromatograph was a Varian 2700 with an alkali flame-ionisation detector (rubidium sulphate). Nitrogen was used as the carrier gas at a flow-rate of 23 ml min^{-1}. Air and hydrogen flow-rates were optimised to give the best detector response. The chart speed of the 1-mV recorder was 0.8 cm min^{-1} and the amplifier ranges were 4×10^{-12} and 8×10^{-12} A mV^{-1}.

The main column used was a 3 m × 2 mm i.d. glass column packed with 0.2% Carbowax 1500 on 60–80-mesh Carbopack C. The injector temperature was 200 °C, the detector temperature 200 °C and the oven temperature 70 °C, isothermal. In order to confirm the presence of acrylonitrile in a sample, another glass column of 3 m × 2 mm i.d. was used, packed with 20% Carbowax 20M on Chromosorb W, 60–80 mesh. The instrumental conditions were the same except for the oven temperature, which was 50 °C, and the nitrogen flow-rate, which was 15 ml min^{-1}. The retention times for acrylonitrile and propionitrile on the alternative column were 1.7 min and 2.3 min, respectively.

Procedure

Preparation of samples

Samples of carbonated beverages were shaken in a stoppered Erlenmeyer flask until most of the carbonic acid had been evolved. Samples of the plastic materials were cut into small pieces.

Determination of acrylonitrile in beverages

A 3-ml volume of the homogenised sample was weighed into a glass vial, 2.0 ml of

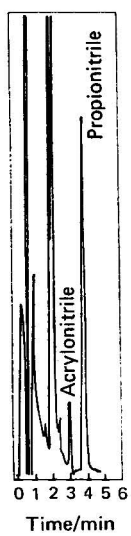

Fig. 1. Beer from a glass bottle spiked with 0.03 mg of acrylonitrile per kilogram. Propionitrile was added as internal standard. Attenuation 4×10^{-12} A mV^{-1}.

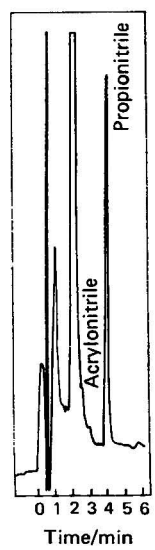

Fig. 2. Beer sample from Barex bottle with propionitrile added as internal standard. Attenuation 8×10^{-12} A mV^{-1}.

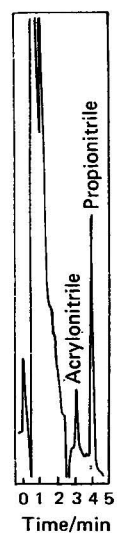

Fig. 3. Plastic sample from Barex bottle with propionitrile added as internal standard. Attenuation 8×10^{-12} A mV^{-1}.

propionitrile (the internal standard), at a concentration of about 0.5 μg ml^{-1} in distilled water, and 3.0 ml of distilled water were added and the bottle was closed with a rubber seal and cap. The vial was shaken well and placed in an oven at 90 °C for at least 30 min. The sample was then ready for gas chromatography (Fig. 2).

Determination of acrylonitrile in plastic containers

The plastic sample (0.3–0.5 g) was weighed into a glass vial, 4.0 ml of propionitrile in propylene carbonate (at a concentration of about 1.5 μg ml^{-1}) and 4.0 ml of propylene carbonate were added and the bottle was then closed with a rubber seal and cap. The vial was placed in an oven at 90 °C until all the plastic had dissolved and then left at room temperature overnight. Prior to the gas-chromatographic determination, the glass vial containing the polymer sample was placed in an oven at 90 °C for at least 30 min (Fig. 3).

Gas-chromatographic determination

A 1-ml volume of gas was drawn from the gaseous phase in the glass vial using a warm (90 °C) gas-tight syringe, and injected into the chromatograph.

Blank determinations

The solutions and solvents were tested by headspace gas chromatography prior to use to ensure freedom from peaks that would interfere with the determination of acrylonitrile (Figs. 4, 5 and 6).

Preparation of calibration graphs

Acrylonitrile standard solutions of concentrations 0.01, 0.03, 0.05, 0.10 and 0.15 μg ml^{-1}

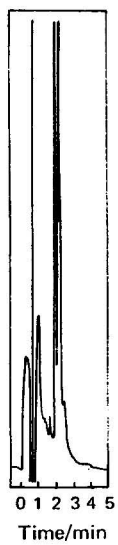

Fig. 4. Beer sample from a glass bottle. Attenuation 8 × 10^{-12} A mV^{-1}.

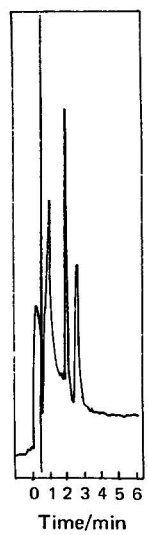

Fig. 5. Carbonated soft drink from a glass bottle. Attenuation 8 × 10^{-12} A mV^{-1}.

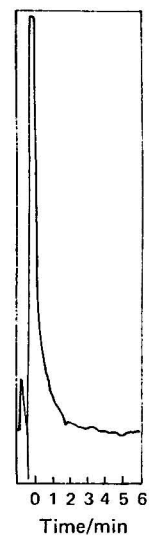

Fig. 6. Blank determination of purified propylene carbonate. Attenuation 8 × 10^{-12} A mV^{-1}.

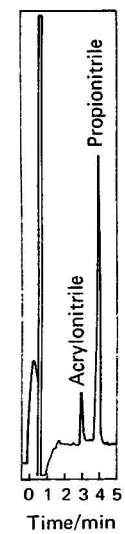

Fig. 7. Purified propylene carbonate spiked with 0.12 μg of acrylonitrile per millilitre, corresponding to 0.96 mg kg^{-1} in plastic. Propionitrile was added as internal standard. Attenuation 8 × 10^{-12} A mV^{-1}.

were prepared in distilled water. A 3.0-ml volume of acrylonitrile standard solution, 2.0 ml of propionitrile solution in distilled water at a concentration of about 0.5 μg ml^{-1} and 3.0 ml of liquid (similar to the sample, but which has never been in contact with any plastic, for example, beer from a glass bottle) were pipetted into a vial. The vial was then sealed, shaken and placed in an oven at 90 °C for at least 30 min. The chromatographic determination was then carried out as described above (Fig. 1).

Acrylonitrile standard solutions of concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 μg ml^{-1} were prepared in purified propylene carbonate. A 4.0-ml volume of acrylonitrile standard solution and 4.0 ml of propionitrile solution in propylene carbonate, at a concentration of about 1.5 μg ml^{-1}, were pipetted into a vial. The vial was then sealed, shaken and heated in the oven at 90 °C for 30 min. A 1-ml volume of the gaseous phase from the headspace was injected into the chromatograph as described above (Fig. 7). Although the peak heights in Fig. 7 are approximately the same as in Fig. 1, the amounts of acrylonitrile and propionitrile vary and proportionally represent substantially different amounts, because the solubilities of acrylonitrile and propionitrile in propylene carbonate are different from the solubilities in water.

Duplicate determinations from each acrylonitrile standard solution were made in addition to duplicate injections from each vial. The concentrations of the standard solutions in the vials correspond to 0.01–0.15 mg kg^{-1} in the beverages and 2–10 mg kg^{-1} in the plastics. For each pair of injections of the standard solutions, the mean of the peak-height ratios for acrylonitrile and propionitrile were calculated (y) and plotted against the mass ratio of acrylonitrile to propionitrile of each standard solution in the same vial (x) in order to construct calibration graphs.

Quantification

The linear equation $y = kx + l$ was used to calculate the acrylonitrile content in beverages and plastics.

$$\text{Amount of acrylonitrile in sample (mg kg}^{-1}) = \frac{(y - l)W_{is}}{W_s k}$$

where y = mean of the two acrylonitrile to propionitrile peak-height ratios from the gas chromatograms of two headspace injections of the sample solution; l = intercept on the y-axis; W_{is} = amount of internal standard added (μg); W_s = amount of sample weighed out (g); and k = slope of the straight line.

Results and Discussion

The proposed method was used to examine twelve samples of beer and four samples of carbonated soft drinks packed in Barex. The residual acrylonitrile in the packages ranged from 2 to 5 mg kg^{-1} and in some samples of beer and soft drinks trace amounts of acrylonitrile (<0.005 mg kg^{-1}) were found (Fig. 2). The equations used to calculate the acrylonitrile content in beverages and plastics were $y = 2.434x + 0.001$ and $y = 2.254x - 0.017$, respectively. The linear regression was 0.9999 for both calibration graphs. The acrylonitrile standard solutions in distilled water remained stable for approximately 1 week in a refrigerator.

The accuracy of the determinations in plastics was tested. The relative standard deviation calculated on ten determinations on Barex plastic from beer bottles was 2.9% with an acrylonitrile concentration of 2.8 mg kg^{-1} and 3.5% with an acrylonitrile concentration of 1.8 mg kg^{-1}. The method is also suitable for the determination of acrylonitrile in simulating solvents such as water, 3% acetic acid and 10% ethanol, which are often used in specific migration tests. The determination of acrylonitrile with this method is easy, rapid and, due to the use of the nitrogen-sensitive detector, very sensitive.

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Determination of Ethylenethiourea in Ethylenebisdithiocarbamate Fungicides: Comparison of High-performance Liquid Chromatography and Gas - Liquid Chromatography

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A rapid, sensitive method is described for the determination of ethylenethiourea (imidazolidine-2-thione) in ethylenebisdithiocarbamate fungicides. High-performance liquid chromatography is used with an ultraviolet spectrophotometric detector. The results are compared with those obtained using gas - liquid chromatography. All fungicide samples assayed contained ethylenethiourea, and gas - liquid chromatography indicated higher concentrations than high-performance liquid chromatography.

Keywords: Ethylenethiourea determination; ethylenebisdithiocarbamate fungicides; high-performance liquid chromatography; gas - liquid chromatography

Ethylenethiourea (ETU) (imidazolidine-2-thione) may occur in formulations both as a manufacturing impurity and as a product of degradation during storage.[1,2] The presence of ETU in commercial formulations is of concern because of its possible carcinogenic and teratogenic properties.[3,4] However, deficiencies in available data have inhibited any firm conclusions being reached in respect of these properties.

The routine method of analysis for the active ingredient in ethylenebisdithiocarbamate (EBDC) formulations is based on the determination of carbon disulphide after acid hydrolysis.[5,6] This technique is not applicable in the presence of copper and the Dubosq method[7] is then used. The latter method is based on hydrolytic cleavage using hydriodic acid but ETU interferes. As ETU may be present in formulations to the order of several per cent., this interference can be significant and a correction must be made for the ETU content.

A number of workers have reported methods for the assay of ETU. Johnson and Tyler[8] determined ETU in aqueous extracts from a number of EBDC fungicides using paper chromatography. All fungicides based on ethylenebisdithiocarbamic acid were reported to contain ETU. Thin-layer chromatography was used by Czegledi-Janko and Hollo[9] to examine the degradation of zineb and maneb. Fishbein and Fawkes[10] also reported a thin-layer chromatographic method for the determination of the degradation products of EBDC fungicides. Thin-layer chromatography was used by Bontoyan and Looker[2] as a screening method for detecting ETU prior to gas - liquid chromatographic analysis of EBDC fungicide formulations maintained under conditions of elevated temperature and humidity.

It was thought that high-performance liquid chromatography would give high specificity, speed and sensitivity, and form the basis of a method by which the results obtained by gas - liquid chromatography could be critically assessed.

Experimental

Liquid Chromatograph

A Waters Associates, Model 6000, constant-volume solvent-delivery system was used. A variable-wavelength ultraviolet monitor (Cecil Instruments, Model CE 212), fitted with a 10-μl flow cell and set at 240 nm, was used as a detector.

Preparation of Column

A stainless-steel column tube, 180 × 4.6 mm i.d., was washed with chloroform and

methanol and polished on the inner surface. One end was fitted with a $\frac{1}{4} \times \frac{1}{16}$ in Crawford Patent column end fitting, and the other end was coupled to a 400-mm pre-column through a $\frac{1}{4} \times \frac{1}{4}$ in Swagelok union. Spherisorb CN, 5 μm (Phase Separations Ltd.), was packed into the column from a slurry in ethanol under a pressure of 5000 lb in^{-2}. The pre-column was removed and the analytical column was prepared for stop-flow injection by removing the top few millimetres of packing and inserting a disc of stainless-steel fine-mesh gauze, of 8 μm nominal porosity, a plug of silanised glass-wool and a top plug of porous PTFE.

Sample Injection

A Varian Associates stop-flow injector was used and samples were injected on to the stainless-steel fine-mesh gauze fitted on top of the packing. A needle guide was incorporated in the injector to ensure that the samples were introduced on to the centre of the top of the column.

Conditions for Gas - Liquid Chromatography

Analyses were carried out on a Pye 104 gas - liquid chromatograph, fitted with a flame-ionisation detector, under the following operating conditions:

Column	1000 × 4 mm i.d., glass
Column packing	Chromosorb WHP, 2% of Carbowax 20M TPA on Chromosorb W, 80–100 mesh
Column oven temperature	210 °C
Detector oven temperature	250 °C
Injection zone temperature	230 °C
Carrier gas	Nitrogen, free from oxygen
Flow-rates: nitrogen	50 cm^3 min^{-1}
hydrogen	50 cm^3 min^{-1}
air	600 cm^3 min^{-1}

Reagents

Ethylenethiourea. Recrystallised, obtained from Robinson Brothers Ltd.

ETU standard solution. Prepare a standard solution in methanol, containing 0.7 g l^{-1} of ETU.

Ethanol, absolute.

Methanol. Analytical-reagent grade.

Hexane. Spectrograde (Fisons).

Mobile phase for liquid chromatography. Prepare a solution containing 35% *V*/*V* of absolute ethanol in hexane.

Procedure

Weigh accurately about 0.5 g of EBDC, or a mass equivalent to 0.5 g of active ingredient, into a 15-cm^3 centrifuge tube. Add 10.0 cm^3 of methanol from a pipette, stopper the tube and shake it vigorously for 15 min on a wrist-action shaker. Filter the resulting mixture through a filter-paper that retains particles of at least 5 μm, for example, Whatman No. 42, and collect the filtrate. Inject 4 μl of the filtrate on to the top of the liquid chromatograph using a flow-rate of 0.8 ml min^{-1}. Complete the analysis as soon as possible. Calculate the ETU content of the sample by comparing the peak height with that obtained from a 4-μl injection of standard solution.

To ensure that the response of the detector is within the linear range, dilute the sample until the peak height obtained is equal to or less than that obtained with the standard. Gas - liquid chromatographic analyses are undertaken using the same solution and the same injection volume as are used for liquid-chromatographic determinations.

Results and Discussion

A number of procedures were investigated for extracting ETU from EBDC fungicides. A cold-extraction technique was adopted in order to reduce the risk of production of ETU. Optimum speed and repeatability were obtained by shaking the fungicides with solvent for

15 min. Duplicate analyses of a sample of ziram fortified with ETU (3%) gave recoveries of approximately 90%.

The choice of the solvent was limited by the low solubility of ETU. The use of water has been reported; however, it was found that this extraction solvent resulted in unacceptably broad peaks on the liquid chromatograph. Methanol and methanol - chloroform have also been used for extraction and these were investigated. Both systems resulted in good peaks on the liquid chromatograph and were shown to have similar extraction efficiencies with respect to ETU. However, methanol - chloroform co-extracted greater amounts of other substances and therefore methanol was adopted as the extraction solvent.

ETU has a sharp absorbance at 240 nm and this was adopted as the wavelength setting on the UV monitoring system. ETU was readily eluted from a Spherisorb ODS system, but adequate retention could not be obtained. A bonded stationary phase exhibiting polar characteristics appeared likely to be suitable and Spherisorb CN was selected. With hexane - ethanol (65 + 35) as the mobile phase, ETU is eluted from a Spherisorb CN packed column with a good peak shape and optimum resolution. Ethylenethiuram monosulphide (ETM) elutes shortly before ETU, but is not extracted efficiently by methanol and does not absorb strongly at 240 nm. The procedure was applied to both technical samples and formulations of zineb, maneb, mancozeb and Vondozeb (a co-ordination product of EBDC with zinc and manganese ions). The results obtained are shown in Table I. Figs. 1 and 2 show typical chromatographic traces obtained from analyses of these compounds. With the procedure described, 0.01% of ETU can be determined in the four active ingredients investigated.

TABLE I

ETHYLENETHIOUREA CONTENT OF ETHYLENEBISDITHIOCARBAMATE FUNGICIDES

Figures are expressed as percentages.

Fungicide	Results by high-performance liquid chromatography	Results by gas - liquid chromatography
Maneb technical 1	0.1, 0.1	0.2, 0.2
Maneb technical 2	1.2, 1.3	2.8, 2.9
Maneb formulation 1	0.7, 0.7	1.2, 1.3
Maneb formulation 2	1.1, 1.1	2.0, 2.0
Maneb formulation 3	1.0, 1.1	1.8, 1.9
Zineb technical 1	1.1, 1.2	1.5, 1.5
Zineb technical 2	1.5, 1.4	2.1, 2.2
Zineb formulation	2.1, 2.0	2.5, 2.4
Maneb - zineb technical	0.4, 0.4	0.6, 0.6
Maneb - zineb formulation	1.0, 1.0	1.2, 1.3
Mancozeb technical 1	0.1, 0.1	0.1, 0.1
Mancozeb technical 2	0.2, 0.2	0.4, 0.4
Mancozeb technical 3	0.2, 0.2	0.4, 0.4
Mancozeb technical 4	0.2, 0.2	0.4, 0.4
Mancozeb formulation	0.2, 0.2	0.2, 0.3
Vondozeb technical 1	1.0	1.6
Vondozeb technical 2	1.3, 1.3	2.1, 2.1
Vondozeb technical 3	1.4, 1.5	2.9, 2.6
Vondozeb formulation	0.9, 1.0	1.5, 1.3

High-performance liquid chromatographic traces obtained from analyses of extracts of maneb samples showed that maneb yields greater concentrations of co-extractives than the other EBDC fungicides examined (Fig. 1). This was also observed by Czegledi-Janko and Hollo[9] when analysing a number of EBDC fungicides by thin-layer chromatography. Mancozeb samples contained comparatively little ETU and less co-extractives (Fig. 1), which agrees with the findings of Bontoyan and Looker.[2]

Gas - liquid chromatographic analysis of all samples gave two peaks (Fig. 2), the second eluting shortly after ETU. A correlation between the area of this peak and the rate of

formation of ETU from EBDCs was noted by Bontoyan and Looker.[2] They concluded that ETU may arise as a result of two decomposition routes, a direct route from the parent EBDC and formation via intermediate degradation products. A mechanism for the formation of ETU through degradation products, but not directly from the parent EBDC, was proposed by Marshall.[11] This was based on studies of the thermal decomposition of EBDCs in aqueous media.

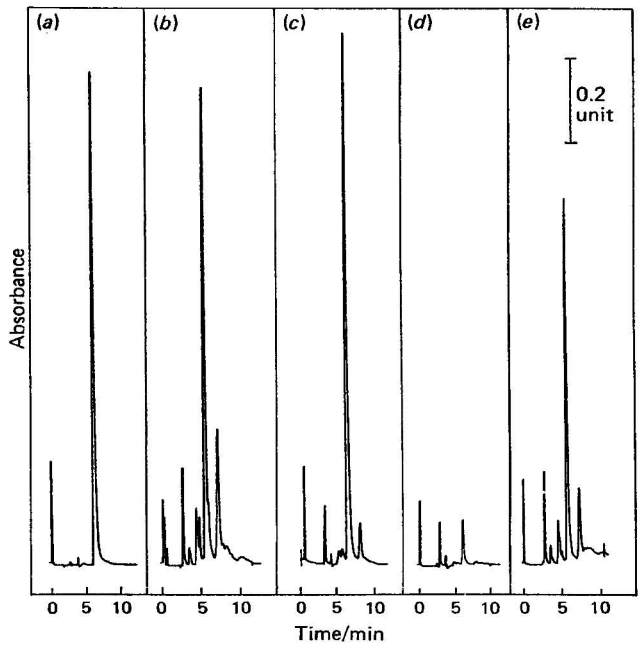

Fig. 1. Typical liquid chromatograms obtained from 4-μl injections of: (*a*) ETU standard; (*b*) maneb technical; (*c*) zineb technical; (*d*) mancozeb technical; and (*e*) Vondozeb technical.

The analysis of an aqueous solution of nabam and an aqueous suspension of zineb, by injection on to a gas chromatograph, was investigated by Zielinski and Fishbein.[12] These experiments gave rise to peaks attributable to ETU rather than peaks directly attributable to the EBDCs. They concluded that this was due to the formation of ETU on sample injection, although ETU already present in the sample could have contributed to the peaks observed.

A sample of ETM, which high-performance liquid chromatographic analysis indicated contained no apparent ETU, when dissolved in chloroform - methanol and injected on to the gas chromatograph yielded a significant peak with the same retention time as ETU. It was observed by Marshall[11] that ETM can give rise to ETU at elevated temperatures and our findings are in agreement with that observation. It is apparent that ETU is formed from one or more precursors, present as degradation products of EBDCs or as impurities, on injection in methanolic solutions on to a gas chromatograph. It is significant that those samples which contained the greatest concentration of co-extractives, based on high-performance liquid chromatographic analyses, gave rise to the largest difference between the gas - liquid and high-performance liquid chromatographic results for ETU content.

Samples extracted with chloroform - methanol (1 + 1) gave the same result for ETU content, when assayed by high-performance liquid chromatography, as those obtained following extraction with methanol (Table II). However, on injection on to the gas - liquid chromatograph, higher results for ETU were observed than those obtained following

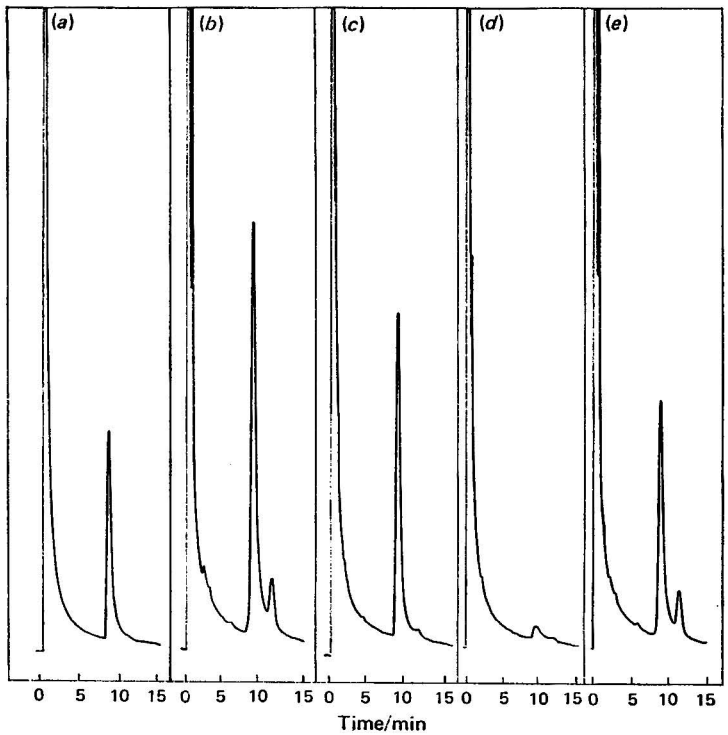

Fig. 2. Typical gas - liquid chromatograms obtained from 4-μl injections of: (*a*) ETU standard; (*b*) maneb technical; (*c*) zineb technical; (*d*) mancozeb technical; and (*e*) Vondozeb technical.

comparative methanol extractions. This serves to illustrate further that degradation of ETU precursors is occurring during gas - liquid chromatography, with possible contributions from unresolved extraneous peaks.

TABLE II

ETHYLENETHIOUREA CONTENT OF ETHYLENEBISDITHIOCARBAMATE FUNGICIDES USING CHLOROFORM - METHANOL AS THE EXTRACTION SOLVENT

Figures are expressed as percentages.

Fungicide	Results by high-performance liquid chromatography	Results by gas - liquid chromatography
Maneb technical 1	0.1, 0.1	0.2, 0.2
Maneb technical 2	1.2, 1.2	4.3, 4.2
Maneb formulation 2	1.1, 1.1	3.3, 3.1
Maneb formulation 3	1.1, 1.1	2.9, 2.6
Zineb technical 1	1.2, 1.2	1.4, 1.4
Maneb - zineb formulation ..	0.9, 1.0	1.4, 1.4
Mancozeb technical 1	0.1, 0.1	0.1, 0.1
Vondozeb technical 1	0.9	2.6
Vondozeb technical 2	1.2	2.7

Conclusion

If ETU analyses are carried out by gas - liquid chromatography, results will be obtained that reflect both the sample content of ETU and the ETU that may be formed by thermal decomposition from other compounds present. The use of high-performance liquid chromatography offers a rapid analytical technique that yields a truer estimate of the ETU content.

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Fluorimetric Determination of Acetohexamide in Plasma and Tablet Formulations Using 1-Methylnicotinamide

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A sensitive method is described for the fluorimetric determination of acetohexamide in plasma or in tablets by means of its reaction with 1-methylnicotinamide, which is shown to be a useful reagent for the determination of ketonic compounds. The limit of detection is approximately 0.2 $\mu g\ ml^{-1}$ and the relative standard deviation is 3.1% for 2 $\mu g\ ml^{-1}$ in plasma. Acetoacetic acid usually does not interfere, but can be separated, if necessary, from acetohexamide by means of a washing technique. No interference is caused by the presence of insulin, other (non-ketonic) oral hypoglycaemic drugs, acetone or pyruvic or α-ketoglutaric acid.

Keywords: Acetohexamide determination; plasma; tablets; 1-methylnicotinamide reagent; spectrofluorimetry

The finding[1] that the oral hypoglycaemic compound acetohexamide can exist in at least two polymorphic forms made it of interest to develop a sensitive and relatively specific method for the routine determination of plasma levels of the unchanged drug, especially in view of a recent report[2] that marked variations in bioavailability can exist between different batches of tablets containing the related sulphonylurea tolbutamide. Serum concentrations of acetohexamide can be measured colorimetrically by a modification[3] of Spingler's procedure.[4] Both the drug and its metabolite, hydroxyhexamide, can be determined in serum or plasma by isotope dilution analysis,[5] or by a two-component spectrophotometric procedure[6] utilising measurements at 247 and 228 nm, a disadvantage of the last method being that blank absorbance values are high. A more sensitive gas - liquid chromatographic method has been proposed by Kleber *et al.*[7] for which the calibration concentrations of the drug and its metabolite range from 5 to 40 $\mu g\ ml^{-1}$ in plasma.

The method described here is suitable for plasma samples containing 0.5–2.5 $\mu g\ ml^{-1}$ of acetohexamide. It introduces a new use for 1-methylnicotinamide as a reagent for the fluorimetric determination of ketones, with which it is known[8] to react readily, after treatment with an alkali in order to convert it into the highly reactive α-carbinol. The reaction has previously been applied only to determining 1-methylnicotinamide by condensation in the presence of an alkali with a variety of different methyl ketones. Huff and Perlzweig[8] and Carpenter and Kodicek[9] have described similar procedures for 1-methylnicotinamide, which involve condensation in aqueous alkali, followed by acidification to about pH 0.5 with hydrochloric acid, a short period of heating and finally the addition of potassium dihydrogen orthophosphate in order to buffer the mixture at about pH 2. In another modification, Clark *et al.*[10] converted the 1-methylnicotinamide into a fluorescent derivative by treatment with acetophenone in alcoholic potassium hydroxide, and then acidified the solution with 99% formic acid. The acidification in each procedure reversibly changed the fluorescence from greenish blue to blue and enhanced it.

The method developed for acetohexamide in plasma can also be applied to the determination of the drug in tablets, and is simpler and less time consuming than the high-performance liquid chromatographic method of Beyer,[11] or the Salim and Hilty spectrophotometric assay,[12] which is the official method of the United States Pharmacopeia.[13] Other techniques that have been proposed for tablet preparations include non-aqueous titration,[14,15] colorimetry using either cobalt acetate[16] or 2,4-dinitrophenylhydrazine[17] and polarography.[18]

Experimental

Apparatus

Fluorimetric measurements were carried out on a Perkin-Elmer, Model 1000, fluorescence spectrophotometer in 1 × 1 cm silica cells with a 371-nm filter at an angle of 20° for excitation and an emission wavelength setting of 437 nm, slit width N and scale expansion ×1 or ×2.

The infrared spectra were recorded from potassium bromide discs using a Perkin-Elmer, Model 357, grating spectrometer.

Spectrophotometric measurements were made using a Unicam SP500 Series 2 spectrophotometer and 1-cm silica cells.

Ultrafiltration of plasma solutions of acetohexamide was carried out using Amicon Centriflo Membrane Cones CF 50A with conical supports and 50-ml centrifuge tubes.

Reagents

All reagents were of analytical-reagent grade, and were checked before use for the presence of fluorescent contaminants. De-ionised water was used in the preparation of all solutions.

Acetohexamide. Kindly supplied by Eli Lilly and Company Ltd.

1-Methylnicotinamide iodide reagent, 3% m/V in 10^{-4} M hydrochloric acid. This solution should be freshly prepared. Dissolve 9 g of nicotinamide in 50 ml of dimethylformamide and add 10 ml of methyl iodide. Allow the mixture to stand, preferably overnight, then separate the product by filtration, wash it with about 50 ml of dimethylformamide and dry it in air (melting-point 205–208° C).

Formic acid solution, 50% V/V. Prepared from 98–100% *m/m* formic acid.

Hydrochloric acid, 2 M and 10% m/m.

Sodium hydroxide solutions, 0.01, 0.1 and 5 M.

Chloroform.

Preparation of hydroxyhexamide

A solution of 1 g of acetohexamide in 60 ml of 2% *m/V* sodium hydroxide solution was prepared and 150 mg of sodium tetrahydroborate(III) were added and dissolved by shaking. The solution was left to stand for 1 h at room temperature and then acidified with 40 ml of 10% *m/m* hydrochloric acid. The product was separated immediately by filtration, washed with approximately 150 ml of water and recrystallised from dilute ethanol to give 0.7 g of hydroxyhexamide {*N*-[(cyclohexylamino)carbonyl]-4-(1-hydroxyethyl)benzenesulphonamide}, melting at 146–149 °C. The compound was identified by thin-layer chromatography on pre-coated silica gel 60 F_{254} plates with chloroform - formic acid (97 + 3) as the solvent system[6] and by nuclear magnetic resonance and infrared absorption spectrometry. Thin-layer chromatography showed a trace amount of acetohexamide in the product that was difficult to remove by further recrystallisation. The infrared spectrum (Fig. 1) showed the presence of an OH band at 3520 cm^{-1}. The slight shoulder at 1690 cm^{-1} provided evidence that a carbonyl group had been reduced when the spectrum was compared with that of

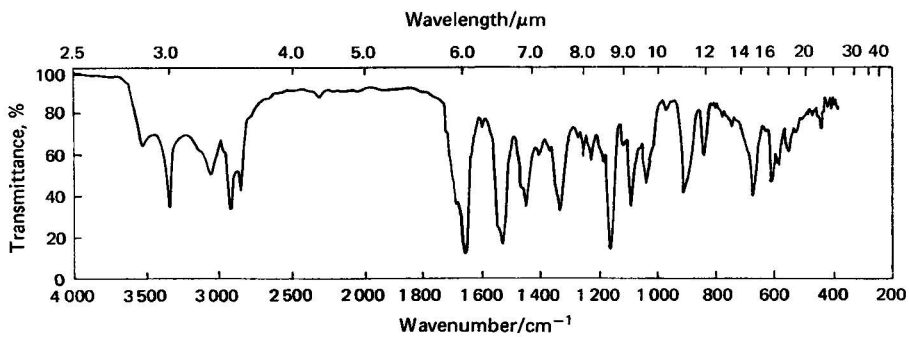

Fig. 1. Infrared spectrum of hydroxyhexamide.

acetohexamide polymorph B,[1] as this shows a clear doublet with peaks at 1690 and 1665 cm^{-1}. Attempts to prepare hydroxyhexamide on a small scale by catalytic hydrogenation, which has been used by previous workers,[19,20] were not successful as the reduction proceeded too far, and the product was *N*-[(cyclohexylamino)carbonyl]-4-ethylbenzenesulphonamide.

Procedures

Assay of acetohexamide in plasma

Plasma extraction. Mix 2.0 ml of plasma with 1.5 ml of 2 M hydrochloric acid in a glass-stoppered tube. Add 20.0 ml of chloroform, shake thoroughly and allow to stand for at least 30 min in order to allow the phases to separate. Transfer the bulk of the chloroform layer into a stoppered centrifuge tube and centrifuge. Measure 5.0 ml of the chloroform extract into a 10-ml calibrated flask and evaporate to dryness in a stream of nitrogen at room temperature. (Up to three 5-ml portions of the chloroform extract can be evaporated to dryness, and subjected separately to the fluorimetric procedure to improve the confidence limit of the assay.)

Fluorimetric procedure. Dissolve the residue in the 10-ml flask in 0.10 ml of 0.01 M sodium hydroxide solution, add 0.10 ml of 1-methylnicotinamide reagent, followed immediately by 0.10 ml of 5 M sodium hydroxide solution and mix thoroughly. Exactly 2 min after addition of the 5 M sodium hydroxide solution make up to volume with 50% *V*/*V* formic acid. Measure the fluorescence intensity (maximum value) after 1.5–2 h. Correct the observed fluorescence by subtracting the fluorescence intensity measured using the same procedure on a drug-free plasma sample taken from the same subject prior to drug administration.

Calculation. Determine the concentration of acetohexamide in the plasma by reference to a calibration graph obtained by carrying out the assay procedure using 2.0-ml aliquots of standard solutions of acetohexamide in 0.01 M sodium hydroxide solution or in plasma containing 0, 0.5, 1.0, 1.5, 2.0 and 2.5 $\mu g\ ml^{-1}$ of drug. [Fluorescence intensity values after subtraction of the appropriate blank (zero drug concentration) are the same, irrespective of whether the drug is dissolved in plasma or in aqueous alkali.] If necessary, dilute the test fluorescent solution with 50% *V*/*V* formic acid, in order to bring the fluorescence intensity within the range of the calibration graph. Determine the approximate concentration of acetohexamide in the plasma by reference to the calibration graph. Repeat the assay using a dilution of the plasma in 0.01 M sodium hydroxide solution accurately prepared to contain about 2.0 $\mu g\ ml^{-1}$ of acetohexamide. Carry out a plasma blank determination using an equivalent dilution in 0.01 M sodium hydroxide solution of the drug-free plasma sample.

Assay of acetohexamide in tablets

Sample preparation. Weigh and powder 20 tablets. Accurately weigh an amount of the powder equivalent to 500 mg of acetohexamide into a 100-ml calibrated flask, add 60 ml of 0.1 M sodium hydroxide solution and shake for 30 min. Make up to volume with 0.1 M sodium hydroxide solution, mix and filter, and discard the first 20 ml of filtrate. Dilute 5.0 ml of filtrate to 50.0 ml with water. Transfer 1.0 ml of this solution into a 100-ml calibrated flask, add 0.01 M sodium hydroxide solution to volume and mix.

Fluorimetric procedure. To 1.0 ml of the final dilute solution in a 100-ml calibrated flask, add 1.0 ml of 1-methylnicotinamide reagent followed immediately by 1.0 ml of 5 M sodium hydroxide solution and mix thoroughly. Exactly 2 min after addition of the 5 M sodium hydroxide solution, make up to volume with 50% *V*/*V* formic acid. Measure the fluorescence intensity (maximum value) after 1.5–2 h.

Calculation. Determine the concentration of acetohexamide in the final dilute solution by reference to a calibration graph, obtained by carrying out the fluorimetric procedure on 1.0-ml aliquots of standard solutions of acetohexamide in 0.01 M sodium hydroxide solution containing 0, 2.0, 4.0, 6.0, 8.0 and 10.0 $\mu g\ ml^{-1}$ of drug. Calculate the amount of acetohexamide in milligrams per tablet using the expression

$$\text{Mass per tablet/mg} = \frac{m_1 \times C_s \times 100}{m_2}$$

where C_s μg ml^{-1} is the concentration of acetohexamide in the final sample solution and m_1 and m_2 g are the average mass of the tablets and mass of sample taken, respectively.

Results and Discussion

Factors Affecting the Fluorimetric Procedure

The significance of the reagent concentrations and reaction times selected for the recommended method was shown by carrying out the fluorimetric procedure described for the assay of acetohexamide in tablets, using a 10 μg ml^{-1} solution of acetohexamide, and varying the concentration of the different reagents individually. Changing the concentration of 1-methylnicotinamide iodide in the reagent solution over a range from 0.5 to 10% *m/V* showed that a concentration of at least 2–3% was necessary for the highest fluorescence intensity to be obtained [Fig. 2(*a*)], and that when reagent concentrations were reduced to 1 or 0.5%, fluorescence readings decreased to 78 or 52% of the maximum, respectively. When using a 3% reagent concentration, maximum fluorescence develops within 1.5 h and remains stable, decreasing by not more than about 2% of its value over 24 h. When the reagent concentration is increased to 5 or 10%, maximum fluorescence is measured after 1 h, after which time readings decrease gradually because of quenching caused by a yellow colour that slowly develops in the solution after acidification. This quenching effect can be avoided by extracting the aqueous solution with dichloromethane immediately after acidification and measuring the fluorescence in the organic layer at the usual wavelength settings.

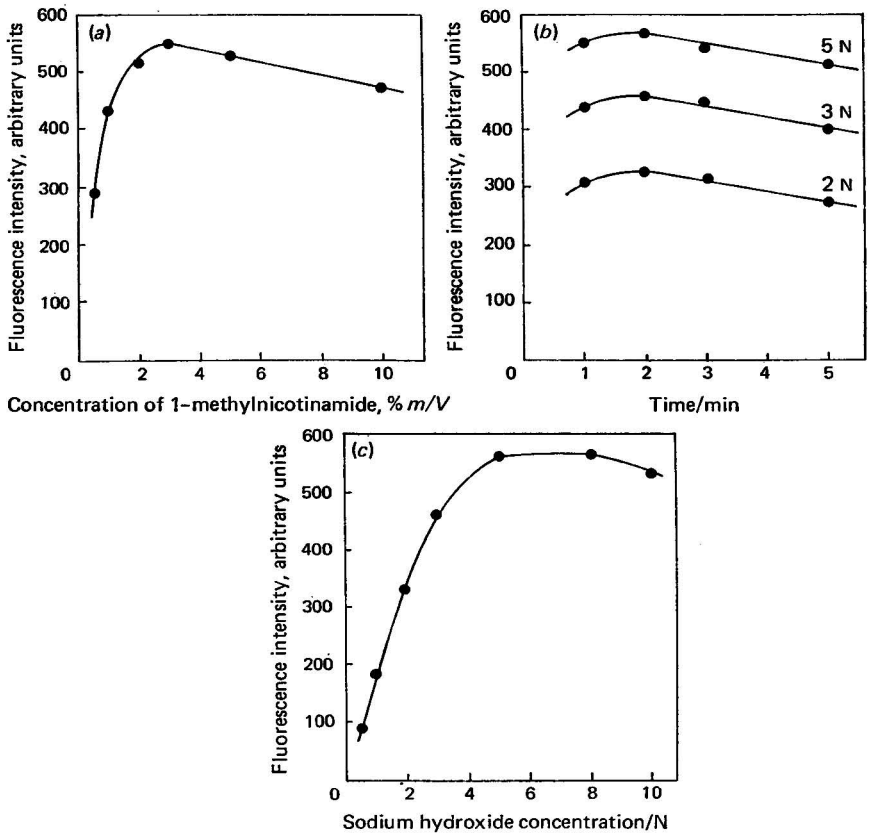

Fig. 2. Influence on fluorescence intensity developed from 10 μg of acetohexamide of: (*a*), 1-methylnicotinamide iodide reagent concentration; (*b*), duration of reaction with 2, 3 or 5 N sodium hydroxide solution; and (*c*), normality of sodium hydroxide solution.

By following this procedure it was shown that there is no significant change in fluorescence intensity when the 1-methylnicotinamide concentration is increased from 3 to 5 or 10%. Substituting 1-methylnicotinamide chloride for the iodide does not result in any enhancement of fluorescence, and the iodide was therefore used throughout the work as it is easier to prepare.

The fluorescence intensity is also influenced by the duration of the reaction in alkaline solution, as well as by the concentration of alkali used. Fig. 2(*b*) shows that the fluorescence intensity reaches a maximum when the reaction in alkali is allowed to proceed for 2 min, but that it is reduced if a longer reaction time is used. This observation is the same irrespective of whether the fluorescence is measured in aqueous solution, or after extraction into dichloromethane. The highest fluorescence readings are obtained when the sodium hydroxide reagent concentration is 5 M or above [Fig. 2(*c*)]. With the higher alkali concentrations (8 or 10 M), however, the fluorescence is slightly less stable because of the quenching effect already mentioned. The fluorescence intensity is about ten times higher in acidic than in alkaline solution, and the final fluorimetric measurement is therefore made after acidification with formic acid. This acid produced a higher fluorescence than hydrochloric acid, possibly because with the latter a heating step is necessary in order to develop the fluorescence. The optimum concentration of formic acid in the final solution is approximately 50% *V*/*V*. With concentrations higher than this a yellow colour again develops and the fluorescence is less stable; with lower concentrations fluorescence develops to the same extent but more slowly.

Precision and Accuracy

The calibration graph for the assay in plasma was linear, and fluorescence intensity measurements (arbitrary units), after subtraction of the blank value, ranged from 130 for 0.5 μg ml^{-1} to 653 for 2.5 μg ml^{-1} of acetohexamide in human plasma or in 0.01 M sodium hydroxide solution. In order to test the precision of the procedure, eight replicate determinations were made on each of two plasma solutions containing 1.0 and 2.0 μg ml^{-1} of acetohexamide. The relative standard deviations of the assay were calculated to be 3.7 and 3.1%, respectively. The accuracy of the procedure was tested by assaying a sample of plasma to which had been added 40.0 μg ml^{-1} of acetohexamide and comparing the results obtained by using the fluorimetric method and the spectrophotometric procedure of Smith *et al.*[6] The average recovery (see Table I) was 99.2% by the fluorimetric method, while by the spectrophotometric procedure it was 93.0% calculated by means of the Smith equation, or 90.0% calculated directly using an $A^{1\%}_{1cm}$ value of 538.9 at 247 nm, which had been determined for the acetohexamide sample used. The lower recovery by the spectrophotometric procedure, which does not apply a correction for the losses in the extraction steps involved, is in accordance with recoveries of 91 and 94% reported by Smith *et al.* The spectrophotometric procedure is not sufficiently sensitive to allow a comparison of the two methods of assay at lower levels of drug concentration in plasma. Defining the detection limit as the amount giving twice the background (blank) fluorescence, the limit calculated for acetohexamide in plasma is 0.2 μg ml^{-1}. The calibration graph was also linear for the assay of tablets, and fluorescence intensity measurements (arbitrary units), after subtraction of the blank value, ranged from 113 for 2.0 μg ml^{-1} to 564 for 10.0 μg ml^{-1} of acetohexamide in

TABLE I

COMPARISON OF THE FLUORIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF PLASMA CONTAINING 40 μg ml^{-1} OF ADDED ACETOHEXAMIDE

Fluorimetric method		Spectrophotometric method[6]	
Acetohexamide found/μg ml^{-1}	Recovery, %	Acetohexamide found/μg ml^{-1}	Recovery, %
39.6	99.0	37.3	93.3
39.7	99.3	37.6	94.0
39.7	99.3	36.8	92.0
		36.1	90.0
		37.6	94.0
	Mean: 99.2		Mean: 93.0

0.01 M sodium hydroxide solution. In order to test the precision and accuracy of the fluorimetric procedure, results were compared with those obtained by the spectrophotometric method of the United States Pharmacopeia.[13] Five portions, each equivalent to about 500 mg of acetohexamide, were taken from the same powdered sample of tablets, accurately weighed, and made up to 100 ml in 0.1 M sodium hydroxide solution in the usual way. Duplicate aliquots of the filtered solution were assayed by each method. The results are shown in Table II.

TABLE II

COMPARISON OF THE FLUORIMETRIC AND USP METHODS FOR THE ASSAY OF A COMMERCIAL SAMPLE OF ACETOHEXAMIDE TABLETS

Average mass per tablet, 0.653 g; nominal content of acetohexamide, 500 mg.

	Fluorimetric method		USP method	
Sample mass/g	Acetohexamide found per tablet/mg	Percentage of stated amount	Acetohexamide found per tablet/mg	Percentage of stated amount
0.6690	500	100.0	500	100.0
	508	101.6	509	101.8
0.6509	493	98.6	503	100.6
	495	99.0	512	102.4
0.6444	509	101.8	505	101.0
	503	100.6	504	100.8
0.6481	505	101.0	504	100.8
	511	102.2	503	100.6
0.6665	514	102.8	501	100.2
	506	101.2	502	100.4
Mean	504	100.9 ± 1.1	504	100.9 ± 0.5
Relative standard deviation	1.3%		0.7%	

Specificity

The procedure can be used for the determination of acetohexamide in the presence of insulin and other commercially available oral hypoglycaemic drugs, as none of these contains a ketone grouping. The fluorimetric reaction is also not shown by lactic or β-hydroxybutyric acids, or by hydroxyhexamide, which is the main route of metabolism for acetohexamide in man.[21]

Acetone and acetoacetic, pyruvic and α-ketoglutaric acids produce fluorescent derivatives in the reaction with 1-methylnicotinamide. When, however, solutions of these compounds, in the concentrations shown in Table III, were tested by the assay procedure for acetohexamide in plasma, only acetoacetic acid was extracted with chloroform and remained behind after evaporation of the solvent in an amount sufficient to make it liable to interfere

TABLE III

FLUORESCENCE MEASUREMENTS MADE BY SUBJECTING SOLUTIONS OF POSSIBLE INTERFERING COMPOUNDS TO THE ASSAY PROCEDURE FOR ACETOHEXAMIDE IN PLASMA

Compound	Concentration in solution/ mg ml^{-1}	Fluorescence intensity,* arbitrary units
Acetoacetic acid	5.2	474
Acetone	8	0
β-Hydroxybutyric acid ..	6	0
Hydroxyhexamide	0.1	0
α-Ketoglutaric acid	0.1	0
Lactic acid	0.36	0
Pyruvic acid	0.01	10

* Blank fluorescence subtracted.

with the assay. The acetoacetic acid can be separated from the acetohexamide by washing an aliquot of the chloroform extract twice, each time with an equal volume of distilled water. Repeated assays using this washing technique showed that, on average, 93% of the chloroform-extracted acetohexamide will remain in the chloroform layer, the remainder passing into the aqueous washings. Experience has shown that interference, if any, from acetoacetic acid in the assay procedure for plasma is likely to be slight. Plasma samples were taken from ten diabetic patients who were not receiving any acetohexamide, but were being controlled either by diet alone, or by treatment with insulin, chlorpropamide or metformin. The average plasma blank reading (arbitrary units) was 27 (range 23–30) by the normal assay procedure, 23 (range 20–25) when the chloroform was washed twice with water and 18 (range 16–19) when aliquots of the chloroform extracts were washed once with an equal volume of 0.5 M sodium carbonate solution before evaporation. The mean blank fluorescence (reagent blank) when the procedure was carried out using 0.01 M sodium hydroxide solution in place of plasma was also 18 (range 17–19). Blanks obtained using plasma samples from non-diabetic patients were in the same ranges.

Binding of Acetohexamide to Plasma

Attempts to separate acetohexamide from plasma proteins by ultrafiltration showed that the drug is largely bound by these proteins. Solutions of the drug in human plasma containing 10 and 100 $\mu g\ ml^{-1}$ of acetohexamide were assayed by the fluorimetric procedure. Samples (about 5 ml) from each solution were also subjected to ultrafiltration by centrifuging for about 30 min in Centriflo membrane cones. The ultrafiltrate collected from each solution was then subjected to fluorimetric assay. It was found that the binding of acetohexamide was 96.6% for the 10 $\mu g\ ml^{-1}$ and 95.7% for the 100 $\mu g\ ml^{-1}$ solution. These figures support the measurements of binding of the same drug to various human proteins made by Judis[22] by means of equilibrium dialysis, which showed that acetohexamide can be bound 88% to albumin, and to a lesser extent to fibrinogen I and α-globulin IV-4.

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Polarographic Determination of Trace Elements in Food from a Single Digest

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The determination of 12 trace elements, namely copper, zinc, mercury, lead, cadmium, iron, tin, chromium, arsenic, antimony, selenium and tellurium, in admixture by means of a cathode-ray polarograph is described. The elements were investigated in concentrations ranging from 0.1 to 20 p.p.m. by means of normal, reverse-sweep or resistance - capacitance derivative techniques. The last technique could not be used for mercury, although it was used for all the other elements in concentrations less than 1 p.p.m. The complete determination took 4–7 h, and was applied to various kinds of food, such as bread, meat and vegetables.

Keywords: *Trace element determination*; *food analysis*; *polarography*; *single digest*

The determination of trace elements in food is of great importance, as some of them have nutritional significance, whilst others are toxic. The aim of this work was to establish a method that is accurate at an adequate level, is not very expensive, is rapid in comparison with other available techniques and covers a wide area of applicability.

Solvent extraction has been used for a long time for the separation of trace elements. The technique is not characterised by specificity, a fact that can be turned to advantage when the method is combined with polarography.

In the method recommended here, the sample is first digested with sulphuric and nitric acids and the pH then adjusted to a suitable value. Mercury(II), copper(II), zinc(II), cadmium(II) and lead(II) are removed in the form of dithizone (diphenylthiocarbazone) complexes.[1]

Concentrated hydrochloric acid is added to the aqueous phase, after which iron(III), tin(IV) and antimony(III), if present, can be extracted in the form of salts of cupferron (ammonium *N*-nitrosophenylhydroxylamine).[2] The pH of the resulting aqueous phase is adjusted with ammonia solution, after which chromium(VI) is extracted with sodium diethyldithiocarbamate in chloroform.[3] The aqueous phase remaining is digested with sulphuric and nitric acids and, after complete destruction of the organic material, tellurium(IV), selenium(IV), antimony(V) and arsenic(III) are determined. A flow diagram of the procedure is shown in Fig. 1.

Experimental

Apparatus

The work was carried out with an A 1660 Davis differential cathode-ray polarograph manufactured by Southern Analytical Limited.

The pH was measured with a Digital 110 Expanded Scale pH meter manufactured by Corning Scientific Instruments.

Reagents

Nitric acid, concentrated, relative density 1.42 (*AnalaR*).
Sulphuric acid, concentrated, relative density 1.84 (*AnalaR*).
Hydrochloric acid, concentrated, relative density 1.18 (*AnalaR*).
Potassium hydroxide solution, 3 M.
Ammonia solution, 6 M.
Hydrochloric acid, 4 M.
Sulphuric acid, 2 M.
Dithizone solution. Prepared by solution of 50 mg of dithizone in 1 l of chloroform.
Cupferron solution, 10% *m/V*. Prepared by solution of 10 g of cupferron in 100 ml of distilled water.

* Present address: Department of Food Chemistry, University of Athens, Navarinou 13, Athens(144), Greece.

Sodium diethyldithiocarbamate solution, 5% *m/V*. Prepared by solution of 5 g of sodium diethyldithiocarbamate in 100 ml of distilled water.
Ammonium citrate solution, 20% *m/V*.
Potassium iodide solution, 2% *m/V*.
Potassium disulphite solution, 5% *m/V*.
Potassium chloride solution, 1 M.
Hydrogen peroxide, 30%.
Ethyl acetate.

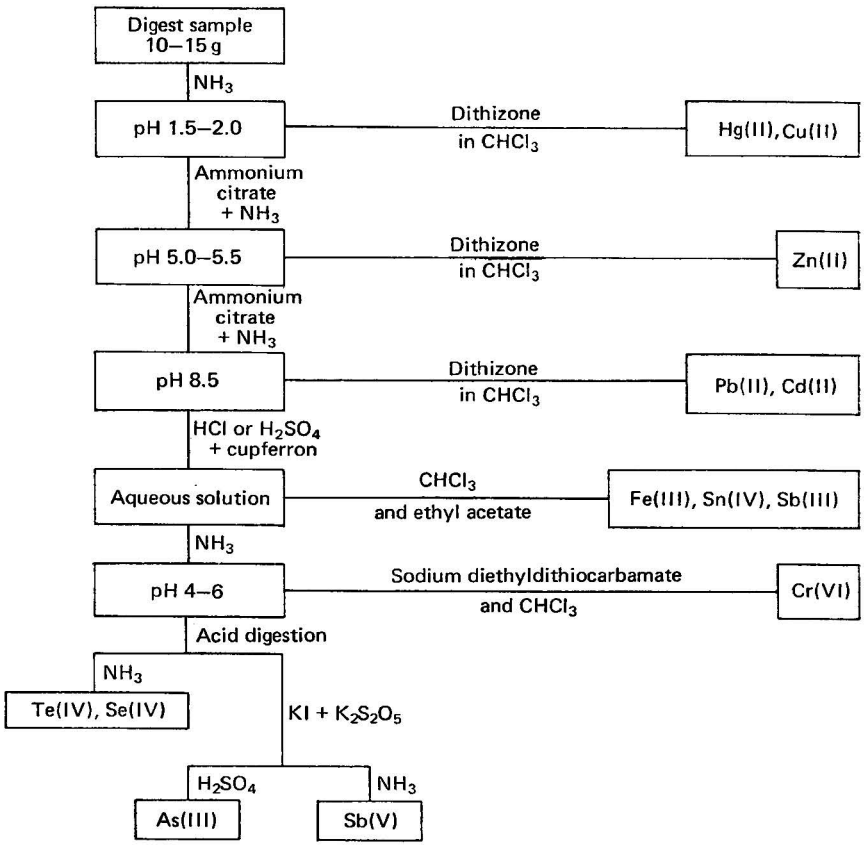

Fig. 1. Flow diagram of the procedure.

Procedure

The sample (10–15 g) was weighed accurately and transferred into a 600-ml Kjeldahl flask. Concentrated sulphuric acid (20–25 ml) and concentrated nitric acid (20–25 ml) were added and the mixture was heated in a fume cupboard (see Note 1). When the liquid turned brown, small amounts of concentrated nitric acid were added dropwise until complete decolorisation occurred. After decolorisation, heating was continued until the fumes of nitric acid had disappeared. This usually happened in 10–20 min according to the mass of sample.

When the flask had cooled, the solution was transferred into a beaker with 40–50 ml of water and 6 M ammonia solution was added, with stirring, until the pH value became 1.5–2.0. The solution was cooled and transferred into a 500-ml separating funnel, after which extraction with 50-ml portions of dithizone in chloroform (extract 1) was used for the removal of

mercury(II) and copper(II). The extraction was continued until the organic layer did not change in colour. To the aqueous phase, 1 ml of 20% *m/V* ammonium citrate solution was added (see Note 2), the pH then being adjusted to between 5 and 5.5 with 6 M ammonia solution.

The solution was transferred into a separating funnel and zinc(II) was extracted with 50-ml portions of the solution of dithizone in chloroform (extract 2). Once again, the colour of the organic layer was used as the criterion for the completeness of the extraction.

Subsequently, 1 ml of 20% *m/V* ammonium citrate solution was added and the pH was adjusted to 8.5 with 6 M ammonia solution. Lead(II) and cadmium(II) were then similarly extracted with dithizone in chloroform (extract 3).

Hence, all metals forming dithizonates were removed from the solution. The chloroform extracts, 1 and 2, were mixed and, after evaporation of the chloroform, digested with a concentrated nitric acid - sulphuric acid mixture (1 + 1). After cooling, dilution and adjustment to pH 5.0 with 6 M ammonia solution, mercury(II), copper(II) and zinc(II) were determined polarographically in 1 or 2 M ammonium sulphate solution. Alternatively, the digestion could be carried out with concentrated sulphuric acid and 30% hydrogen peroxide.

For the determination of cadmium(II) and lead(II), extract 3 was evaporated and the residue was digested with the nitric acid - sulphuric acid mixture (1 + 1) as above, after which the solution was diluted, and 1 M potassium chloride solution and 6 M ammonia solution were added until a solution 0.1 M in potassium chloride, 0.1 M in sulphuric acid and 0.8 M in ammonium sulphate was obtained with a pH of 1.6. This solution was then examined polarographically according to the details in the manufacturer's manual. Once again, concentrated sulphuric acid and 30% hydrogen peroxide could be used as a digestion mixture.

Alternatively, the determination could be carried out as follows. The chloroform extracts 1, 2 and 3 were mixed and, after evaporation of the chloroform, digested with the concentrated nitric acid - sulphuric acid mixture (1 + 1). After cooling, dilution and adjustment to pH 5.0 with 6 M ammonia solution, mercury(II), copper(II), zinc(II) and cadmium(II) were determined polarographically in 1 or 2 M ammonium sulphate solution.

For the determination of lead(II) in a portion of the above solution, 1 M potassium chloride solution was added together with enough sulphuric acid to give a solution 0.1 M in potassium chloride, 0.1 M in sulphuric acid and 0.8 M in ammonium sulphate with a pH of 1.6. This solution was then examined polarographically for lead(II).

After the extraction of dithizonates, the aqueous phase was treated with either concentrated hydrochloric acid or concentrated sulphuric acid, so that the latter constituted 10% by volume of the resulting mixture. An excess of 10% *m/V* aqueous cupferron solution was added, after which iron(III), tin(IV) and antimony(III) were extracted with two 50-ml portions of chloroform followed by similar portions of ethyl acetate (see Note 3).

From the combined organic layers, the solvents were evaporated; then 25 ml of concentrated nitric acid and 30 ml of 30% hydrogen peroxide were added. The mixture was boiled until only a small volume remained. If a brown colour developed, a few drops of 30% hydrogen peroxide were added cautiously. The digestion was continued until the mixture became colourless. After the destruction of the organic material, 50 ml of 4 M hydrochloric acid were added and the solution was partly neutralised with 33.3 ml of 3 M potassium hydroxide solution. The mixture was transferred into a 100-ml calibrated flask and diluted to the mark with water, so that it became 1 M in potassium chloride and 1 M in hydrochloric acid. This solution was examined polarographically.

The acidic aqueous phase remaining after the extraction of the cupferronates was adjusted with 6 M ammonia solution to a pH of 4–6, and an excess of 5% *m/V* aqueous sodium diethyldithiocarbamate solution was added. The mixture was shaken and chromium(VI) was extracted with several 50-ml portions of chloroform until the aqueous layer was clear and colourless.

The organic layer was digested with concentrated nitric acid and 30% hydrogen peroxide in the manner described above. Twenty-five millilitres of 4 M hydrochloric acid were added, and the mixture was neutralised with 3 M potassium hydroxide solution. It was then transferred into a 100-ml calibrated flask and diluted to the mark with water, so that it became 1 M with respect to potassium chloride. This solution was investigated polarographically, the peak potential, E_p, being −1.25 V.

Alternatively, a mixture of concentrated nitric and sulphuric acids in equal proportions by volume could be used. In this instance, the neutralisation was carried out with 6 M ammonia solution and the solution was diluted until it became 1–2 M with respect to ammonium sulphate.

The aqueous phase remaining from the extraction of chromium was evaporated to a small volume and then digested with a concentrated nitric acid - sulphuric acid mixture (1 + 1). After this digestion, a convenient amount (10–50 ml) of hydrochloric acid (1 + 1) was added and the solution was split into two portions of equal volume.

The first of these portions was evaporated until fumes appeared, cooled, diluted and neutralised with 6 M ammonia solution to pH 8.5. It was then suitably diluted to yield a solution 1 M with respect to ammonium sulphate. Tellurium(IV) and selenium(IV) were determined polarographically in this mixture.

To the second portion, 2% *m/V* potassium iodide and 5% *m/V* potassium disulphite solutions were added in excess and the mixture was heated until white fumes appeared. The flask was cooled and the solution, suitably diluted to a known volume, was divided into two equal parts. To the first part, distilled water was added until the solution was 1 M in sulphuric acid. Arsenic(III) was then determined polarographically. The second part was diluted and neutralised with 6 M ammonia solution to pH 8.5. After suitable dilution, antimony(III), originally present as antimony(V), was determined polarographically in a solution 1 M in ammonia and 1 M in ammonium sulphate.

NOTES—

1. In the presence of chromium(III), the addition of 2 ml of 3% *m/V* ammonium peroxodisulphate solution is recommended.
2. The addition of 20% *m/V* ammonium citrate solution was necessary to prevent the precipitation of some metals. Ammonium oxalate solution was also tried, but was not effective in the presence of tin(IV) at high pH. It was found that the extraction of tin(IV) was unaffected by the presence of ammonium citrate solution.
3. The use of ethyl acetate for the extraction of tin(IV) was necessary, as a complete recovery could not be achieved with chloroform alone.

Results

A sample solution containing all the elements under consideration was digested according to the method described above. The solution was diluted to 1 l, and the method was applied to 10 ml of the diluted solution. The determination was carried out twice, and the results are shown in Table I.

TABLE I

DETERMINATION OF ELEMENTS IN ADMIXTURE

Metal ion	Concentration, p.p.m.	Supporting electrolyte	Peak potential/V	Peak height, scale divisions: Found	Peak height, scale divisions: Theoretical	Recovery, %
Cu(II) ..	7.21	1 M $(NH_4)_2SO_4$	−0.23	1.10, 1.00	1.10	100.00, 90.91
Hg(II) ..	16.69	1 M $(NH_4)_2SO_4$	−0.05	1.10, 1.05	1.15	95.65, 91.30
Zn(II) ..	15.82	1 M $(NH_4)_2SO_4$	−1.30	2.60, 2.60	2.63	98.86, 98.86
Pb(II) ..	10.26	0.1 M KCl - 0.1 M H_2SO_4 - 0.8 M $(NH_4)_2SO_4$	−0.52	1.10, 1.10	1.80	61.10, 61.10
Cd(II) ..	11.37	0.1 M KCl - 0.1 M H_2SO_4 - 0.8 M $(NH_4)_2SO_4$	−0.73	1.65, 1.65	1.70	97.06, 97.06
Fe(III) ..	11.20	1 M HCl - 1 M KCl	−1.05	1.16, 1.00	1.07	108.41, 93.46
Sn(IV) ..	9.47	1 M HCl - 1 M KCl	−0.50	0.55, 0.52	0.50	110.00, 104.00
Cr(VI) ..	15.64	1 M $(NH_4)_2SO_4$	−1.35	2.50, 2.50	2.50	100.00, 100.00
As(III) ..	9.23	1 M H_2SO_4	−0.75*	14.20, 14.00	15.00	94.67, 93.33
Se(IV) ..	17.44	1 M NH_3 - 1 M $(NH_4)_2SO_4$	−1.50	0.60, 0.60	0.60	100.00, 100.00
Sb(III) ..	10.00	1 M NH_3 - 1 M $(NH_4)_2SO_4$	−0.61†	2.20, 2.00	2.20	100.00, 90.91
Te(IV) ..	13.07	1 M NH_3 - 1 M $(NH_4)_2SO_4$	−1.04† −0.65	3.75, 3.80	4.00	93.75, 95.00

* Resistance - capacitance derivative circuit used.
† Tellurium(IV) was determined by means of the peak at −1.04 V. In the determination of antimony(III) a correction was made for the overlapping tellurium peak.

From this table, it can be seen that all the elements can be determined accurately with recoveries ranging from 90.9 to 100%, except for lead(II), where the recovery was 55.56%. This discrepancy was due to the fact that some lead(II) was lost by precipitation in the form of sulphate. Lead must be present at a concentration less than 7 p.p.m. in order to prevent this loss.

TABLE II

DETERMINATION OF THE ELEMENTS IN BEEF SAUSAGE WITH PORK

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†
Cr(VI)	20.93	20.15	12.13‡	11.65	16.96‡ 18.96	17.70	3.65‡	6.10	5.80	6.25	1.09‡	1.02	1.04‡	1.21	1.11‡	1.20
Sn(IV)	13.40	12.20	6.78	7.05	10.30	10.72	3.55	3.70	3.50	3.80	0.57	0.62	0.70‡	0.73	0.68‡	0.72
Fe(III)	14.98	14.42	8.04‡ 7.58	8.34	13.58‡	12.67	4.35‡	4.35	4.15‡	4.40	0.83‡	0.73	0.77‡	0.86	0.80‡	0.86
Pb(II)	9.08	13.22	5.66§	7.64	8.60‡	11.61	4.03‡	4.00	4.065‡	4.10	0.67‡	0.67	0.76‡	0.79	0.76‡	0.78
Cd(II)	13.54§ 14.08	14.64	8.47‡ 7.97‡	8.47	12.87§ 12.06	12.87	4.10‡	4.35	4.50‡	4.60	0.74‡	0.74	0.88‡	0.88	0.87‡	0.87
Hg(II)	21.50	21.50	14.20	12.43	17.80	18.88	5.95	6.50	6.25	6.65	1.03	1.09	1.47	1.29	1.46	1.28
Cu(II)	8.99	9.28	5.37‡ 5.86§	5.37	8.15§ 8.55‡	8.15	2.90‡	2.80	3.00‡	2.90	0.54‡	0.47	0.56‡	0.56	0.52‡	0.55
Zn(II)	19.74	20.38	11.49‡ 11.35	11.79	17.25‡ 17.47‡	17.91	6.15‡	6.15	6.65‡	6.33	1.16‡	1.03	1.37	1.22	1.29‡	1.22
As(III)	12.39‡	11.88	6.32‡	6.87	9.81‡	10.44	3.42‡	3.60	3.52‡	3.70	0.72‡	0.65	0.63‡	0.71	0.63‡	0.71
Sb(III)	12.89‡	12.89	6.09‡ 8.28	7.45	11.28‡	11.32	4.15‡	3.80	4.10‡	4.00	0.59‡	0.65	0.73‡	0.77	0.70‡	0.77
Te(IV)	15.28	16.84	9.49‡ 8.85‡¶	9.74	13.62‡	14.79	4.75‡	5.10	4.85‡	5.12	0.90‡	0.86	1.07‡	1.01	1.10‡	1.00
Se(IV)	25.28‡	22.47	12.99 12.35‡	11.99	20.32‡	19.74	6.40‡	6.80	6.45‡	6.95	1.22‡	1.14	1.24‡	1.34	1.26‡	1.34

* The concentrations are expressed in p.p.m. of sample mass.
† F = found; E = expected.
‡ Resistance - capacitance derivative circuit was used.
§ Reverse voltage was used.
¶ Calculated at different values of E_p (peak potential).

TABLE III

DETERMINATION OF THE ELEMENTS IN BEEF

Footnotes as in Table II.

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†
Cr(VI)	12.64	11.96	13.57‡¶	15.27	10.05‡¶	10.49	5.55‡	5.90	6.10‡	6.50	1.68‡	1.57	0.97‡	1.09	1.08‡	1.12
	11.06‡		13.26‡		9.83						1.78					
Sn(IV)	6.54	7.24	9.77	9.25	5.80	6.35	3.40	3.60	3.80	3.95	0.88	0.95	0.60	0.65	0.64	0.68
Fe(III)	7.37	8.11	10.54‡	10.93	6.23	7.51	4.05‡	4.20	4.65‡	4.675	1.19‡	1.12	0.73‡	0.78	0.77‡	0.80
	8.88‡				8.23‡											
Pb(II)	5.81‡	7.84	11.13	10.02	4.59‡	6.88	3.85‡	3.85	4.25‡	4.30	0.84	1.03	0.68‡	0.71	0.70‡	0.73
Cd(II)	8.69‡	8.69	10.09§	11.10	6.93§	7.62	4.20‡	4.20	4.70‡	4.75	1.23‡	1.14	0.77‡	0.79	0.81‡	0.81
	7.90§		12.68‡		7.84‡						1.20					
Hg(II)	10.93	12.76	15.13	16.29	12.42	11.19	5.95	6.30	6.75	6.95	2.14	1.67	1.09	1.16	1.08	1.19
Cu(II)	5.85‡	5.51	7.36§	7.04	4.83§	4.83	2.60‡	2.70	2.90‡	3.00	0.72‡	0.72	0.50‡	0.50	0.49‡	0.52
	5.61§		7.48‡		5.11											
Zn(II)	12.55‡	12.10	14.00‡	15.45	10.20	10.61	5.95‡	5.95	6.90‡	6.60	1.49‡	1.59	1.14‡	1.10	1.18‡	1.13
	12.32		14.75		9.61‡											
As(III)	7.28‡¶	7.05	9.01‡¶	9.01	6.70	6.19	3.10‡	3.45	3.65‡	3.85	1.02‡	0.93	0.60‡	0.64	0.66‡	0.66
	6.65‡		8.86‡		6.68‡											
Sb(III)	8.50	7.65	11.63	9.77	8.04	6.71	3.50‡	3.75	3.93‡	4.17	1.09‡	1.00	0.64‡	0.69	0.64‡	0.72
Te(IV)	10.65	9.94	15.19	12.76	9.97	8.77	6.20‡	6.55	6.90‡	7.30	1.47‡	1.31	0.83‡	0.91	0.86‡	0.94
Se(IV)	13.88	13.33	20.43‡	17.03	13.19	11.69	4.75‡	4.90	5.40‡	5.45	1.63‡	1.75	1.21‡	1.21	1.17‡	1.25
			15.76		12.12‡											

TABLE IV

DETERMINATION OF THE ELEMENTS IN PORK LUNCHEON MEAT

Footnotes as in Table II.

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†
Cr(VI)	12.02 12.02‡	11.54	12.30 12.30‡	12.73	12.99§	13.65	5.85‡	5.45	5.45‡	5.60	1.01‡	1.09	0.91‡	0.98	0.87‡	0.94
Sn(IV)	6.72	6.99	7.41	7.71	7.95	8.27	3.15	3.30	3.10	3.32	0.63	0.66	0.54	0.59	0.55	0.57
Fe(III)	8.85‡	8.26	9.76‡ 9.11	9.11	8.88 9.63‡	9.77	3.80‡	3.90	3.85‡	4.00	0.65‡	0.78	0.65‡	0.70	0.65‡	0.67
Pb(II)	5.60‡	7.54	6.19	8.35	6.63	8.95	3.45‡	3.60	3.50‡	3.65	0.72‡	0.72	0.61‡	0.64	0.61‡	0.61
Cd(II)	7.70‡ 8.39§	8.39	9.25 9.25‡	9.25	9.93§ 9.03‡	9.93	3.95‡	3.95	4.00‡	4.05	0.77‡	0.79	0.71‡	0.71	0.68‡	0.68
Hg(II)	11.25	12.31	12.80	13.58	13.32	14.57	6.00	5.85	6.10	5.95	1.08	1.16	0.96	1.05	0.94	1.00
Cu(II)	5.05‡	5.32	5.54 5.86‡	5.86	6.86 6.29‡	6.29	2.45§	2.50	2.55‡	2.55	0.54‡	0.50	0.42‡	0.45	0.43‡	0.43
Zn(II)	19.11 12.55‡	11.68	13.58 13.63‡	12.88	14.67 14.32‡	13.81	5.95‡	5.55	5.93‡	5.75	1.17‡	1.10	0.99‡	0.99	0.93‡	0.95
As(III)	6.48‡	6.81	7.26‡	7.51	7.89‡	8.05	3.20‡	3.20	3.15‡	3.30	0.60‡	0.64	0.52‡	0.58	0.55‡	0.55
Sb(III)	7.13§	7.38	7.73‡	8.14	7.94‡	8.73	3.30‡	3.50	3.38‡	3.60	0.76‡	0.70	0.59‡	0.63	0.57‡	0.60
Te(IV)	9.12‡¶ 8.69‡	9.65	9.80	10.64	10.38‡ 11.42‡	11.42	4.30‡	4.60	4.25‡	4.60	0.91‡	0.91	0.96‡	0.82	0.86‡	0.78
Se(IV)	13.42 12.42‡	12.87	15.96 14.94‡	14.19	16.90 17.90‡	15.22	6.25‡	6.10	6.20‡	6.20	1.31‡	1.22	1.23‡	1.09	1.08‡	1.04

TABLE V

DETERMINATION OF THE ELEMENTS IN BREAD

Footnotes as in Table II.

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†
Cr(VI)	14.67‡¶ 14.67‡	13.57	13.74 13.74‡	14.99	13.57 15.42‡	14.81	7.35‡	7.80	7.30‡	7.85	1.02‡	0.95	1.16‡	1.35	1.19‡	1.39
Sn(IV)	7.75	8.22	9.77	9.07	8.11	8.97	5.25	4.70	5.10	4.75	0.55	0.58	0.79	0.82	0.775	0.84
Fe(III)	10.06‡	9.72	11.10‡	10.73	9.64 11.61‡	10.60	5.90‡	5.55	5.95‡	5.60	0.62§ 0.61‡	0.68	0.86‡	0.96	0.92‡	0.99
Pb(II)	5.24	8.90	7.18‡	9.83	7.20‡	9.72	5.10‡	5.10	4.95‡	5.15	0.68‡	0.63	0.84‡	0.88	0.87‡	0.91
Cd(II)	10.67‡	9.87	10.83§ 10.03	10.89	9.79§ 10.77‡	10.77	5.80‡	5.65	5.65‡	5.70	0.69‡	0.69	0.92‡	0.98	0.97‡	1.01
Hg(II)	15.78	14.48	18.27	15.99	18.40	15.80	7.60	8.30	7.90	8.40	1.16	1.02	1.36	1.44	1.40	1.48
Cu(II)	5.86‡ 6.46	6.25	7.20§ 6.10	6.90	7.67‡ 6.94§	6.82	3.40‡ 3.49§	3.60	3.40	3.60	0.41‡	0.44	0.71‡	0.62	0.64‡	0.64
Zn(II)	12.50 13.18§	13.73	14.40§ 16.24	15.16	15.55‡ 15.26	14.99	7.55§ 7.45‡	7.85	7.95‡	7.95	0.90‡	0.96	1.28‡	1.36	1.41‡	1.41
As(III)	7.34‡	8.01	8.25‡ 9.72	8.84	9.02‡ 8.25‡	8.74	4.50‡	4.60	4.55‡	4.65	0.53‡	0.56	0.79‡	0.79	0.76‡	0.82
Sb(III)	9.20 8.07	8.68	10.46‡	9.59	10.53	9.48	5.30‡	4.98	5.12‡	5.00	0.62‡	0.61	0.80‡	0.86	0.82‡	0.89
Te(IV)	12.81¶ 10.40	11.35	14.32 11.27‡	12.53	13.26	12.38	6.20‡	6.50	5.95‡	6.55	0.90‡	0.80	1.12‡	1.12	1.06‡	1.16
Se(IV)	14.38	15.14	19.65‡	16.71	17.20	16.52	9.45‡	8.70	10.00‡	9.25	1.06‡	1.06	1.68‡	1.50	1.55‡	1.55

TABLE VI

DETERMINATION OF THE ELEMENTS IN MIXED VEGETABLES

Footnotes as in Table II.

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†
Cr(VI)	7.11 6.80¶ 6.80‡	7.11	19.73‡ 19.37‡¶	21.14	22.39 18.42‡	20.15	4.20‡	4.50	4.25‡	4.40	0.75‡	0.88	0.69‡	0.77	0.66‡	0.71
Sn(IV)	3.91	4.30	12.30	12.79	11.09	12.20	2.55	2.75	2.55	2.65	0.55	0.53	0.43	0.46	0.41	0.43
Fe(III)	4.85§ 4.63‡	5.09	14.40§ 13.75‡	15.13	13.11‡	14.42	3.05‡	3.25	2.95‡	3.15	0.59‡	0.63	0.53‡	0.55	0.49‡	0.51
Pb(II)	3.58‡	4.66	9.70‡	13.86	9.25‡	13.22	2.85‡	2.95	5.75‡	5.77	0.53‡	0.58	0.48‡	0.50	0.45‡	0.47
Cd(II)	5.17‡ 4.76§ 4.71	5.17	14.15 13.96§ 15.36‡	15.36	15.42 13.36‡	14.65	3.30‡	3.30	3.15‡	3.20	0.64‡	0.64	0.60‡	0.57	0.54‡	0.52
Hg(II)	8.55	7.58	20.94	22.55	24.26	21.50	4.95	4.83	4.85	4.70	0.94	0.94	0.75	0.82	0.69	0.76
Cu(II)	2.97§ 3.84	3.27	9.83‡ 8.85§	9.74	8.62	9.28	1.90‡	2.10	1.80‡	2.00	0.44‡	0.41	0.33‡	0.35	0.31‡	0.33
Zn(II)	7.52§ 6.36‡	7.19	20.23	21.38	18.54	20.39	4.85‡	4.60	4.45‡	4.45	0.83‡	0.89	0.77‡	0.77	0.75‡	0.72
As(III)	4.11‡	4.19	13.30‡	12.47	11.89	11.89	2.40‡	2.60	2.45‡	2.60	0.52‡	0.52	0.47‡	0.45	0.44‡	0.42
Sb(III)	5.15	4.55	14.02	13.52	12.89	12.89	2.60‡	2.90	2.65‡	2.80	0.53‡	0.56	0.45‡	0.49	0.43‡	0.49
Te(IV)	5.54	5.94	19.63	17.67	16.84‡	16.84	3.80‡	3.80	3.55‡	3.65	0.92‡	0.98	0.59‡	0.68	0.55‡	0.60
Se(IV)	8.32§ 7.60‡	7.92	22.63‡¶ 21.63‡	23.57	23.97‡ 23.67	22.47	4.75‡	5.05	4.65‡	4.90	0.79‡	0.74	0.75	0.85	0.75‡	0.80

TABLE VII

DETERMINATION OF THE ELEMENTS IN JELLY

Footnotes as in Table II.

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†	F†	E†
Cr(VI)	7.98¶ 10.11‡	8.78	10.94	10.50	13.25‡ 13.25	12.72	4.05‡	4.30	4.10‡	4.40	1.93‡	2.075	0.78‡	0.88	0.85‡	0.92
Sn(IV)	4.81	5.32	5.87	6.36	7.40	7.70	2.45	2.60	2.47	2.65	1.33	1.26	0.49	0.54	0.52	0.56
Fe(III)	6.29‡	6.29	6.71‡	7.52	8.28 9.76‡	9.11	2.70‡	3.05	2.90‡	3.15	1.38‡	1.48	0.65‡	0.63	0.64‡	0.66
Pb(II)	4.43‡ 4.03	5.76	4.77	6.89	6.49‡	8.34	2.65‡	2.65	2.875‡	2.90	1.41‡	1.36	0.56‡	0.58	0.58‡	0.60
Cd(II)	6.19‡ 5.80§	6.38	7.21§ 8.03‡	7.63	8.43‡ 8.41§	9.25	3.025‡	3.10	3.15‡	3.20	1.42‡	1.51	0.68‡	0.64	0.67‡	0.67
Hg(II)	7.36	9.37	9.60	11.21	15.50	13.57	4.30	4.60	4.45	4.70	2.52	2.21	0.89	0.94	0.98	0.98
Cu(II)	3.69	4.05	4.60‡ 5.22	4.84	6.08‡ 5.86§	5.86	1.90‡	1.97	2.00‡	2.05	1.03‡	0.96	0.41‡	0.41	0.42‡	0.42
Zn(II)	8.54§ 9.43	8.88	11.12‡ 11.39	10.63	12.06‡ 12.16	12.87	4.50‡	4.35	4.45‡	4.45	2.23‡	2.10	0.98‡	0.89	0.97‡	0.93
As(III)	5.54	5.18	6.93‡	6.19	7.20‡	7.50	2.55‡	2.55	2.85‡	2.60	1.15‡	1.22	0.51‡	0.52	0.53‡	0.54
Sb(III)	6.31	5.62	7.63	6.72	7.40	8.14	2.50‡	2.75	2.65‡	2.80	1.21‡	1.33	0.52‡	0.56	0.55‡	0.59
Te(IV)	6.85¶ 7.01	7.34	7.98‡¶ 7.90‡	8.78	9.30‡¶ 9.30‡	10.63	3.25‡	3.50	3.50‡	3.70	1.64‡	1.73	0.68‡	0.74	0.71‡	0.77
Se(IV)	9.36¶ 9.41	9.79	11.36‡ 11.71	11.71	16.36 16.68‡	14.18	4.30‡	4.80	4.60‡	4.90	2.38‡	2.31	1.10‡	0.98	1.03‡	1.03

The method was applied to various kinds of food. For this purpose, a portion of food (10–20 g) was weighed accurately, and known amounts of the elements concerned were added. After treatment in accordance with the method described above, the metals were determined polarographically.

The foods used were beef sausage with pork, fresh meat (beef), pork luncheon meat, bread, mixed vegetables, jelly, orange and lemon drink. The results are shown in Tables II, III, IV, V, VI, VII and VIII, respectively.

TABLE VIII

DETERMINATION OF THE ELEMENTS IN ORANGE - LEMON DRINK

Footnotes as in Table II.

Metal ion	Concentration, p.p.m.*		Concentration, p.p.m.*		Concentration, p.p.m.*	
	F†	E†	F†	E†	F†	E†
Cr(VI)	0.17‡	0.16	0.86‡	0.78	0.15‡	0.16
Sn(IV)	0.10	0.10	0.49	0.47	0.09	0.10
Fe(III)	0.11‡	0.11	0.55‡	0.56	0.12‡	0.11
Pb(II)	0.09‡	0.10	0.49‡	0.51	0.10‡	0.10
Cd(II)	0.11‡	0.11	0.57‡	0.57	0.11‡	0.11
Hg(II)	0.18	0.17	0.855	0.83	0.18	0.17
Cu(II)	0.08‡	0.07	0.37‡	0.36	0.07‡	0.07
Zn(II)	0.15‡	0.16	0.72‡	0.78	0.17‡	0.16
As(III)	0.10‡	0.09	0.47‡	0.46	0.09‡	0.09
Sb(III)	0.11	0.10	0.52‡	0.50	0.09‡	0.10
Te(IV)	0.12‡	0.13	0.62‡	0.65	0.14‡	0.13
Se(IV)	0.15	0.17	0.83‡	0.87	0.18‡	0.17

Discussion

The elements were investigated in a range of concentrations varying from 21 to 0.1 p.p.m. Mercury(II) was determined by the normal polarographic process, because the mercury peak is in the region of zero voltage and the resistance - capacitance derivative circuit does not give accurate results. Tin(IV) was also determined by the normal process. The elements most accurately determined were cadmium(II), lead(II) (for concentrations less than 7 p.p.m.), antimony(III), arsenic(III), zinc(II) and tellurium(IV). Copper(II) was determined with less accuracy, whilst chromium(VI), selenium(IV), iron(III), mercury(II) and tin(IV) gave results which were even less accurate.

The errors in the determinations of chromium(VI), selenium(IV), iron(III) and tellurium(IV) could be attributed in part to the fact that the peaks of these elements were close to the hydrogen peak, with which they partly overlapped. Mercury(II) gave a peak near the point of zero voltage (E_p = −0.05 to −0.07 V) and, because of this, it could not be determined accurately. The same was true of copper(II) (E_p = −0.20 V), although to a lesser degree.

The time required for the complete analysis varied from 4 to 7 h, according to the concentrations of the elements. The reason for this was that the time required for the extraction of copper(II), mercury(II), lead(II), zinc(II) and cadmium(II) with dithizone increased considerably when the concentrations were greater than 10 p.p.m.

The use of the resistance - capacitance derivative graph was necessary for most of the elements at concentrations below 1.2 p.p.m.

Conclusion

The procedure possesses considerable advantages of time and cost over most other methods designed for the simultaneous determination of 12 elements.

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Apparatus for the Automatic Preparation of Soil Extracts for Mineral-nitrogen Determination

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An apparatus is described that automatically prepares samples and feeds an AutoAnalyzer system. It consists of a reagent adder, which adds the correct volume of extractant for an approximately weighed amount of soil, and a sample preparation unit, which mixes, filters, dilutes and loads samples on to an AutoAnalyzer sampler. The results obtained using the apparatus were in good agreement with those obtained by manual sample preparation.

Keywords: *Mineral-nitrogen determination*; *soil analysis*; *automatic extraction*

The Microbiology Group at the Weed Research Organization analyses about 3500 samples for mineral-nitrogen each year. The manual preparation of such large numbers of soil samples for the determination of ammonium- and nitrate- plus nitrite-nitrogen on an AutoAnalyzer is tedious, time consuming and prone to considerable operator error.

As accurate weighing of soil samples and addition of a fixed volume of extractant are two of the slowest jobs involved, it was decided that a considerable saving of skilled labour could be made by building a modified version of the reagent adder manufactured by the British Sugar Corporation Ltd. for use in their Tarehouse Laboratories. The remainder of the system was improved by designing and building a sample preparation unit (SPU) that would automatically stir and filter the mixture, dilute the extract and load it on to an AutoAnalyzer sample tray.

Apparatus

Reagent Adder

The reagent adder (Fig. 1) consists of a basic beam balance (Denward Instruments Ltd.) modified to weigh between 19 and 21 g and tared to use standard-mass plastic beakers. One balance pan has been removed and replaced with a palladium probe connected to the equipment electronics. This probe is suspended in the neck of a specially manufactured pipette with a precision-bore neck (T. W. Wingent Ltd., Cambridge) designed such that a 1-g variation from 20 g on the balance gives a difference of 2 ml in the pipette. The working of the equipment is dependent on the use of an electrolytic solution and component values were chosen for the liquid in use (2 M potassium chloride solution).

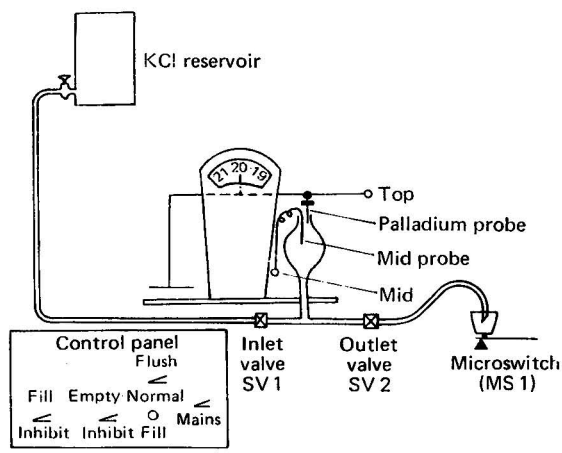

Fig. 1. Layout of the reagent adder.

A schematic drawing of the electronic circuit is shown in Fig. 2. When the mains switch S1 is closed, power is applied to the mains transformer whose nominal output of 20 V is rectified to produce a supply, allowing for losses in the transformer and rectifiers, of approximately 24 V d.c.; S2, the Inhibit empty switch, and S3, the Inhibit fill switch, are shown in the inhibit position, and S4, the Normal - Flush switch, is shown in the flush position. During normal operation these switches are closed. When power is applied to the circuit, the two resistors (R1 and R2) apply sufficient current to the two transistors (in a Darlington pair) to turn on the solenoid valve SV1 and its associated indicator lamp LP1. The potassium chloride solution rises in the pipette until it reaches the mid probe (Fig. 1), when the low-impedance path through the liquid removes the drive from the transistors and valve SV1 turns off. Between 19 and 21 g of moist soil are placed in a beaker on the balance pan, then push-button PB1 is operated. This applies power to the relay RA/2 and the mid probe is disconnected by contact RA2, thus re-establishing drive to the transistors and turning SV1 on again. The relay is latched on by contact RA1. The pipette then continues to fill until the liquid reaches the palladium probe, when the potassium chloride solution again removes drive from the transistors, thus turning off SV1 and RA2, which is unlatched. Indicator lamp LP1 also goes out, showing that the liquid has reached the required level. The beaker is removed from the balance pan and placed on a platform, which operates microswitch MS1. This applies power to valve SV2 and removes the transistor drive, via S4, from the Fill valve circuit, allowing the contents of the pipette to drain into the beaker.

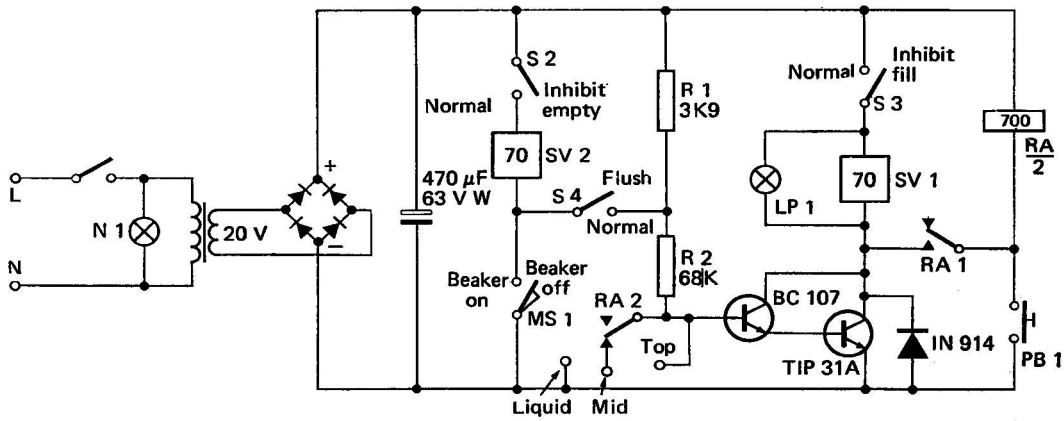

Fig. 2. Circuit diagram of the reagent adder.

As soon as MS1 has been operated the next sample is weighed out, while the pipette is draining. If part of the pipework is to be drained, S2 or S3 is operated as appropriate. If the whole of the system is to be drained or washed out then switch S4 is opened and the microswitch MS1 is closed, thus applying power to SV1 and SV2 at the same time. It is important that the system should be thoroughly washed out after use because if potassium chloride solution is left in the system it crystallises and blocks the valves.

Sample Preparation Unit (SPU)

After addition of the reagent, a magnetic follower (length 40 mm) is placed in each beaker, and the beakers are fitted into cups on the conveyor on the SPU (B in Fig. 3). The beakers are carried over four rows of revolving magnets to give a total of 48-min stirring. Subsequently, the beakers stand, unstirred, for 12 min and then tip into filter-funnels (C in Fig. 3) around the periphery of a 10-sided Perspex table. The soil extract, filtered through a Whatman No. 1 filter-paper, is collected in a beaker (D in Fig. 3) fitted to a similar table lying below the filter-table. The tables rotate on a common spindle until the sample reaches a sample pick-up arm (E in Fig. 3) and is drawn up, via a peristaltic pump (F in Fig. 3), and is automatically diluted when necessary and dispensed via another arm (G in Fig. 3) into the cups on an AutoAnalyzer tray (H in Fig. 3).

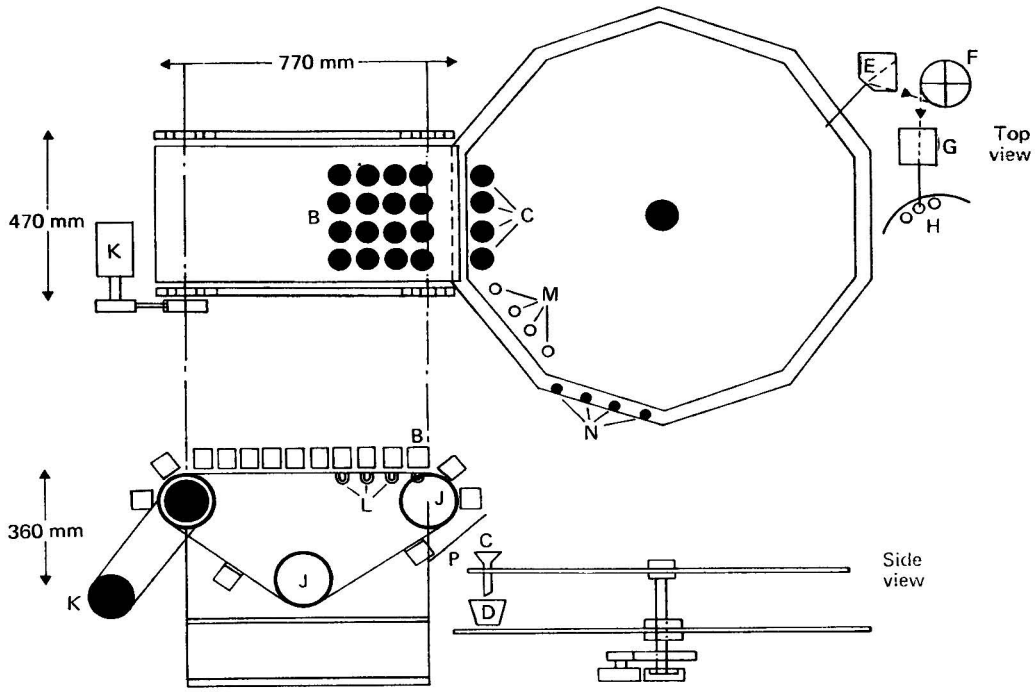

Fig. 3. Layout of the sample preparation unit.

The frame of the conveyor is constructed from angle-iron. The conveyor consists of two lengths of Renolds chain with a 30-mm pitch, joined by 30 aluminium slats (350 × 25 mm) at right-angles to the chain. Each slat carries four plastic cups into which 100-ml plastic beakers fit tightly. The conveyor, in a triangular configuration with gear wheels at the angles (J in Fig. 3), is driven by a Parvalux 9.0 N m torque motor (K in Fig. 3) running at 5 rev min^{-1}.

The stirring mechanism consists of 16 horseshoe magnets (Gallenkamp, Cat. No. XJP-780-T) set in four rows of four (L in Fig. 3) and attached to ball races by spindles, each of which carries a toothed pulley. The magnets are rotated by a toothed drive belt, and their polarities are arranged such that the magnetic field helps the turning and, therefore, only a small motor (Parvalux SD8S, 50 W) is required to drive them.[1]

The two 10-sided Perspex tables are fixed 150 mm apart on a vertical spindle driven by a motor (95 W). The top disc is 0.965 m across the flats, 9 mm thick and each of the 10 sides has four holes drilled to hold 63 mm diameter polypropylene funnels (M in Fig. 3). The funnels are fitted with rubber grommets (9.5 × 6.3 mm), which sit flush on the upper table to prevent unfiltered sample from running down the outside of the funnel and dripping into the beaker below. The lower disc has depressions drilled in it to locate the collection beakers, under the funnels (D in Fig. 3). The collection beakers are 100-ml plastic beakers that have been cut off at approximately 35 mm to facilitate entry of the pick-up arm. At the edge of the upper surface of the lower disc are cams (N in Fig. 3), which are positioned to contact a microswitch that controls the position of the tables at each movement. The movements of the Perspex tables and the conveyor are co-ordinated so that as each beaker moves over the end of the conveyor it tips its contents into a funnel aligned with it. A sheet of polyethylene draped from a bar (P in Fig. 3) prevents cross-contamination of the samples as they move round. The sample pick-up unit is situated approximately one third of the way round the table (E in Fig. 3) and is designed to pick up the sample from the table, flush with air, pick up a wash solution and then flush with air again until it picks up the next sample. It consists of an arm connected to a 12-V car windscreen-wiper motor with

limit switches to stop movement at the end of the track. For sampling or washing, the arm is lowered into the sample or wash solution by means of an electromagnet, and is returned to its upper position after sampling, by means of a spring. The sample is picked up through a small-bore polyethylene tube, the suction being provided by tubing on the peristaltic pump of the AutoAnalyzer. If required, the sample can be diluted at this stage using the pump and AutoAnalyzer coils, and it is then pumped to the AutoAnalyzer sample tray via a dispenser unit (G in Fig. 3). The dispenser unit is similar to the pick-up unit, but has a simple horizontal movement from the AutoAnalyzer sample tray to waste.

The timing and switching sequence of the SPU operates on a 3-min cycle, which is started and maintained by the presence of a 110-V a.c. supply, that is switched by the timing cam on a Technicon AutoAnalyzer. Individual timings, which are all given as time after initiation, are controlled by standard plug-in (octal) timers and the auxiliary switching by standard plug-in (octal) relays. Schematic drawings of the circuit are shown in Fig. 4. The two halves of the circuit are drawn separately for clarity only. Fig. 4 (*a*) is the timing circuit and Fig. 4 (*b*) is the low-voltage switching circuit. The presence of mains voltage at the inlet powers timer TF, which closes the 110-V circuit. When the 110-V supply from the AutoAnalyzer switches on, relay RA operates and connects the mains supply to the timer circuit and the 12-V d.c. supply to the low-voltage circuit. Power is thus supplied to the pick-up solenoid via pin 4 and the pick-up arm drops into the sample on the lower table.

After 60 s TA stops and the +12-V supply is transferred from the pick-up solenoid to the pick-up motor via pin 3. This allows the pick-up arm to spring back to its upper position. The motor swings the pick-up arm to the wash position where a double-limit switch removes power from the motor and connects the +12-V supply to relay RD. This relay latches on and removes the +12 V from pin 3 for the remainder of the cycle. At the same time mains voltage is applied to timer TG and via contact TG1 to the table motor. The table rotates and allows microswitch C (MSC) to slide off the table cam (N in Fig. 3) and close. This powers RB, which by-passes TG1. TG cuts out while the table is in motion so that when the next cam position is reached MSC removes the +12 V from RB, which in turn removes power from the table motor, thus stopping the table at the appropriate point.

At every fourth movement of the table, one corner of the table momentarily closes microswitch MSB, which powers RC. This applies mains voltage to the conveyor motor and a cam on the conveyor allows microswitch MSA to close before MSB opens again, thus maintaining power to RC. This allows the conveyor to continue to move to its next position when the next cam opens MSA and RC removes power from the conveyor motor.

After 75 s TD makes contact TD1, enabling contact TE1 to apply +12 V to the dispenser motor, thereby driving the dispenser arm to the sample position, where it is stopped by a limit switch.

Timer TE is initiated by mains power from the other contact on TD.

After 90 s TB completes its cycle and contact TB1 is made, enabling TC1 to apply +12 V to the pick-up solenoid via pin 2, thus lowering the pick-up arm into the wash solution. Contact TB2 applies mains voltage to TC.

After 115 s TE switches, TE1 applies +12 V to the dispenser motor via pin 3 and the dispenser arm drives to the waste position, where it is stopped by a limit switch.

After 120 s TC completes its cycle, TC1 removes +12 V from the pick-up solenoid, allowing the pick-up arm to return to its upper position, and applies +12 V to the pick-up motor, which drives the pick-up arm to its original position over the disc, where a limit switch stops it.

The equipment remains in this position for the remainder of the 3 min. The end of the sequence is signified by a short interruption in the 110-V supply from the AutoAnalyzer, which causes a similar break in the 12 V and mains supplies, thus re-setting the timers. When the 110-V supply is re-applied, by the timing cam of the AutoAnalyzer, the sequence re-starts.

Results

Tests were carried out to compare the efficiency of extraction results obtained using a manual weighing and sample preparation method, and the reagent adder and sample preparation unit. Extracts of four replicates of 10 soils were prepared by each method and

analysed for nitrate- plus nitrite-nitrogen by a diazotisation and coupling reaction with sulphanilic acid and *N*-(1-naphthyl)ethylenediamine and ammonium-nitrogen by an indophenol method. These methods are described fully by Greaves *et al.*[2]

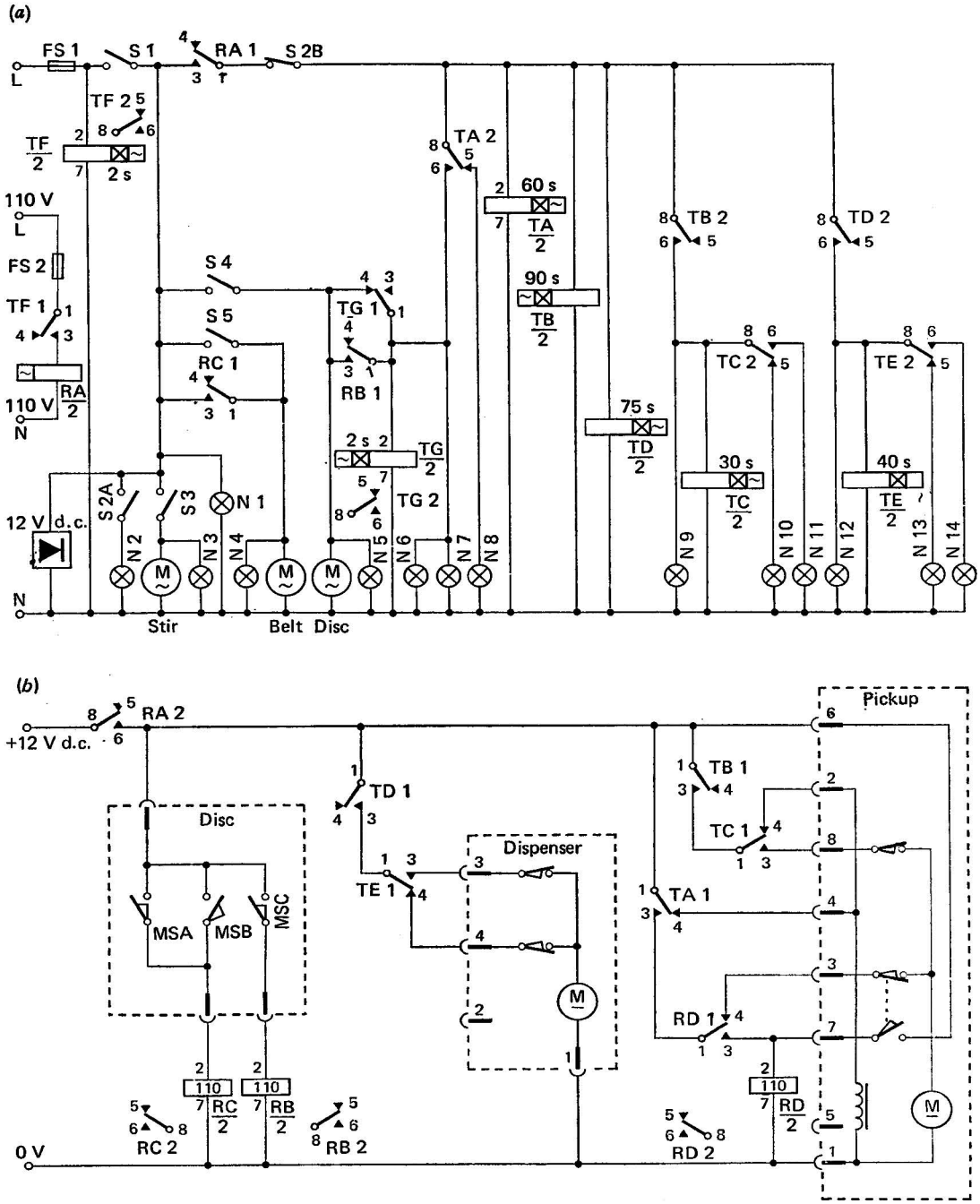

Fig. 4. Circuit diagrams of the sample preparation unit.

Results for the nitrate plus nitrite determinations (Table I) show that there was very little difference in the amounts extracted with either technique.

TABLE I

DETERMINATION OF NITRATE- PLUS NITRITE-NITROGEN IN SOIL AFTER EXTRACTION BY MANUAL AND AUTOMATIC TECHNIQUES

	Manual preparation			Automatic preparation			Automatic — manual		
Sample	Mean of 4 determinations/ μg g^{-1} dry soil	Standard deviation/ μg g^{-1}	Coefficient of variation, %	Mean of 4 determinations/ μg g^{-1} dry soil	Standard deviation/ μg g^{-1}	Coefficient of variation, %	Difference/ μg g^{-1}	Standard error/μg g^{-1}	t
1	23.15	0.671	2.9	23.26	0.587	2.5	0.11	0.446	0.25
2	14.00	0.303	2.2	13.54	0.220	1.6	−0.46	0.187	2.46
3	8.38	0.120	1.4	8.18	0.160	2.0	−0.20	0.100	2.03
4	38.00	1.281	3.4	36.82	0.913	2.5	−1.17	0.786	1.49
5	19.08	0.426	2.2	19.04	0.323	1.7	−0.04	0.268	0.15
6	4.80	0.293	6.1	4.82	0.223	4.6	0.01	0.184	0.07
7	10.08	0.086	0.9	10.54	0.250	2.4	0.46	0.132	3.49
8	47.45	0.426	0.9	47.66	0.798	1.7	0.20	0.452	0.45
9	14.80	0.172	1.2	15.28	0.215	1.4	0.47	0.138	3.44
10	57.94	1.536	2.7	58.49	0.538	0.9	0.54	0.814	0.67

Ammonium-nitrogen (Table II) extracted by the automatic method was slightly higher than that extracted manually, and was significantly higher when less than 2 μg of ammonium-nitrogen per gram of dry soil was present. This may be due to the timing for the filtering of the samples and loading the AutoAnalyzer being shorter than with the manual method. The regression coefficient for results of automatic against manual methods is significantly less than 1 at $P = 0.01$, the relationship between the two being

$$NH_4^+{}_{Auto} = 0.241 + 0.9285(NH_4^+{}_{Manual})$$

TABLE II

DETERMINATION OF AMMONIUM-NITROGEN IN SOIL AFTER EXTRACTION BY MANUAL AND AUTOMATIC TECHNIQUES

	Manual preparation			Automatic preparation			Automatic — manual		
Sample	Mean of 4 determinations/ μg g^{-1} dry soil	Standard deviation/ μg g^{-1}	Coefficient of variation, %	Mean of 4 determinations/ μg g^{-1} dry soil	Standard deviation/ μg g^{-1}	Coefficient of variation, %	Difference/ μg g^{-1}	Standard error/μg g^{-1}	t
1	6.93	0.264	3.8	6.53	0.155	2.4	−0.40	0.153	2.64
2	4.41	0.261	5.9	4.33	0.088	2.0	−0.08	0.138	0.59
3	3.10	0.082	2.6	3.20	0.051	1.6	0.09	0.048	1.93
4	4.28	0.101	2.4	4.37	0.278	6.4	0.09	0.148	0.58
5	2.49	0.113	4.5	2.42	0.118	4.9	−0.07	0.082	0.82
6	0.48	0.024	5.1	0.56	0.025	4.4	0.08	0.018	4.69
7	3.18	0.030	0.9	3.37	0.136	4.0	0.18	0.070	2.65
8	1.46	0.017	1.2	1.63	0.062	3.8	0.17	0.032	5.20
9	0.55	0.024	4.3	0.74	0.025	3.4	0.19	0.017	11.28
10	1.48	0.024	1.7	1.61	0.015	0.9	0.13	0.014	8.89

Discussion

The equipment described in this paper has now been used for the preparation of over 4000 samples for determining ammonium- and nitrate- plus nitrite-nitrogen in soil and has proved reliable.

A comparison of the labour-intensive stages in the manual and automatic sample preparations is shown in Table III. The equipment has reduced the labour requirement by approximately 60%.

The SPU has also been used for the extraction of available phosphate from soil, although analytical problems have, at present, made it impossible to automate the loading of the AutoAnalyzer for this analysis.

We thank Mr. D. F. A. Horsley and Mr. P. R. Leaton of the British Sugar Corporation Ltd. for their guidance and advice in building the reagent adder, Mr. R. W. Foddy for constructing the equipment and Mr. R. C. Simmons who designed the prototype circuits and did the original wiring.

TABLE III

COMPARISON OF STAGES REQUIRING LABOUR IN THE MANUAL AND AUTOMATIC PREPARATION OF SOIL SAMPLES FOR DETERMINATION OF AMMONIUM- AND NITRATE- PLUS NITRITE-NITROGEN ON AN AUTOANALYZER

Stage	Manual preparation	Stage	Automatic preparation
1	Accurate weighing into bottles	1	Rough weighing into pre-tared beakers
2	Accurate addition of extractant		
3	Stoppering bottles		
4	Placing on shaker	2	Placing on conveyor belt
5	Removing from shaker		
6	Transport to filter area		
7	Unstoppering bottles		
8	Setting up filters	3	Setting up filters
9	Filtering		
10	Diluting		
11	Loading AutoAnalyzer tray		

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SHORT PAPERS

Spectrophotometric Determination of Dequalinium Chloride in Pharmaceutical Preparations

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Keywords: *Dequalinium chloride determination*; *spectrophotometry*; *picric acid*

Dequalinium chloride [decamethylenebis(4-aminoquinaldinium chloride)] is a quaternary ammonium compound with antibacterial and antifungal properties. It is commonly used in the form of lozenges and paints for the treatment of infections of the mouth and throat. The compound is described in the British Pharmacopoeia[1] and the method of assay therein is based on non-aqueous titration with perchloric acid in 1,4-dioxan. There are no official methods of assay for preparations containing dequalinium chloride. The usual amount of dequalinium chloride in lozenges is 0.25 mg and a method of assay has not hitherto been developed for the accurate determination of such small amounts.

Picric acid has been used for the spectrophotometric determination of residual quaternary ammonium cations[2]; the coloured complex formed is extracted into chloroform and the absorbance is measured at 365 nm. Picric acid has also been used for the determination of some cationic surfactants[3]; the extractant is 1,2-dichloroethane and the absorbance is measured at 375 nm.

In the proposed method a complex is formed from picric acid and dequalinium chloride at pH 5–9. The yellow complex is extracted into 1,2-dichloroethane and the absorbance measured spectrophotometrically at the maximum, at 342 nm.

The proposed method has been applied successfully to the determination of dequalinium chloride in lozenges (0.25 mg) and paint (0.5% *m/V*). For paint, an alternative direct ultraviolet absorption method has also been found to be applicable as the only excipient in the paint is propane-1,2-diol, which does not show ultraviolet absorption in the range 220–350 nm.

For identification purposes and for detection of other related compounds in the sample, a thin-layer chromatographic method has also been proposed.

Experimental

Apparatus

Spectrophotometer. A Pye Unicam, Model SP8000, spectrophotometer was used.

Reagents

Picric acid solution, 0.12% *m/V in water.*

Buffer solution, pH 7. Dilute 250 ml of 0.2 M potassium dihydrogen orthophosphate and 150 ml of 0.2 M sodium hydroxide to 1 l with water.

Acidified iodoplatinate solution. Dissolve 0.25 g of hexachloroplatinic(IV) acid hexahydrate ($H_2PtCl_6.6H_2O$), 5 g of potassium iodide and 2 ml of hydrochloric acid (sp. gr. 1.16) in sufficient water to produce 100 ml.

Procedures

Spectrophotometric determination of dequalinium chloride with picric acid

Determination in lozenges. Weigh and powder 20 lozenges. Transfer an amount of powder equivalent to 0.25 mg of dequalinium chloride into a 100-ml separating funnel, add 20 ml of water, 20 ml of pH 7 buffer solution and 1 ml of picric acid solution. Extract the solution with three 15-ml portions of 1,2-dichloroethane and filter each extract through a

small pledget of cotton-wool into a 50-ml calibrated flask. Adjust to the mark with the same solvent and measure the absorbance at 342 nm in a 1-cm cell against a blank solution prepared in the same way but omitting the sample.

Calculate the amount of dequalinium chloride present in the sample by reference to a calibration graph prepared by applying the same procedure to 5.0–20.0-ml volumes of a standard solution of dequalinium chloride containing 0.02 mg ml^{-1}, each aliquot being diluted to 20 ml with water before addition of the buffer solution and picric acid.

Determination in paint. Dilute a volume of sample equivalent to 10 mg of dequalinium chloride to 1 l with water. Pipette 20 ml of the dilute solution into a 100-ml separating funnel and proceed as described above for lozenges.

Direct ultraviolet absorption method for determination of dequalinium chloride in paint

Dilute a volume of sample with water to a drug concentration of 0.01 mg ml^{-1}. Measure the absorbance at 326 nm concomitantly with a standard solution of dequalinium chloride containing 0.01 mg ml^{-1}.

Identification of dequalinium chloride by thin-layer chromatography

Identification in lozenges. Shake an amount of powdered lozenges equivalent to 0.25 mg of dequalinium chloride with 2 ml of 95% *V/V* ethanol in a stoppered centrifuge tube. Centrifuge, then spot 0.04 ml of the supernatant liquid on to a 0.25-mm silica gel G plate.

Identification in paint. Spot a volume of paint containing 5 μg of dequalinium chloride on to a silica gel G plate. Spot on to the plate a volume of standard solution equivalent to about 5 μg of dequalinium chloride, and develop the plate using either of the following mobile phases: (*i*) butan-1-ol - glacial acetic acid - water (3 + 1 + 1); (*ii*) 2-methylpropan-1-ol - glacial acetic acid - water - acetic anhydride (2 + 1 + 1 + 1).

Locate the spots on the plate by spraying with acidified iodoplatinate solution.

Results and Discussion

The yellow complex extracted into 1,2-dichloroethane exhibits maximum absorption at 342 nm. The colour was found to be stable for at least 1 h. Beer's law was obeyed over the concentration range 2–14 μg ml^{-1} in 1,2-dichloroethane.

The effect of variation of pH on the development of colour is shown in Fig. 1. The colour is stable over the pH range 5–9. Solutions buffered at pH 7 were used throughout the work.

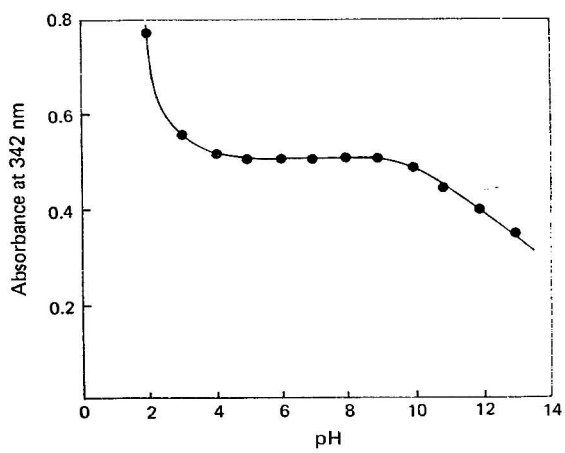

Fig. 1. Effect of pH on development of colour of complex formed from dequalinium chloride and picric acid.

Chloroform was examined as a possible extraction solvent. However, the coloured complex was found to be only partially soluble in chloroform and quantitative extraction of the complex was, therefore, not possible.

The method was applied to two brands of lozenges and to one brand of paint. Lozenge 1 and the paint were British-made products and lozenges 2 and 3 were different batches of a local (Hong Kong) product. The local product was manufactured with overages of dequalinium chloride of 10% for lozenge 2 and 5% for lozenge 3. The results obtained are given in Table I.

TABLE I

DETERMINATION OF DEQUALINIUM CHLORIDE IN COMMERCIAL PREPARATIONS

Preparation	Amount of drug		Recovery (amount found/ labelled amount), %
	Labelled	Found*	
Lozenge 1	0.25 mg	0.260 (±0.001) mg	104.0 (±0.4)
Lozenge 2	0.25 mg	0.280 (±0.002) mg	112.0 (±0.8)
Lozenge 3	0.25 mg	0.266 (±0.001) mg	106.4 (±0.4)
Paint	0.5% *m/V*	0.553 (±0.002)% *m/V*	110.6 (±0.4)
		0.550 (±0.000)% *m/V*†	110.0 (±0.0)†

* Mean of 10 determinations; values in parentheses are standard deviations of individual results.

† Result obtained from direct ultraviolet absorption method.

The accuracy of the method was tested by adding known amounts of dequalinium chloride to the samples and carrying out analyses before and after addition. Recoveries of dequalinium chloride added to the samples are listed in Table II. These experiments indicated a maximum error of about ±2%.

TABLE II

RECOVERY OF ADDED DEQUALINIUM CHLORIDE FROM PREPARATIONS

Preparation	Amount of dequalinium chloride/μg		Recovery, %
	Added	Recovered*	
Lozenge 1	200	201.1 (±1.2)	100.6 (±0.6)
Lozenge 2	100	100.4 (±0.8)	100.4 (±0.8)
	200	202.2 (±2.0)	101.1 (±1.0)
Lozenge 3	100	99.9 (±0.6)	99.9 (±0.6)
	200	200.5 (±1.0)	100.3 (±0.5)
Paint	100	100.7 (±0.7)	100.7 (±0.7)
	200	202.4 (±2.0)	101.2 (±1.0)

* Mean of 10 determinations; values in parentheses are standard deviations of individual results.

Recovery experiments were also performed on a mixture prepared in the laboratory according to the formula used by the local manufacturer:

Dequalinium chloride	5 mg
Sucrose	13.1 g
Lactose	4.7 g
Acacia gum	0.9 g

The recovery was found to be 101.2% (average of 10 determinations), with a standard deviation of 1.0%.

Thin-layer chromatography served for both identification of dequalinium chloride and

establishing the absence of other related compounds. No quaternary ammonium compounds, other than dequalinium chloride, or amines were detected in the samples.

The colouring matter present in the two brands of lozenges tested was Sunset Yellow FCF [C.I. 15985 (1956), E.E.C. No. E110], which was found not to interfere in the determination. The other common excipients encountered in dequalinium chloride lozenges are sucrose, lactose and acacia gum, and in paint the solvent is propane-1,2-diol. All of these substances were also found not to interfere in the proposed method of assay for dequalinium chloride.

The authors thank Dr. A. J. Nutten, Government Chemist of Hong Kong, for permission to publish this paper.

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Spectrophotometric Determination of Isoprenaline Sulphate and Methyldopa Using Chloranil

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Keywords: *Isoprenaline sulphate determination*; *methyldopa determination*; *chloranil reagent*; *spectrophotometry*; *charge-transfer complex*

The official methods[1] for the determination of isoprenaline sulphate and methyldopa are based on measurement of the colour produced when these compounds are treated with iron salts in the presence of an alkaline buffer. Other spectrophotometric methods based on the use of sodium molybdate,[2] 4-nitrobenzenediazonium chloride,[3] thiosemicarbazide[4,5] and sodium hexanitritocobaltate(III)[6] have also been reported.

Feigl[7] and Feigl *et al.*[8] reported that chloranil (tetrachloroquinone) forms coloured condensation products with primary and secondary aryl amines, amino acids, phenols and naphthalene. Birks and Slifkin[9] reported that some amino acids form n - π charge-transfer complexes with chloranil in aqueous ethanol (50% V/V) buffered at certain pH values. Al-Sulimany and Townshend[10] described a procedure for the determination of various amino acids using chloranil. Recently, Al-Ghabsha *et al.*[11] investigated the reaction of chloranil with a wide range of amines and described a method for their determination.

This paper deals with the use of chloranil for the spectrophotometric determination of isoprenaline sulphate and methyldopa.

Experimental

Apparatus

Beckman double-beam spectrophotometer, Model 24.

Reagents

Chloranil solution. A saturated solution of chloranil in ethanol.

Buffer solution, pH 9. A 0.05 M solution of disodium tetraborate.

Prepared drug solution. Dissolve 20 mg of methyldopa or isoprenaline sulphate in 20 ml of 0.1 M hydrochloric acid. Neutralise with 0.05 M disodium tetraborate solution to pH 7 and dilute to 100 ml with water in a calibrated flask.

General Procedure and Preparation of Calibration Graph

Pipette 1–5-ml aliquots of the prepared drug solution into a 25-ml calibrated flask. Add exactly 5 ml of chloranil solution and 2 ml of 0.05 M disodium tetraborate solution. Mix and dilute to volume with water. Prepare a blank solution using 2 ml of 0.05 M disodium tetraborate solution and 5 ml of chloranil solution and dilute to 25 ml with water. Heat both solutions in a water-bath at 65 °C for 30 min. Measure the absorbance in 1-cm cells at 354 nm for isoprenaline sulphate and 358 nm for methyldopa.

Procedure for Tablets

Weigh and powder 20 tablets. Extract an accurately weighed amount of the powder, equivalent to about 100 mg of isoprenaline sulphate or methyldopa, with 0.1 M hydrochloric acid by washing through a filter-paper into a 100-ml calibrated flask. Dilute the extract to the mark with 0.1 M hydrochloric acid. Pipette 20 ml of this solution into a 100-ml calibrated flask, neutralise with 0.05 M disodium tetraborate solution to pH 7 and dilute to the mark with water. Proceed as described under General Procedure using 2 ml of the final neutral solution. Calculate the amount of isoprenaline sulphate or methyldopa from the appropriate calibration graph.

Results and Discussion

Chloranil, in aqueous alcoholic solution buffered at pH 9, reacts with isoprenaline and methyldopa to form complexes with maximum absorption at 354 and 358 nm, respectively. The absorbances of the complexes were found to be stable for 1 h. Under the described experimental conditions, a linear correlation was obtained between absorbance, A_{1cm}, and concentration, C, of isoprenaline sulphate and methyldopa over the range 0.2–1 mg per 25 ml. On extrapolation to zero concentration, the graphs have a small positive intercept on the absorbance axis. The two linear equations were found to be $A_{1cm} = 0.022 + 0.710\ C$ for isoprenaline sulphate and $A_{1cm} = 0.024 + 1.104\ C$ for methyldopa. The small positive intercept in these two equations may have originated from a minor non-specific side-reaction of the phenolic groups in the alkaline buffer used. The apparent molar absorptivities for isoprenaline sulphate and methyldopa were found to be 4.9×10^3 and 6.6×10^3 l mol^{-1} cm^{-1}, respectively. The relative standard deviation for the determination of 0.448 mg of isoprenaline sulphate and 0.460 mg of methyldopa were 0.50 and 0.92%, respectively (five separate determinations).

Chloranil solution in ethanol is yellow, but it slowly becomes violet when added to disodium tetraborate solution of pH 9. The absorbances of the blank solution against solvent (alcohol - buffer solution) at 354 and 358 nm were found to be 0.710 and 0.527, respectively. The violet colour was developed equally in the blank and test solutions. However, in view of these relatively high absorbance readings the use of a double-beam spectrophotometer is preferable in order to obtain precise results.

The reaction mechanism can be explained by analogy with the reaction of adrenaline and noradrenaline with chloranil.[11] According to the suggestions of Al-Sulimany and Townshend,[10] and El-Ghabsha *et al.*,[11] it is assumed that the reaction involves the formation

TABLE I

DETERMINATION OF ISOPRENALINE SULPHATE AND METHYLDOPA IN COMMERCIAL TABLETS

Tablet	Fraction of nominal content, %*	
	Proposed method	Official method
Isoprenaline sulphate (20 mg per tablet) ..	100.0 ± 0.9	100.7 ± 0.6
Methyldopa (250 mg per tablet) ..	99.4 ± 0.7	100.5 ± 0.5

* Results expressed as mean of six separate determinations ± standard deviation.

of a molecular complex between the primary aliphatic amine of methyldopa or the secondary aliphatic amine of isoprenaline sulphate with a reaction product of chloranil at pH 9.

The method has been applied to the determination of isoprenaline sulphate and methyldopa in purchased tablets. In the absence of any knowledge of the composition of the excipients and fillers in the tablets, the results obtained were compared with those obtained by using the official methods.[1] Concordant results were obtained on using both methods (Table I). This agreement indicated that tablet fillers and excipients did not interfere in the proposed method. The official methods suffer from the disadvantage that the colour produced is based on the phenolic group of the compounds and requires the use of elaborate buffer solutions.

It should be emphasised that the present method can be applied to any compound with an $-NH_2$ or –NH– group but it can only be applied to tablets containing one amino compound.

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Spectrophotometric Determination of Microgram Amounts of Hydroquinone, Pyrogallol and Resorcinol

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Keywords: *Hydroquinone determination*; *pyrogallol determination*; *resorcinol determination*; *spectrophotometric microdetermination*; *sodium carbonate*

Advances in polyhydroxyphenol chemistry are a result of the development of new experimental techniques, especially methods for detection and determination of these compounds. Small-scale procedures are valuable for establishing the concentration and the constitution of polyhydroxyphenols in plant products.

Various spectrophotometric and colorimetric methods[1–5] have been reported for the small-scale determination of polyhydroxyphenols. A spectrophotometric method[6] has been developed for the determination of catechol, resorcinol and phloroglucinol with the use of potassium iodate in dilute nitric acid.

In this investigation hydroquinone, pyrogallol and resorcinol were determined spectrophotometrically, after heating with sodium carbonate solution in a boiling water-bath for about 15 min. The method is convenient and precise.

Experimental

Reagents

All reagents were of AnalaR grade.

Sodium carbonate solution, 0.5% *m/V*.

Sample solution. Stock solutions of hydroquinone, pyrogallol and resorcinol were prepared by dissolving accurately weighed amounts of the samples in distilled water in 100-ml calibrated flasks. Aliquots of these solutions were used to give a range of 0.5–250 μg.

Apparatus

Absorbance measurements were carried out with a Beckman, Model DU, spectrophotometer using 1-cm matched silica cells.

Preparation of Calibration Graph

Aliquots of solution containing 5–250 μg of the polyhydroxylphenol were transferred into different stoppered test-tubes and 5 ml of 0.5% sodium carbonate solution were added to each. The tubes and contents were thoroughly shaken and placed in a boiling water-bath for about 15 min. The reaction mixture in each tube was then cooled and transferred quantitatively into a 25-ml calibrated flask and diluted to the mark with water. The absorbance of hydroquinone, pyrogallol and resorcinol solutions were measured at 320, 340 and 440 nm, respectively, and plotted against concentration of the phenol.

Results and Discussion

The absorbance is proportional to the concentration over the range 5–100 μg of hydroquinone, 5–50 μg of pyrogallol and 20–200 μg of resorcinol. The Beer - Lambert law is not obeyed outside these ranges although colours are produced at a lower concentration of the samples.

Determinations of the concentrations of unknown samples of hydroquinone, pyrogallol and resorcinol were carried out by measuring the absorbance and reading off the concentration from a calibration graph (Fig. 1). Results obtained with different concentrations of the phenols are shown in Table I. The determination of other mono-, di- and trihydroxyphenols has not been possible with this method. The determination of hydroquinone, pyrogallol or resorcinol by this method was not possible if more than one of these compounds was present in the same solution. The presence of carbohydrates (glucose, galactose, etc.) was the only interference investigated. In the presence of these compounds the colour developed was not constant.

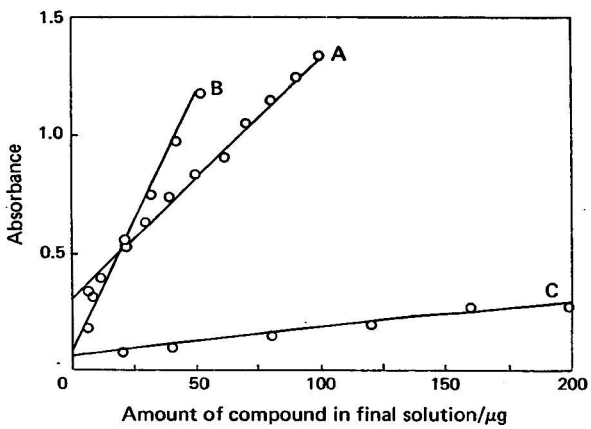

Fig. 1. Graph of absorbance *versus* amount of compound in final solution. A, Hydroquinone; B, pyrogallol; C, resorcinol. For wavelengths used see Table I.

It is thought that the development of a yellow colour on heating the sample with sodium carbonate is caused by the oxidation of the polyhydroxyphenols to the corresponding quinones. This opinion is supported by examination of the ultraviolet spectrum of the oxidation product from hydroquinone, which is found to be the same as that of *p*-benzoquinone.

The effect of oxygen was investigated. On passing a slow stream of oxygen through the solution, while heating in the water-bath, it was found there was no effect on either the time required for the development of the colour or the absorbance. The sensitivity and the range over which the Beer - Lambert law was obeyed was also unaffected. However, under oxygen-free conditions it was observed that the colour did not develop even after heating the reaction mixture for a long period in a boiling water-bath. Hence, the atmospheric oxygen is sufficient for the development of the colour under the proposed reaction conditions. Moreover, without the use of sodium carbonate the atmospheric oxygen alone does not give a constant colour within the described reaction time.

TABLE I

DETERMINATION OF HYDROQUINONE, PYROGALLOL AND RESORCINOL WITH SODIUM CARBONATE SOLUTION

Compound	Wavelength/ nm	Range of validity of Beer's law/ μg	Amount taken/ μg	Absorbance	Standard deviation
Hydroquinone	320	5–100	5	0.360 0.360 0.365	0.55
			40	0.720 0.730 0.730	0.97
			80	1.140 1.140 1.190	1.14
Pyrogallol	340	5–50	7	0.245 0.240 0.240	0.83
			28	0.720 0.730 0.720	0.41
			49	1.20 1.20 1.25	1.66
Resorcinol	440	20–200	30	0.105 0.105 0.110	1.90
			120	0.205 0.215 0.205	1.46
			180	0.280 0.280 0.275	1.09

The use of other alkaline reagents such as sodium hydrogen carbonate, ammonia solution and sodium hydroxide gave similar colours under the described reaction conditions. With the use of sodium hydrogen carbonate the sensitivity of the method is much lower than that with sodium carbonate. The colour developed with ammonia and sodium hydroxide is not constant even after 30 min and the absorbance continues to increase with increase in reaction time.

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Simple Procedure for the Determination of Total Carbon and its Radioactivity in Soils and Plant Materials

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Keywords: *Soil organic carbon determination; plant carbon determination; ^{14}C radioactivity measurement; chromic acid digestion*

Carbon in soil and plant materials can be determined by wet- and dry-combustion methods.[1,2] In both instances, soil and plant carbon is converted into carbon dioxide, absorbed in alkali and determined either by titration against a standard acid or by weighing. These methods involve large apparatus, are expensive and time consuming, and therefore cannot be adapted to the routine analysis of a large number of samples. This paper describes a simple procedure for the simultaneous determination of total carbon and its radioactivity.

Experimental

A wet-combustion method modified from that developed for the measurement of the radioactivity of leaves treated with carbon-14[3,4] was used. The digestion apparatus consisted of a McCartney bottle (i.d. 22 mm, capacity 28 cm^3) with an aluminium screw-cap fitted with a 2.5 mm thick neoprene seal, and a test-tube (i.d. 13 mm, capacity 8 cm^3). The digestion mixture, modified from that of van Slyke and Folch,[5] was prepared by dissolving 25 g of chromium trioxide in 100 cm^3 of concentrated sulphuric acid - orthophosphoric acid (2 + 1). This mixture was heated to 145–150 °C, then cooled and tightly stoppered to prevent the absorption of moisture from the atmosphere.

A suitable amount of soil (particle size less than 0.15 mm) or plant material, containing less than 10 mg of carbon, was weighed into a McCartney bottle and 5 cm^3 of chromic acid digestion mixture were added rapidly. With the aid of forceps the test-tube, containing 5.0 cm^3 of 0.4 M sodium hydroxide solution, was lowered into the bottle, which was immediately stoppered tightly. Appropriate blanks were prepared simultaneously. The bottles were autoclaved at 121 °C and approximately 105 kPa for 1 h, then left overnight at room temperature. The test-tube was removed from the bottle, its outside washed free of acid and the sodium hydroxide solution was transferred into a graduated tube (capacity 36 cm^3, length 150 mm) fitted with a C19/17 Quickfit or similar stopper. The volume was made up to 10.0 cm^3 with carbon dioxide free distilled water and the tube was stoppered.

For the purpose of measuring radioactivity when the soil or plant samples were labelled with carbon-14, 1.0 cm^3 of the sodium hydroxide solution from the stoppered tube was transferred into a 20-cm^3 glass scintillation vial. After adding 10 cm^3 of scintillation solution [a mixture of toluene (2 volumes), containing 5 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) per litre, and Triton X-100 (1 volume)[6]], the vial was capped tightly and shaken. A stable, clear, homogeneous emulsion was formed at room temperature and, after storage overnight in the dark, the sides of the vial were wiped to remove any smudges or radioactive contaminants and counting was carried

out in an ambient-temperature liquid scintillation counter. The counting efficiency, calculated by spiking the mixture with [^{14}C]hexadecane of known specific activity, was found to be 74%. The radioactivity measured was corrected for the background and efficiency and the total and specific activity of the sample were calculated.

For the determination of the total carbon in the samples, 1 cm^3 of saturated barium chloride solution and 0.05 cm^3 of 1% phenolphthalein solution (prepared in ethanol) were added to the remaining 9.0 cm^3 of the sodium hydroxide solution in the tube (10.0 cm^3 if no radioactivity measurements were required). The mixture was titrated against 0.10 M hydrochloric acid until the colour of the solution changed from red to colourless. By using a magnetic stirrer and titrating under reflected light, the end-point in the titration was reproducible. Alternatively, the sodium hydroxide solution can be titrated to pH 8.3 using an automatic titrator or by the procedure suggested by Bundy and Bremner.[7] From the volume of 0.10 M hydrochloric acid used for the titration of the sample and the blank, the amount of organic carbon in the sample can be calculated.

1 cm^3 of 0.10 M hydrochloric acid $\equiv$ 0.60 mg of carbon

Results and Discussion

The chromic acid digestion mixture oxidised all of the carbon in glucose, benzoic acid (benzenecarboxylic acid), hydroquinone (1,4-dihydroxybenzene) and adenine (6-aminopurine) (Table I). There was no significant effect on the oxidation of the carbon in these compounds or soil and plant samples if the proportions of concentrated sulphuric acid and orthophosphoric acid in the acid mixture were changed from 2 + 1 to 3 + 2. However, if the amount of chromium trioxide was decreased below 20 g per 100 cm^3 of the acid mixture, adenine was not completely oxidised.

TABLE I

CARBON CONTENTS OF GLUCOSE, BENZOIC ACID, HYDROQUINONE AND ADENINE

Sample	Amount taken (dry mass)/mg	Carbon content determined: mg	Carbon content determined: Percentage of theoretical value
Glucose	20.0	7.90 ± 0.02	98.8
Benzoic acid	15.0	10.22 ± 0.05	99.0
Hydroquinone	15.5	9.68 ± 0.03	98.7
Adenine	20.0	8.82 ± 0.03	99.3

The operation from the addition of the chromic acid digestion mixture to the closing of the McCartney bottle prior to autoclaving should not take more than 10 s, during which period the loss of carbon even from glucose did not exceed 2%. The loss of carbon from organic compounds and soil and plant materials was reduced and the period of operation was extended to 20 s when the chromic acid digestion mixture was cooled to 4 °C, which reduced the rate of reaction in comparison with that at room temperature (20 °C).

TABLE II

EFFECT ON RECOVERY OF CARBON OF THE AMOUNT OF SOIL AND PLANT MATERIALS USED

Sample	Amount (dry mass)/mg	Carbon content: mg	Carbon content: %	Percentage of the maximum carbon content	Least-square difference (P = 0.05), %
Soil	250	0.985	0.394	96.5	
	500	2.040	0.408	100.0	
	750	3.045	0.406	99.5	2.5
	1000	4.050	0.398	97.5	
	1500	5.820	0.388	95.1	
	2000	6.921	0.346	84.8	
Plant material ..	10	2.882	38.8	99.2	
	15	5.835	38.9	99.5	1.5
	20	7.820	39.1	100.0	
	25	9.652	38.6	98.7	

Studies on the effect of the size of the soil samples showed that the carbon recovery was low when the carbon content of the sample did not exceed 1 mg or the amount of soil taken exceeded 1 g (Table II). No significant effect on the amount of plant material per sample was observed. However, the amount of carbon per sample preferably should not exceed 10 mg.

When the soil was mixed with carbon-14 labelled plant material, the recoveries of total carbon and carbon-14 activity were complete (Table III). Hence both total carbon and its radioactivity in soil and plant samples can be measured simultaneously.

TABLE III

RECOVERY OF CARBON FROM SOIL MIXED WITH ^{14}C-LABELLED PLANT MATERIAL

Sample	Carbon content, %	^{14}C activity*		Recovery of carbon	
		Total activity/ Bq	Specific activity/ Bq per mg C	Total C, %	^{14}C activity, %
Soil (1 g)	0.406 ± 0.012	—	—	—	—
Plant material (20 mg)	39.10 ± 0.77	215 ± 2	27.5 ± 0.3	—	—
Soil + plant material†	0.446 ± 0.012	10.7 ± 0.2	2.4 ± 0.05	101.8 ± 2.0	99.5 ± 1.9

* Each carbon-14 activity measurement was repeated at least four times and counted to at least 10^4 counts per sample with a counting efficiency of 74%.

† 500 mg of plant material were mixed with 500 g of air-dried soils; 1 g of sample was taken for determination of carbon.

In the wet-digestion methods of Walkley - Black[8] and Schollenberger[9] for the determination of organic carbon in soil, iron(II) and oxides of manganese interfere.[1] The presence of the former leads to over-estimates and of the latter to under-estimates of the organic carbon content of soils.[10] These interferences are eliminated in the present procedure because the carbon oxidised from the sample is measured directly.

Organic carbon can be determined in calcareous soils after the carbonates have been removed by treatment with sulphuric acid - iron(II) sulphate solution[1] and the samples oven dried at 105 °C. However, as in all other wet-combustion methods, chloride ions interfere.[1] Interference from small amounts of chloride ions (up to 4 mg of Cl^- as KCl or NaCl) was reduced by adding 2.5% of mercury(II) oxide or silver(I) sulphate to the acid digestion mixture.

When amounts of organic carbon in 17 soils varying from 0.30 to 14.42% were determined by the present procedure and by the Walkley - Black method,[1] the results were closely correlated [equation (1)]. The regression equation

$$y = 1.21x - 0.03 \quad \ldots \quad (1)$$

$r = 0.997$, $P < 0.001$, where y and x are the percentages of organic carbon in the soils determined by the present procedure and by the Walkley - Black method, respectively, showed that the present procedure determined 21% more organic carbon than the Walkley - Black method. Assuming that the present method measured the total organic carbon in these soils, the recovery of organic carbon by the Walkley - Black method was 100/1.21 = 83%, which falls within the range of values reported by Allison.[11] However, this procedure needs to be calibrated with the dry-combustion procedure[1] for the determination of total carbon in soils.

Conclusion

The present method can be used for the routine analysis of soil and plant samples for carbon and carbon-14 radioactivity measurements. For the determination of organic carbon in samples that contain carbonates, pre-treatment of the samples with sulphuric acid - iron(II) sulphate solution is required. Iron(II) and oxides of manganese do not interfere.

The interference by small amounts of chlorides (up to 4 mg of Cl^- per sample) can be reduced by adding mercury(II) oxide or silver(I) sulphate to the digestion mixture, although samples that contain large amounts of chloride ions should be pre-treated in order to remove chlorides.[12]

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Determination of Lead in Columbite Concentrates by Atomic-absorption Spectrometry After Sulphide Separation

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Keywords: *Lead determination*; *columbite concentrates*; *atomic-absorption spectrometry*; *sulphide separation*

Spectrographic,[1–4] chromatographic[5] and polarographic[6] methods have been used to determine lead in niobium and tantalum metal and their oxides. In the determination of lead in columbite concentrates by atomic-absorption spectrometry, the problem arises that the ore is not completely decomposed by common acids. Further, niobium and tantalum compounds are easily hydrolysed, especially in the presence of mineral acids, forming white precipitates of hydrated earth acids that coprecipitate lead, and this often leads to low results. It was found that if potassium pyrosulphate[7] was used to decompose the ore and the melt leached with tartaric acid, the dissolved lead could be precipitated as sulphide, using copper sulphide as collector. After filtering the mixture of lead sulphate and lead sulphide and dissolving it in concentrated hydrochloric and nitric acids, lead was determined by atomic-absorption spectrometry.

Experimental

Instrumental

A Varian Techtron AA4 instrument was used with the following settings: wavelength, 217.0 nm; lamp current, 5 mA; slit width, 200 μm; and air - acetylene flame (air pressure, 15 p.s.i.g.; acetylene pressure, setting 3 on the flow meter). A standard ASL hollow-cathode lamp was used as the line source.

Reagents

All reagents were of analytical-reagent grade.

Potassium pyrosulphate.

Standard lead solutions, 1000 and 100 μg ml⁻¹. Prepare the 1000 μg ml⁻¹ solution by dissolving 0.3197 g of anhydrous lead nitrate in 200 ml of distilled water. Prepare the 100 μg ml⁻¹ solution by dilution.

Copper solution. Dissolve approximately 0.2 g of metallic copper in a mixture of about 6 drops of concentrated nitric acid and 4 ml of concentrated hydrochloric acid. Dilute to 200 ml with distilled water.

Tartaric acid solution, 10% m/V. Dissolve 100 g of L(+)-tartaric acid in distilled water, filter the solution into a 1-l calibrated flask and dilute to volume with distilled water.

Procedure

Weigh accurately 0.25 g of columbite sample (less than 200 mesh) into a silica crucible. Add about 8–10 g of potassium pyrosulphate and fuse the mixture for about 45 min in a low to medium Bunsen-burner flame. Cool the melt and extract it into 50 ml of 10% *m/V* tartaric acid solution by boiling with continuous swirling until the melt is completely dissolved. Dilute the resulting solution to 200 ml with distilled water, add 5 ml of copper solution and pass hydrogen sulphide through the solution for about 15 min. Add some ashless paper pulp, filter through Whatman No. 40 (11-cm) filter-paper and wash with water saturated with hydrogen sulphide. Transfer the residue into a porcelain crucible and ignite it at low heat until all of the carbon has been burnt away. Dissolve the residue by warming it gently in the porcelain crucible with 5–10 drops of concentrated hydrochloric acid followed by 3–4 drops of concentrated nitric acid (if the sample contains bismuth, use 3 ml of concentrated hydrochloric acid in order to prevent hydrolysis of the bismuth). Filter the solution through Whatman No. 40 filter-paper into a 100-ml calibrated flask, wash with distilled water and dilute to volume with distilled water. Measure the absorbance at 217.0 nm against a blank prepared in the same manner as the sample.

Preparation of Calibration Graph

Transfer by pipette 1–10-ml aliquots of standard 100 μg ml⁻¹ lead solution into a series of silica crucibles and evaporate the solutions to dryness on an asbestos sheet on a hot-plate. Fuse the residue with 8–10 g of potassium pyrosulphate and then follow the method described under Procedure.

Interferences

Elements likely to be present in columbite samples were investigated for their interference effects. Known amounts of diverse ions were added to 500 μg of lead. Errors corresponding to less than about twice the standard deviation found for the determination of lead in pure solutions were considered to indicate the absence of interference effects.

Results and Discussion

The lead content in the samples examined had not been determined previously. The additions method was used in the analyses. Two portions of columbite sample, each of 0.25 g, were weighed accurately into two silica crucibles, one of which contained a known amount of standard lead that had previously been transferred by pipetting in a standard lead solution and evaporating to dryness in the silica crucible on an asbestos sheet on a hot-plate. The sample solution was prepared according to the method described under Procedure. As shown in Table I, the recovery of lead was satisfactory.

No interference was observed in the presence of 140 mg of niobium, 50 mg of tantalum, zinc, tin(II), calcium, manganese(II), tungsten, cadmium, copper(II) or iron(III), 30 mg of titanium(IV), 5 mg of cobalt, nickel or chromium(VI), or 2 mg of strontium, bismuth(III), arsenic(III) or mercury(II). However, 2 mg of antimony(III) or barium could not be tolerated.

TABLE I

RECOVERY AND DETERMINATION OF LEAD IN COLUMBITE SAMPLES

All results are averages of duplicate determinations.

Sample reference	Lead found in sample/ μg	Lead added to sample/ μg	Total lead found/ μg	Lead recovered/ μg	Error, %
682(1)232	325	—	—	—	—
	325	100	430	105	+5
	325	200	535	210	+5
	325	500	830	330	+1
682(1)L108	425	—	—	—	—
	425	200	625	200	0
682(1)195	260	—	—	—	—
	260	200	450	190	−5
682(1)L85	525	—	—	—	—
	525	150	665	140	−6.7
682(1)L91	498	—	—	—	—
	498	150	655	157	+4.7

The atomic-absorption spectrometric method described is simple and rapid. By carrying out a preliminary sulphide separation niobium and tantalum are removed from the matrix, together with relatively large amounts of potassium pyrosulphate and tartaric acid.

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Direct Determination of Calcium, Magnesium and Zinc in Lubricating Oils and Additives by Atomic-absorption Spectrometry Using a Mixed Solvent System

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Keywords: *Lubricating oil analysis*; *calcium determination*; *magnesium determination*; *zinc determination*; *atomic-absorption spectrometry*

Continued interest in the testing of lubricants has centred on the various additives used and the determination of their metallic constituents. There are some spectroscopic methods for the determination of calcium, magnesium and zinc salts in oils in which an organic solvent is used as a medium for spraying directly into the flame. Alternatively, preliminary ashing to remove organic matter followed by dissolution of the residue in a mineral acid may be

more suitable, but this takes a longer time. The use of organic solvents was studied in this work and the results are compared with those obtained by established methods.

Since the work of Allan[1,2] and Robinson,[3] organic solvents have been used to increase the sensitivity of atomic-absorption determinations. In addition, the use of organic solutions offers a rapid means of analysis in comparison with conventional atomic-absorption spectrometric techniques. The most widely used simple solvent is 4-methylpentan-2-one but xylene, cyclohexane, 2,2,4-trimethylpentane and others have also been applied.

The use of mixed solvent systems as homogeneous solvents has become more widely used recently. Inorganic salts can be used in these solvent systems as standards instead of the much less readily available, more expensive and less stable organic metal salt solutions. Cyclohexanone - butan-1-ol - ethanol - concentrated hydrochloric acid - water (10 + 6 + 4 + 1 + 1)[4,5] and 95 parts of 2-methylpropan-2-ol - toluene (3 + 2) plus 5 parts of water[6] as both oil- and water-compatible mixed solvent systems and aqueous inorganic salt solutions as calibration solutions were applied by Holding and co-workers.

Inorganic salt standards dissolved in dimethyl sulphoxide and a mixed solvent (toluene - glacial acetic acid, 7 + 3 *V/V*) were used by Guttenberger and Harold[7] for the determination of different metals using an air - acetylene flame.

The atomic-absorption determination of calcium, magnesium and zinc in fresh and used lubricating oils and additives in an air - acetylene flame using inorganic salt standards and a mixed solvent system is described in this paper.

Experimental

Holding and co-workers' mixed solvent systems[4–6] were tried, but with some additives stable solutions were not obtained. Some other mixed solvents, such as toluene or benzene - propan-2-ol, methanol or ethanol - water (1 + 3 + 1) and aqueous inorganic salts were also tried as calibration solutions and proved to be good solvents. However, the results were sometimes erratic, because the standards and samples had dissimilar structures. Even the addition of strong mineral acids, *e.g.*, concentrated hydrochloric acid, could not eliminate the system errors of 20–40% relative.

A clear-burning flame was obtained with Guttenberger and Harold's mixed solvent system (toluene - glacial acetic acid, 7 + 3 *V/V*)[7] if a lean gas mixture containing very small amounts of fuel (close to the point of extinction of the flame) was used.

Inorganic salt standards were dissolved in methanol or ethanol, which are more readily available than dimethyl sulphoxide (used by Guttenberger and Harold[7]). The following mixed solvents were tried: toluene or benzene - propan-2-ol, methanol or ethanol (1 + 4 or 1 + 9). These solvents gave a clear-burning flame and seemed suitable for the determination of calcium, magnesium and zinc. However, systematic errors of 20–40% relative were noticed. Toluene - glacial acetic acid (1 + 4) had excellent burning characteristics, a low spectral background and good solvent properties for lubricating oils and additives. The addition of glacial acetic acid to the solvent resulted in the elimination of the systematic errors.

Apparatus

An MOM, Model 190A, atomic-absorption spectrophotometer with calcium, magnesium and zinc hollow-cathode lamps was used. The determinations were carried out in an air - acetylene flame under the conditions given in Table I.

Stock Solutions

Dissolve appropriate amounts of calcium chloride ($CaCl_2.2H_2O$), zinc acetate [$Zn(CH_3COO)_2.2H_2O$] and magnesium acetate [$Mg(CH_3COO)_2.2H_2O$] in ethanol to give metal concentrations of 500, 500 and 100 μg ml^{-1}, respectively, and standardise the solutions by complexometric titration.

Standard Solutions

Add 0.0, 0.1, 0.2, 0.3 and 0.4 ml of the 500 μg ml^{-1} ethanolic zinc stock solution, 0.0, 0.2, 0.4, . . . and 1.2 ml of the 500 μg ml^{-1} ethanolic calcium stock solution and 0.0, 0.2, 0.4,

TABLE I

OPERATING CONDITIONS

Condition	Calcium	Zinc	Magnesium
Wavelength/nm	422.7	213.8	285.2
Lamp current/mA	4	4	2.5
Slit width/mm	0.04	0.04	0.03
Scale expansion	5×	1×	1×
Burner height/mm	7	5	5

0.6 and 0.8 ml of the 100 μg ml^{-1} ethanolic magnesium stock solution to 100-ml calibrated flasks. Add 20 ml of toluene to each flask and dilute to volume with glacial acetic acid.

Calibration solutions were prepared for the concentration ranges 0.1–2 μg ml^{-1} of zinc, 0.25–6 μg ml^{-1} of calcium and 0.05–0.8 μg ml^{-1} of magnesium. Tests were made of the effect of potassium acetate as an ionisation suppressant on the calibration solutions. Linear calibration graphs were obtained, as shown in Fig. 1.

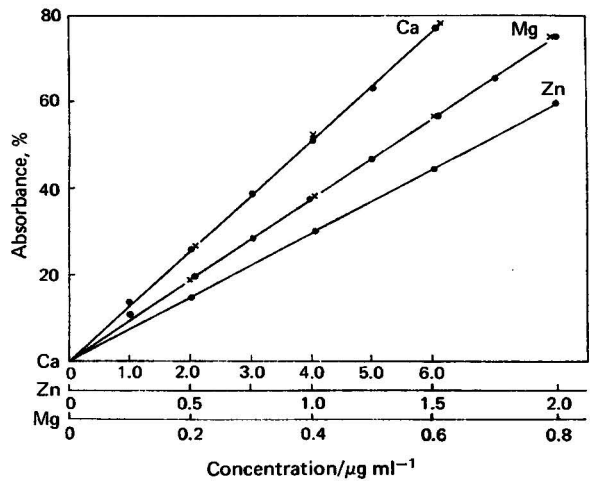

Fig. 1. Calibration graphs: ×, with alkali metal salt; ●, without alkali metal salt.

Sample Solutions

Weigh into a 50-ml beaker an amount of lubricating oil or additives containing approximately 0.3 mg of calcium, 0.1 mg of zinc and 0.05 mg of magnesium. Add 20 ml of toluene and stir the solution. Transfer the solution into a 100-ml calibrated flask with glacial acetic acid and dilute to volume with glacial acetic acid.

Results and Discussion

It is evident that there are certain factors (solvent, inter-element effects, etc.) that can be subject to severe interference, the magnitude of which is dependent on the solvent used. When toluene and glacial acetic acid mixed solvents were used the interferences that result in differences between standards and samples were removed.

Potassium acetate was added as an ionisation suppressant to the mixed solvent. However, the effects of the ionisation suppressant on sensitivity were negligible.

No inter-element effects were found in the determination of calcium and magnesium. Similarly to organic solutions, organophosphates had no effect on the determination of these elements when mixed solvents were used, as has been established for organic solvents by Holding and Matthews.[5]

According to our experience, the addition of ethanol (maximum concentration 1.5% *V*/*V*) to the mixed solvent with the standards caused no interference.

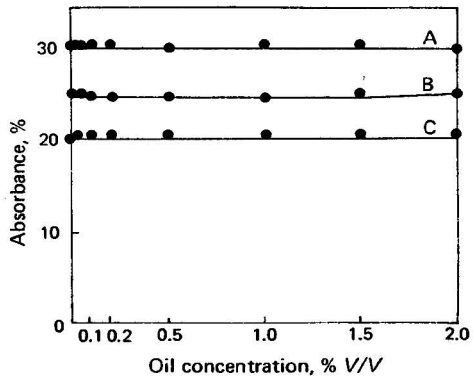

Fig. 2. Effect of oil on absorbance: A, 1.0 μg ml^{-1} of zinc; B, 2.0 μg ml^{-1} of calcium; C, 0.2 μg ml^{-1} of magnesium.

The effects of increasing amounts of oil in the mixed solvent at a constant metal concentration on the absorbance are shown in Fig. 2. The results indicate that the addition of oil to the mixed solvent system during calibration is not necessary.

Different types of lubricating oils and additives were examined in order to compare the results of the determination of metals by three different techniques: direct determination by atomic-absorption spectrometry, atomic-absorption spectrometric determination after ashing and determination by chemical methods. The results of the comparative determinations are given in Table II.

TABLE II

COMPARISON OF RESULTS OF ANALYSES FOR CALCIUM, MAGNESIUM AND ZINC IN LUBRICATING OILS AND ADDITIVES

Element	Sample number	Other elements of interest present	Found, % *m/m*		
			Established methods*	AAS on aqueous solution after ashing	AAS using organic mixed solvent system
Calcium	1	—	12.00 (t)	11.90	11.95
	2	—	15.20 (t, g)	15.05	15.20
	3	—	4.70 (t)	4.65	4.7
	4	P	5.90 (t)	5.85	5.85
	5	P	2.70 (t)	2.80	2.75
	6	P	3.60 (g)	—	3.65
	7	Mg	10.60 (t)	10.60	10.65
	8	Mg	6.75 (t)	6.70	6.72
	9	Mg, Zn, P	1.41 (l, t)	1.40	1.40
Zinc	10	P	5.30 (t)	—	5.40
	11	Ca, Mg, P	1.65 (l, t)	—	1.66
	12	P	2.70 (t)	2.65	2.70
	13	P	7.80 (t, p)	7.85	7.85
	14	P, Ba	0.075 (t)	—	0.075
	15	P, Ba	0.056 (p)	0.056	0.056
	16	P, Ba	0.061 (t)	0.061	0.060
	17	P, Ba, unknown	0.039 (t)	0.039	0.040
	18	P, Ba, unknown	0.031 (t)	—	0.030
Magnesium ..	19	Ca, Zn, P	0.86 (l, t)	0.87	0.87
	20	Ca	0.50 (t)	0.51	0.49
	21	Ca	0.25 (t)	0.26	0.25
	22	Ca	—	0.04	0.04

* t = complexometric titration after ashing; g = gravimetric analysis after ashing; p = polarographic analysis; l = literature value.

The relative standard deviations calculated for ten replicate measurements were 1.5% for zinc at the 1.0 μg ml^{-1} level, 2.0% for calcium at the 3.0 μg ml^{-1} level and 1.5% for magnesium at the 0.5 μg ml^{-1} level.

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Spectrophotometric Determination of Iron by Synergistic Extraction with Isonitrosobenzoylacetone and Pyridine

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Keywords: Synergistic iron extraction; iron determination; spectrophotometry; alloy analysis; isonitrosobenzoylacetone

Isonitrosobenzoylacetone (H-INBA) has been used for the extractive spectrophotometric determination of palladium and ruthenium.[1] In this paper we describe a simple method for the synergistic extraction of iron(II) from an aqueous solution at pH 4 by using H-INBA in combination with pyridine. The extracted blue complex is suitable for the spectrophotometric determination of iron at 590 nm.

Experimental

Apparatus

Absorbance measurements were made on a Zeiss, Model 738636, spectrophotometer, using 1-cm silica cells and pH measurements were made on a Philips pH meter (precision type).

Reagents

Standard iron(II) solution, 5 mg ml^{-1}. Prepared by dissolving 3.51 g of analytical-reagent grade ammonium iron(II) sulphate in 100 ml of distilled water containing 1% of sulphuric acid and standardised titrimetrically.[2] Solutions of lower concentration (50 μg ml^{-1}) were prepared by appropriate dilutions of the stock solution.

Extraction solution. Equal volumes of 0.8 M pyridine in benzene and 0.25% *m/V* H-INBA in benzene.

Sodium thiosulphate solution, 2.5% *m/V*.

Sodium hydroxide solution, 1 M.

Hydrochloric acid, 1 M.

All other reagents were of analytical-reagent grade.

General Procedure

To an aliquot of solution containing 50 μg of iron(II) add 1 ml of 2.5% aqueous sodium thiosulphate solution in order to prevent atmospheric oxidation of the iron and adjust the pH of the solution to 4, in a total volume of 25 ml, with 1 M hydrochloric acid or 1 M sodium

hydroxide solution. Transfer the solution into a separating funnel and shake with 10 ml of extraction solution (5 ml each of 0.25% H-INBA and 0.8 M pyridine solutions in benzene) for 2 min. After separation of the phases measure the absorbance, at 590 nm, of the organic layer against a reagent blank prepared in the same manner and calculate the iron content from a calibration graph obtained by following the above procedure. The calibration graph gave a straight line over the range 20–120 μg of iron per 10 ml of organic phase, indicating that Beer's law is obeyed over this range.

Results and Discussion

The absorption spectrum of the iron(II) - INBA complex extracted with pyridine at pH 4.0 is shown in Fig. 1. In the absence of pyridine the complex was not extracted. Addition of pyridine caused quantitative and rapid extraction of iron. Pyridine thus exerts a synergistic effect on the extraction of the iron(II) - INBA complex.

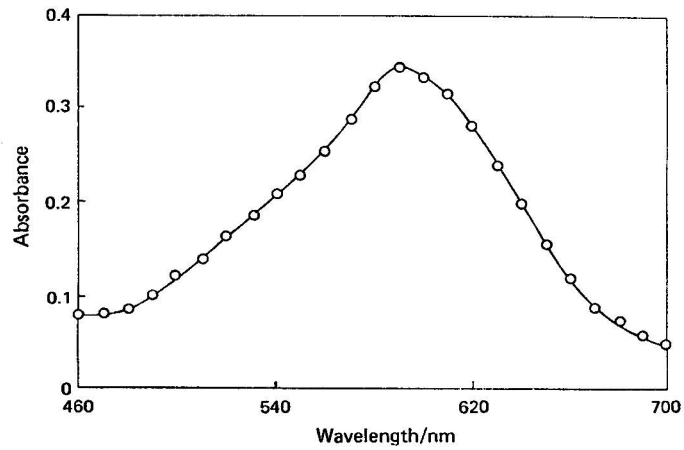

Fig. 1. Absorption spectrum of complex from 50 μg of iron(II), 5 ml of 0.25% H-INBA in benzene and 5 ml of 0.8 M pyridine in benzene, measured against reagent blank.

The extraction of the iron(II) - INBA - C_6H_5N complex commences at pH 3, becomes quantitative between pH 3.8 and 4.4 and then gradually decreases (Table I). The optimum pH range is therefore between 3.8 and 4.4 In all later work the pH of the aqueous solution was adjusted to 4 in order to ensure quantitative extraction of the complex. The complex is stable for 36 h. The Sandell's sensitivity of the colour reaction is 0.014 μg cm^{-2} (at 590 nm) with a molar extinction coefficient of 3.79 $\times$ 10^3 l mol^{-1} cm^{-1}.

TABLE I

DISTRIBUTION RATIO OF IRON(II) - INBA - PYRIDINE COMPLEX BETWEEN BENZENE AND AQUEOUS SOLUTION AS A FUNCTION OF pH

Fifty micrograms of Fe(II) extracted into 5 ml of 0.25% H-INBA and 5 ml of 0.8 M pyridine in benzene.

pH	Amount of iron extracted into benzene, %	Distribution ratio (D)
3	19.86	0.61
3.4	63.23	4.29
3.6	83.81	12.94
3.8–4.4	100.00	∞
4.6	91.18	25.84
4.8	88.22	18.73
5	85.29	14.50
6	72.06	6.44
7	59.24	3.63

The concentrations of H-INBA and pyridine were varied from 0.05 to 0.25% and from 0.1 to 1 M, respectively. It was found that a single extraction with 5 ml each of 0.25% H-INBA and 0.8 M pyridine is sufficient for quantitative extraction of 20–120 μg of iron. The graph of log *D* (distribution coefficient) *versus* log *C* (H-INBA concentration) at constant pyridine concentration has a slope of 1.73. Similarly, the graph of log *D versus* log *C* (pyridine concentration) at constant H-INBA has a slope of 2.3. Hence the probable composition of the extractable species is $Fe(INBA)_2.2C_6H_5N$. This composition is analogous to that formed in other synergistic extractions.[3–7] The extraction reaction, therefore, can be expressed as

$$Fe(H_2O)_6^{2+} + 2H\text{-}INBA \rightleftharpoons Fe(INBA)_2.2H_2O + 2H^+ + 4H_2O$$

$$Fe(INBA)_2.2H_2O + 2C_6H_5N \rightleftharpoons Fe(INBA)_2.2C_6H_5N + 2H_2O$$

Solvents such as toluene and xylene can also be used for the extraction of iron. Similarly, other bases such as β-picoline can be used as a substitute for pyridine.

The precision of the method was checked by measuring the absorbance at 590 nm of six samples each containing 20, 40, 50 and 60 μg of iron. The standard deviation of the mean absorbance was found to be 0.0031 for 50 μg of iron. The coefficient of variation was 0.93%. The results in Table II show that the precision of the method is satisfactory.

TABLE II

PRECISION OF THE METHOD

Amount of iron/μg	Mean absorbance (6 determinations)	Standard deviation	Coefficient of variation, %
20	0.135	0.0015	1.17
40	0.27	0.0015	0.58
50	0.34	0.0031	0.93
60	0.405	0.0035	0.87

Separation and Determination of Iron in Synthetic Mixtures

Iron is found in association with other metals such as chromium, molybdenum, tungsten, manganese, nickel, copper, lead, cobalt, zinc, vanadium, aluminium and titanium in aluminium-, cobalt-, nickel-, titanum- and zinc-based samples. The proposed method for the spectrophotometric determination of iron in synthetic mixtures is made more specific by selective extraction of iron(III) with 4-methylpent-3-en-2-one (mesityl oxide)[8] from a 5 M hydrochloric acid solution.

To iron (5 mg), and the cations mentioned above, in a 10-ml flask is added an adequate amount of concentrated hydrochloric acid to give the desired acid concentration (5 M) in a total volume of 10 ml. The solution is then extracted for 2 min with 10 ml of pure mesityl oxide. This transfers only iron into the organic phase and other cations remain in the aqueous phase. After the separation of the two phases, iron is removed from the organic phase by extraction with two 20-ml portions of water, and these are combined and diluted

TABLE III

DETERMINATION OF IRON IN SYNTHETIC MIXTURES

All results are the means of 3 determinations.

Composition of the mixture/mg	Amount of iron recovered, %	Relative error, %
Fe, 5; Mn, 0.5; W, 0.5	99.12	0.88
Fe, 5; Cu, 1; Zn, 1; Pb, 0.5	98.80	1.20
Fe, 5; Mn, 0.5; Ni, 1; Cr, 0.5; Cu, 2; Mo, 1	98.8	1.20
Fe, 5; Al, 5; V, 0.5	99.12	0.88
Fe, 5; Al, 1; Cu, 1; Mo, 0.5	99.12	0.88
Fe, 5; Cu, 1; Mn, 0.5; Ti, 1; Zn, 3; Ni, 1; Cr, 0.5	98.8	1.20
Fe, 5; Ni, 1; Cu, 1; Mn, 0.5; Co, 2; Cr, 0.5; V, 0.5	98.57	1.43

to 50 ml. An aliquot of this solution containing 50 μg of iron is taken and iron is spectrophotometrically determined as described under General Procedure. Ions such as uranium, palladium, ruthenium(III), gold(III), EDTA, citrate, tartrate and phosphate also do not show any interference. The results of the analysis of seven synthetic mixtures are shown in Table III. The recovery of iron is greater than 98.9%.

Determination of Iron in Steel, Copper-based Alloy and Aluminium Alloy

Two steel samples (NBS 33 b and c), one brass (NML 41) and one aluminium alloy were dissolved[9] and then the proposed method was applied to the separation and determination of iron. The results obtained are shown in Table IV.

TABLE IV

ANALYSIS OF STANDARD SAMPLES

Sample	Composition, elements other than iron, %	Iron content, %		Relative error, %
		Declared	Found	
33 b*	C, 2.24; Si, 2.0; P, 0.11; S, 0.03; Mn, 0.64; Ni, 2.24; Cr, 0.61; Mo, 0.40	91.7	92.0, 92.0, 91.0	0.10
33 c*	C, 3.31; Si, 1.88; S, 0.06; P, 0.11; Mn, 0.86; Ni, 1.98	91.0	91.2, 91.2, 91.4	0.21
41 Brass†	Pb, 2.35; Zn, 40.65; Cu, 56.9 ..	0.009	0.0088, 0.0086, 0.009	2.22
Aluminium alloy	Ni, 2.12; Mn, 1.7; Cu, 4.0; remainder Al	0.03	0.031, 0.030, 0.028	3.33

* Sample from the National Bureau of Standards.
† Sample from the National Metallurgical Laboratory.

Conclusion

The proposed method for the spectrophotometric determination of iron is simple and rapid. A scheme for the separation of iron from manganese, copper, zinc, lead, nickel, chromium, molybdenum, tungsten, aluminium and vanadium has been reported. The results are accurate and the wide applicability of the method has been demonstrated by the satisfactory analysis of a variety of samples.

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Potentiometric Method for the Rapid Determination of Sulphate in the Presence of Chromium(VI)

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Keywords: Sulphate determination; barium ion-selective electrode; chromium(VI); potentiometry

Control of the ratio of chromium(VI) to sulphate is essential for the effective working of chromium(VI) - sulphuric acid electrochemical systems, such as those used for the colouring of stainless steel or the deposition of chromium, requiring rapid and frequent determinations of sulphate, if the optimum properties of the chromium bath are to be maintained. Widely used methods in industry depend on the principles reported by Willard and Schneidewind[1] and Richards and Parker,[2] although it has long been realised that these time-consuming procedures must be followed exactly if reliable results are to be obtained, as these precipitation procedures are susceptible to both positive and negative errors.

An application of a barium(II) ion-selective electrode is described that permits the rapid determination of sulphate in the presence of chromium(VI). Ascorbic acid is used to reduce chromium(VI) and to complex chromium(III). The sulphate ions are then determined by titrating with barium(II) perchlorate. The end-point is determined by following the course of the titration potentiometrically using one of the recently reported electrodes[3,4] that are selective to barium(II) ions as the indicator electrode, in combination with the saturated calomel electrode.

Experimental

Reagents

All of the reagents were of analytical-reagent grade.

L-*Ascorbic acid.*

Sodium hydroxide solution, 30% *m/V.*

Barium(II) perchlorate solutions, 0.05 *and* 0.02 M *in water - propan-2-ol* (3 + 1).

Propan-2-ol.

Apparatus

Measurements were made with a pH meter with a precision of ± 1.0 mV. It was used with the following cell system: Hg - Hg_2Cl_2, saturated KCl | sample solution | membrane, 10^{-2} M $BaCl_2$, AgCl - Ag.

The barium(II) ion-selective electrode was constructed as described by Jaber *et al.*[3] and was used in combination with the calomel electrode.

Procedure

Take an aliquot of the sample containing not more than 40–50 mg of either chromium(VI) or chromium(III) and not less than 2–3 mg of sulphate ions. Dilute to about 100 cm^3 and add about 0.5 g of ascorbic acid. When this has dissolved, adjust the pH to about 3.5 with 30% *m/V* sodium hydroxide solution and add about 20 cm^3 of propan-2-ol. Titrate the solution potentiometrically with the appropriate barium(II) perchlorate solution, previously standardised against standard sulphuric acid.

If the amount of chromium exceeds 50 mg, remove the excess by adjusting the pH of the aliquot to about 10 with sodium hydroxide solution and swirling for about 2–3 min with the appropriate amount of silver(I) benzoate [1 g of chromium(VI) requires 5 g of silver benzoate]. Filter, wash the precipitate with 2–3 portions, each of 15 cm^3, of water and add the washings and the filtrate to about 50 cm^3 of a strong cation-exchange resin

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(H^+ form) in a beaker and percolate for about 5 min to remove silver and sodium ions. Filter and wash the resin with water into a 150-cm^3 tall-form beaker, add 0.5 g of ascorbic acid and continue as described above.

Results and Discussion

It was observed that the response of the electrode did not follow the Nernst equation, giving a slope of approximately 20 mV instead of 29 mV at 20 °C for a ten-fold change in barium-ion activity. However, preliminary titration of sulphuric acid at a pH of about 3.5 in propan-2-ol - water gave reproducible results ($\pm$0.02 cm^3 of both titrants for 2–60 mg of sulphate ions).

The precision and accuracy of the sulphate determinations deteriorated sharply in the presence of chromium(III). Although several commonly cited reductants were used in order to reduce chromium(VI) to chromium(III) and overcome possible inter-ion interference, the results were always low and erratically irreproducible (Table I). These discrepancies were ascribed to the slow dissociation of a chromium(III) - sulphate complex in a slightly acidic environment, preventing the complete precipitation reaction between sulphate and barium(II) ions.

TABLE I

EFFECT OF CHROMIUM(III) ON THE DETERMINATION OF SULPHATE

Method of reducing chromium(VI) to chromium(III)	Amount of sulphate/mg: Theoretical	Amount of sulphate/mg: Found by potentiometry
Hydrochloric acid - acetic acid - ethanol	7.77	5.25
		3.96
		4.35
Hydrochloric acid - ethanol	7.77	5.47
		6.42
Acetic acid - ethanol	7.77	3.42
		5.91
Ethanol	7.77	3.49
		5.14
Hydrogen peroxide	7.77	6.35
		4.13
Sodium nitrite	7.77	4.95
		3.75

Accurate recovery of sulphate, however, was achieved when L-ascorbic acid was used as a reducing agent (Table II). It is tentatively assumed that after reducing chromium(VI) to chromium(III), identified by the redox titration, the L-ascorbic acid (or its oxidation product, dehydroascorbic acid) complexes with chromium(III) ions and thus prevents any complex formation between Cr^{3+} and SO_4^{2-} ions. This assumption is supported by the work of

TABLE II

REDUCTION AND MASKING OF CHROMIUM WITH ASCORBIC ACID

Amount of chromium/mg	Amount of ascorbic acid/mg	Amount of sulphate/mg: Theoretical	Amount of sulphate/mg: Found by the proposed method*
10	50	2.35	2.35
10	150	2.35	2.35
20	220	2.35	2.35
20	250	2.35	2.35
20	300	2.35	2.35
20	200	6.70	6.70
20	200	7.77†	7.96
40	400	9.40	9.35
40	500	9.40	9.45
50	500	11.75	11.72

* Mean of three measurements.
† Gravimetric measurement.

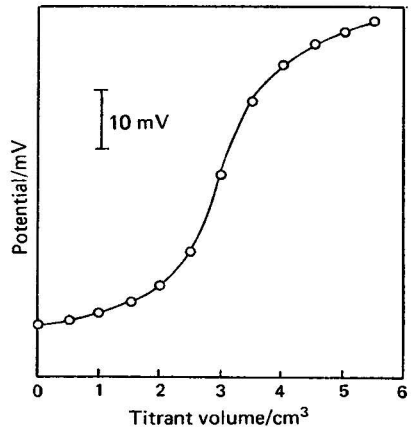

Fig. 1. Potentiometric titration of 6 mg of sulphate in the presence of 40 mg of chromium(VI), with 2×10^{-2} M barium(II) perchlorate.

Přibil and Veselý,[5] who used ascorbic acid to mask chromium(III) in calcium - EDTA titrations. A typical potentiometric titration curve obtained by the described procedure is presented in Fig. 1. Iron and nickel ions and excess of ascorbic acid did not interfere in the determination of sulphate by the proposed potentiometric method (Tables II and III). A sharp decline in the electrode performance was noted when the amount of chromium(VI) and/or chromium(III) was in excess of 50 mg. Elucidation of this observation could not be attempted owing to the absence of chemical data on the chromium - ascorbic acid system and selectivity coefficients of the barium electrode in such a mixed system.

TABLE III

DETERMINATION OF SULPHATE IN THE PRESENCE OF CHROMIUM(VI)

Amount of sulphate/mg			
Theoretical	Found gravimetrically	Found by the proposed method	Amount of other ions present/mg
7.77	8.07	8.03	Iron, 2.24, and ammonium, 0.72
		7.93	
		8.03	
		7.90	
		7.83	
		7.96	
		8.06	
13.84	14.00	13.79	
		13.80	
	43.68	44.13	Nickel, 9.28
		44.67	
	38.10	37.10	Nickel, 12.0
	38.22	37.20	
	37.25*	36.91	
	24.12	24.69	Iron, 4.2, and ammonium, 0.72
	24.03	24.69	
		24.64	

* After recrystallising the preceding precipitate, in which 38.22 mg of sulphate were determined by gravimetry.

Table III records some of the results showing the acceptable precision and accuracy of the proposed procedure, which can be completed in about 10 min compared with the much longer time required for the gravimetric procedure.[6]

The author is indebted to Inco Europe Limited for permission to publish this paper.

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Determination of Thiocyanates by Thermal Decomposition of Silver Thiocyanate

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Keywords: *Thiocyanate determination*; *thermal decomposition of silver thiocyanate*; *halide and thiocyanate determination*

Thiocyanates, in the form of silver thiocyanate, cannot be determined by most methods in the presence of other anions that form insoluble precipitates of silver salts, but they can be determined by thermal analysis as silver thiocyanate in the presence of halogen anions. Alternatively, a thiocyanate and either an iodide, a bromide or a chloride can be determined. The method is relatively simple; the apparatus required is a furnace fitted with temperature control equipment in which a silica vessel containing a porosity 4, sintered-glass crucible and precipitate can be heated.

Thermal Decomposition of Siver Thiocyanate

Experimental

Apparatus

The thermal decomposition studies were carried out using a derivatograph (MON OD 102/1500 °C) with α-alumina as the inert substance. Other operating conditions were: $T_{max.}$ 1000 °C, heating-rate 5 °C min^{-1}, differential thermal analysis (DTA) sensitivity 1/20, thermogravimetric (TG) sensitivity 200 mg, differential thermogravimetric (DTG) sensitivity 1/15 and mass of sample 250 mg.

The X-ray diffraction analysis of the sinters produced by heating silver thiocyanate was carried out with a DRON-1 X-ray analyser using copper radiation and a nickel filter. The intensites of the reflections were measured with a scintillation counter. Diffractograms were recorded with an automatic recorder for 2θ angles from 2 to 70°.

Thermal analysis

Fig. 1 shows thermal curves for silver thiocyanate. Four transformations are represented on the DTA curve, two exothermic ones caused by loss of mass and two endothermic ones. An exothermic transformation follows immediately after the first endothermic peak.

Study of sinters from silver thiocyanate

In order to examine the course of the reactions, sinters from the silver thiocyanate under investigation were prepared under conditions similar to those of the derivatographic determination. Weighed samples of the salt (250 mg) were heated at a rate of 5 °C min^{-1} to the temperatures determined from the thermal analysis curve, *i.e.*, 280 and 600 °C. The loss in mass of each sinter was verified by comparison with the values from the TG curve.

Chemical analysis

The amount of sulphur in sinters was determined by fusion with sodium peroxide and sodium carbonate. Sulphates were determined after dissolving the product in hot dilute hydrochloric acid (1 + 5). Silver was determined by dissolving the sinters in hot dilute nitric acid (1 + 1), evaporating the solution to dryness, dissolving the residue in water and determining silver as silver chloride.

The composition of the gaseous products obtained at 600 °C was also determined. Sulphur dioxide was absorbed in 0.1 M tetrachloromercurate(II), $[HgCl_4]^{2-}$, solution. Carbon dioxide was determined gravimetrically by absorption in Ascarite. Nitrogen was determined by the Dumas method, Körbl's[1] reagent being used as an auxiliary oxidant.

X-ray diffraction analysis

X-ray diffraction patterns of silver thiocyanate and its sinters were made. In Fig. 2 the X-ray diffraction pattern of silver thiocyanate and of its sinters obtained at 280 and 600 °C is presented.

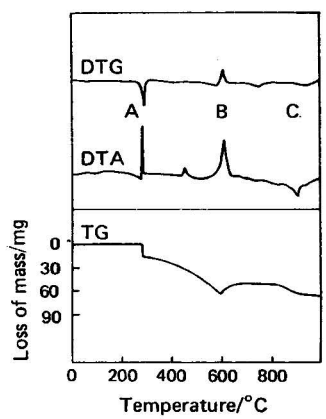

Fig. 1. Thermal curves for silver thiocyanate; A, 280 °C; B, 600 °C; and C, 900 °C.

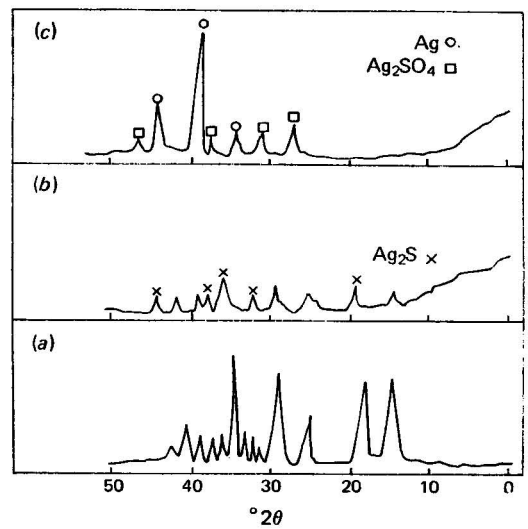

Fig. 2. X-ray diffraction patterns of silver thiocyanate (*a*) and its sinters prepared at 280 °C (*b*) and 600 °C (*c*).

Results and Discussion

The first exothermic transformation at 280 °C corresponds to the reaction

$$5AgSCN + 2O_2 = 2AgCN.3AgSCN + 2SO_2 \quad .. \quad .. \quad .. \quad (1)$$

This reaction is indicated by the loss in mass, the content of sulphur in the sinter and especially by the amount of sulphur dioxide formed (Table I).

TABLE I

DETERMINED AND CALCULATED COMPOSITION OF THE PRODUCTS FORMED DURING DECOMPOSITION OF SILVER THIOCYANATE AT 280 °C

Parameter	Determined value, %	Calculated values for—	
		2AgCN.3AgSCN, %	AgCN.AgSCN, %
Mass loss	8.56	8.09	9.67
Ag content	69.74	70.72	71.95
S content	11.76	12.61	10.69
SO_2 content	14.85	15.54*	19.30†

* SO_2 content calculated according to equation (1).
† SO_2 content calculated according to equation (2).

The AgCN.AgSCN compound was described by Duval.[2] This compound was formed in accordance with the reaction

$$2AgSCN + O_2 = AgCN.AgSCN + SO_2 \quad .. \quad .. \quad .. \quad (2)$$

The calculated mass loss corresponding to the formation of the compound is 9.67%, the sulphur content is 10.69% and the amount of sulphur dioxide released is 19.30%. The determined figures are 8.56, 11.76 and 14.85%, respectively (Table I). A compound of the general formula xAgCN.yAgSCN is probably formed during thermal decomposition of silver thiocyanate; x and y can vary, depending on the conditions under which the sinters are obtained, *e.g.*, during thermal decomposition of $Bi(SCN)_3$ a compound of the formula $Bi(CN)_3.5Bi(SCN)_3$ was formed.[2]

The second transformation corresponds to the formation of silver sulphide at approximately 600 °C, according to the equation

$$2(2AgCN.3AgSCN) + 6O_2 = 5Ag_2S + SO_2 + 5CO_2 + 5N_2 \quad .. \quad .. \quad (3)$$

By adding equations (2) and (3) an equation for the decomposition of silver thiocyanate at 600 °C is obtained:

$$2AgSCN + 2O_2 = Ag_2S + SO_2 + 2CO_2 + N_2 \quad .. \quad .. \quad (4)$$

Such a course for the reaction is proved by mass loss [28.00% as found on the TG curve, 27.73% as calculated from equation (4)], the presence of Ag_2S, found diffractometrically, and the composition of gaseous products. The determined sulphur dioxide content is 19.15% and that calculated from equation (4) is 19.30%. The corresponding figures for carbon dioxide and nitrogen are 25.10 and 26.52%, and 8.23 and 8.44%, respectively.

A by-product of the reaction is silver sulphate, formed as a result of oxidation of the sulphide. This reaction is indicated by the presence of 4.12% of sulphate in the sinter.

The increase in mass, occurring at 620 °C, is caused by the formation of silver sulphate. The presence of this compound was established diffractometrically (Fig. 2). Subsequent mass loss occurring at 850 °C is caused by the decomposition of silver sulphate and formation of metallic silver. The peak occurring on the DTA curve at 970 °C corresponds to the melting-point of silver.

Determination of Thiocyanates

Experimental

Principle

Silver thiocyanate was decomposed by heating at 600 °C and the sulphur dioxide formed was absorbed in sodium tetrachloromercurate(II) solution when the following reaction occurred[4]:

$$[HgCl_4]^{2-} + 2SO_2 + 2H_2O = [Hg(SO_3)_2]^{2-} + 4H^+ + 4Cl^- \quad .. \quad .. \quad (5)$$

The liberated hydrochloric acid was titrated with 0.1 M sodium hydroxide solution and the amount of SCN calculated according to equation (4).

Apparatus

Furnace fitted with temperature control.
Rotameter.
Bubblers to absorb liberated gases.

Reagents

Sodium tetrachloromercurate(II) solution, 0.1 M. Dissolve 27.2 g of mercury(II) chloride and 11.7 g of sodium chloride in water and dilute to 1 l.
Nitric acid, dilute (1 + 2).
Silver nitrate solution, 0.1 M.
Sodium chloride solution, 0.1 M.
Potassium bromide solution, 0.1 M.
Potassium iodide solution, 0.1 M.
Ammonium thiocyanate standard solution, 0.1 M.
Sodium hydroxide standard solution, 0.1 M.

Procedure

One millilitre of nitric acid (1 + 2) was added to 150 ml of the neutral solution containing thiocyanates and, possibly, halogen anions. The solution was heated to boiling and then, with stirring, 0.1 M silver nitrate solution was added dropwise. The solution containing the precipitate was maintained at the boiling-point for 3 min and then cooled and kept in the dark until the precipitate had settled. The precipitate was filtered through a porosity 4, sintered-glass crucible, washed with water, then with ethanol and dried.

The crucible and contents were placed in a fused silica vessel through which a current of air (10 l h^{-1}) could be drawn, and the vessel and contents placed in a crucible furnace. The air, after leaving the silica vessel, was passed through three bubblers, each containing 25 ml of 0.1 M sodium tetrachloromercurate(II) solution. If the amount of thiocyanate taken was more than 85.26 mg the number of bubblers was increased to four. The temperature of the furnace was increased to 600 °C while maintaining the flow of air. The solution from the bubblers was then transferred into a 250-ml conical flask and titrated with 0.1 M sodium hydroxide solution, using methyl red as indicator.

TABLE II

DETERMINATION OF THIOCYANATES BY THERMAL PROCEDURE

Amount of thiocyanate/mg		
Taken	Found	Error, %
113.4	112.7	−0.6
85.03	85.31	−0.3
56.42	55.6	−1.4
28.34	28.2	−0.5
14.17	14.25	−0.6
5.67	5.62	−1.0

TABLE III

DETERMINATION OF MIXTURES OF THIOCYANATES AND IODIDES

Amount of thiocyanate/mg			Amount of iodide/mg			
Taken	Found	Error, %	Taken	Found	Error, %	Ratio of SCN to I
5.69	5.64	−0.87	12.68	12.75	+0.55	1:2.23
14.22	14.35	+0.91	126.8	125.8	−0.78	1:8.9
28.45	28.20	−0.87	126.8	128.8	+1.56	1:4.45
56.91	56.42	−0.86	126.8	128.2	+1.10	1:2.23
85.27	83.98	+1.51	253.7	258.0	+1.69	1:2.97

The results of the determination of thiocyanates are shown in Table II. Table III gives the results of the determination of thiocyanates by the thermal method and iodides by weighing silver iodide and silver thiocyanate. Table IV gives the results of determinations of thiocyanates in the presence of chlorides, bromides and iodides.

TABLE IV

DETERMINATION OF THIOCYANATES IN THE PRESENCE OF CHLORIDES, BROMIDES AND IODIDES

Amount of thiocyanate/mg			Amount of halogen in sample/mg		
Taken	Found	Error, %	Cl	Br	I
17.07	17.03	−0.23	8.8	19.07	31.7
56.91	56.05	−1.51	17.71	39.9	63.4
68.29	67.15	−1.6	35.42	79.9	126.9
85.36	84.25	−1.3	35.42	79.9	126.9

Conclusion

The proposed method is the first thermal method described for the determination of thiocyanates. It is relatively simple. Thiocyanates, in the presence of chlorides, bromides and iodides, can be determined satisfactorily. It is also possible to determine thiocyanates and one of the halogen anions from the amount of sulphur dioxide produced on heating silver thiocyanide and determination of the mass of the precipitate containing silver thiocyanate and silver halide.

References

1. Körbl, J., *Mikrochim. Acta*, 1956, 1705.
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3. Cygański, A., *Roczn. Chem.*, 1977, **51**, 869.
4. West, P. W., and Gacke, G. C., *Analyt. Chem.*, 1956, **28**, 1816.

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Accepted *July 26th*, 1978

Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Selective Determination of Arsenic(III) and Arsenic(V) by Atomic-absorption Spectrophotometry Following Arsine Generation

Keywords: Arsenic(III) and arsenic(V) selective determination; atomic-absorption spectrophotometry; hydride generation

The development of a method for the selective determination of arsenic(III) and arsenic(V) at the parts per billion (10^9) level in water is required. In a direct method for the selective determination of arsenic(III) and arsenic(V) by atomic-absorption spectrophotometry, Aggett and Aspell[1] determined arsenic(III) by maintaining a pH of 4–5, and total arsenic by evolution from 5 M hydrochloric acid by the hydride evolution technique with sodium tetrahydroborate(III).

Normally the determination of arsenic in water is carried out on samples adjusted to about pH 1 by the addition of hydrochloric acid immediately after collection to stabilise the solution from possible adsorption to metal hydroxides. Therefore, although the above method is simple and precise, great care is required in adjusting the pH to 4–5. Thus, it is desirable to develop a method for determining arsenic(III) in the presence of arsenic(V) at a higher acidity.

In the study of arsine generation for atomic-absorption spectrophotometry using sodium tetrahydroborate(III), the selective evolution of arsine from arsenic(III) has been found to take place in 0.25 M hydrochloric acid solutions of both arsenic(III) and arsenic(V) containing appropriate amounts of zirconium(IV) and potassium iodide. A simple and rapid method is described for the differential determination of arsenic(III) and arsenic(V) at the parts per billion level in water utilising this phenomenon.

Experimental

Apparatus

The atomic-absorption equipment, including a long absorption tube (60 × 1.2 cm i.d.) and arsenic measurement unit, was similar to that described previously.[2] Nitrogen was used instead of argon.

Reagents

Zirconium solution, 20 mg ml^{-1}. This was prepared by dissolving zirconium oxychloride in water.

Potassium iodide solution, 20% *m/V*.

Sodium tetrahydroborate(III) solution, 5% *m/V in* 0.1 M *sodium hydroxide solution.*

Procedure for the Determination of Arsenic(III)

The procedure was similar to that published recently.[2] Arsine was generated by injection of 1 ml of sample solution that contained less than 100 ng of total arsenic. A calibration graph was constructed using 0.25 M hydrochloric acid solutions containing 4 mg ml^{-1} of zirconium(IV), 2% of potassium iodide and 0–100 ng ml^{-1} of arsenic(III).

Procedure for the Determination of Total Arsenic

The procedure used was identical with that for the determination of arsenic(III), with the exception that the sample solution was replaced with a 2 M hydrochloric acid solution containing 1% of potassium iodide.

Results and Discussion

In the presence of more than 1 mg ml^{-1} of zirconium(IV) at an acidity of less than 2 M hydrochloric acid, the conversion from arsenic(V) to arsine is more selectively suppressed than that from arsenic(III), although arsenic(III) is partly prevented from being converted into arsine. If 2% of potassium iodide is added after addition of 3–5 mg ml^{-1} of zirconium(IV) in 0.25 M hydrochloric acid, arsenic(III) is quantitatively recovered and can be determined selectively in the presence of arsenic(V) at a level of less than 100 ng ml^{-1} as total arsenic. By this method it is possible to determine arsenic(III) in the presence of arsenic(V) up to a ratio of arsenic(V) to arsenic(III) of 6:1 at a level of 50 ng ml^{-1} of arsenic(III) without interference from the arsenic(V). The absorbance begins to decrease slightly from 60 min after addition of potassium iodide in the presence of zirconium(IV). Therefore, it is necessary to measure the absorbance from between 5 and 50 min after the addition of potassium iodide.

The determination of total arsenic is carried out on a solution in 2 M hydrochloric acid containing 1% of potassium iodide. The amounts of arsenic(V) are calculated from the difference between total arsenic and arsenic(III) results.

The results for the recovery of arsenic(III) and arsenic(V) in mixtures containing known amounts of standard arsenic(III) and arsenic(V) are shown in Table I.

TABLE I
RECOVERY RESULTS

Arsenic added/ng ml^{-1}		Arsenic found/ng ml^{-1}		
As(III)	As(V)	As(III)	Total As	As(V)
0	100	1	100	99
20	20	21	40	19
20	40	20	61	41
20	60	22	80	58
20	80	21	100	79
40	20	40	60	20
40	40	40	80	40
40	60	40	99	59
60	20	60	81	21
60	40	60	100	40
80	20	79	100	21

References

1. Aggett, J., and Aspell, A. C., *Analyst*, 1976, **101**, 341.
2. Nakashima, S., *Analyst*, 1978, **103**, 1031.

Received *December 4th*, 1978

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Book Reviews

CHEMICAL PRINCIPLES WITH QUALITATIVE ANALYSIS. By WILLIAM L. MASTERTON and EMIL J. SLOWINSKI. *Saunders Golden Sunburst Series.* Pp. xli + 873. Philadelphia, London and Toronto: W. B. Saunders Company. 1978. Price £10.50.

General chemistry text books are difficult to review fairly as they are often produced with a particular course or course module in mind and, except in rare instances, seldom perfectly fit courses in different education systems several thousands of miles from their origination. The level of the text under review approximates to the general physical and inorganic chemistry sections of integrated first-year chemistry of a 4-year degree course at a Scottish or Irish University which are provided for candidates without A-level chemistry or with poor grades.

In Part I the basic framework and sequence of topics follow that of earlier editions. Introductory chapters emphasise quantitative experimental aspects of chemistry including thermochemistry and behaviour of gases. Chemical principles are discussed under the headings of electronic structure, periodicity of the elements, chemical bonding and physical properties, concluding with a brief introduction to organic chemistry. Physical chemistry is divided into phase equilibria, solutions, water (sources, pollution, purification and as a solvent), spontaneity of reaction, gaseous equilibria and rates of reaction. Descriptive, mainly inorganic, chemistry is organised around types of reactions but concludes with a discussion of natural and synthetic polymers.

Interesting features include marginal notes that serve to amuse and wake up a student lulled by the clarity and logic of the main text. Historical notes and perspectives on relative atomic masses, periodic table, basic thermodynamics, acid - base theory and on particular chemists such as J. Willard Gibbs, J. N. Lewis and M. Faraday are balanced and factual, except for R. Boyle, who it should be pointed out undertook quantitative work and as far as is known was the first to use the term "chemical analysis." An environmental chemistry flavour is provided by discussion of topics such as the energy crisis, pollution and the depletion of natural resources. The glossary of terms in the basic chemical vocabulary will be helpful to students with no prior knowledge of the subject.

Part II deals with the qualitative analysis of cations and anions following the classical Fresenius scheme but with thioacetamide as the source of hydrogen sulphide. The basic chemistry of the separation and identification reactions is clearly and carefully described. In the Preface the authors stated they believed that qualitative analysis is probably the most interesting way to introduce students to descriptive chemistry. Careful reading and appraisal of this section could well lead to a renaissance of this as a topic in undergraduate courses.

The diagrammatic illustrations are excellent and deservedly given credit on the title page; in addition, the sets of colour photographs on general chemistry and on group separations will act as a link and stimulation to practical work. Illustrative problems and detailed solutions and discussion of approximations, etc., are provided in each chapter, as are graded problems to test student understanding.

The general layout, printing and production are of a high standard.

SI units are not used throughout, *e.g.*, calorie and atmosphere are used for units of energy and pressure, respectively; this feature might debar what is otherwise excellent text in teaching departments that use SI units exclusively. Even if not directly suited to their courses, tertiary-level teachers of chemistry are encouraged to examine this text: they will find much of interest and use in this stimulating student-orientated, first-year text.

D. THORBURN BURNS

MANUAL ON HYDROCARBON ANALYSIS (FORMERLY STP 332 A). Third Edition. Sponsored by ASTM Committee D-2 on Petroleum Products and Lubricants. Pp. xx + 620. Philadelphia: American Society for Testing and Materials. 1977. Price $26 (softback).

The stated purpose of this manual is "to provide a collection of methods and to make them more useful by showing how they fit into a general scheme of analysis." Accordingly, there are well referenced introductory chapters establishing a pattern of analysis by product type. The 88 methods themselves are drawn from various parts of the ASTM Standards, including those dealing with petroleum products, paint solvents, water, electrical insulation (liquids and gases) and spectroscopy and chromatography, together with twelve proposed standards. Although this represents

a much more comprehensive coverage than the Second Edition of the Manual, it is far from complete. The preface states that "many of the sophisticated analytical methods used in petroleum laboratories are not represented" and a number of Standards dealing with such fundamentals as specific gravity, distillation and viscosity are referenced but otherwise omitted.

ASTM Standards are revised annually, and earlier editions of this manual have become rapidly and progressively out of date. About half of the standards included in this Third Edition were published after the issue of the Second Edition in 1968 and all of the others have been subject to re-approval, possibly with amendment. Unless it is intended to issue new editions at far shorter intervals than 9 years, one would anticipate the development of a similar situation with this present publication. Nevertheless, it represents a convenient, economic and authoritative manual of current practice.

J. N. WILSON

VALIDATION OF THE MEASUREMENT PROCESS. Edited by JAMES R. DEVOE. *A Symposium sponsored by the Division of Analytical Chemistry at the 171st Meeting of the American Chemical Society, New York, NY, April* 5–6, 1976. *ACS Symposium Series* 63. Pp. viii + 207. Washington, D.C.: American Chemical Society. 1977. Price $20.

Errors in the results of routine analysis, if not adequately controlled, may easily invalidate conclusions drawn from them. The magnitude of the errors in results reported by analytical chemists has, therefore, always been a topic of interest and importance. However, this subject has received increasing emphasis over the last 20 years or so as a result of two main developments: (*i*) there is increasing need to compare results from different laboratories and countries, and (*ii*) many inter-laboratory studies in a large number of analytical fields have shown that errors are often much larger than would be expected and can be tolerated. Thus, during this period there has been a growing interest in procedures relevant to the estimation and control of analytical errors. The consideration and estimation of errors requires appreciation of statistical concepts and procedures, and it appears to the writer that this aspect of the education and training of the analytical chemist is often given insufficient attention. In this situation, the present book is both helpful and timely in considering several aspects of analytical quality control and in providing references to facilitate further reading.

In Chapter 1 (G. Wernimont, "Statistical Control of Measurement Processes," 29pp.) one of the conclusions of the author is that "Measurements are not valid until we evaluate the performance characteristics of the process which produces them." He discusses in some detail the basic statistical concepts involved in controlling any measurement process, particular attention being paid to the need to ensure that the process is in "statistical control." The use of control charts to check this last point is also discussed. This chapter is a valuable introduction to the basic concepts involved in controlling any measurement process.

In Chapter 2 (J. J. Filliben, "Testing Basic Assumptions in the Measurement Process," 84pp.) the author describes a number of simple, graphical procedures for testing whether or not a measurement process is in statistical control. This chapter shows that complicated, statistical procedures are not inevitable in quality control and that much valuable information can be gained from procedures easily applied by analysts. The chapter is copiously illustrated with examples of the application of the graphical procedures that are intended primarily for checking that the errors in results are random and for estimating the nature of the frequency distribution followed by such errors.

In Chapter 3 (L. A. Currie and J. R. DeVoe, "Systematic Error in Chemical Analysis," 26pp.) the authors emphasise the importance of systematic errors and the need for the quantitative assessment of all potential sources of bias. In the latter connection, the authors make the valuable but often disregarded point that as random errors increase, so the detection of systematic errors becomes more difficult. Examples of systematic errors and their estimation and control are discussed, the examples being taken from various points within the total process: sampling, analysis, reporting results.

In Chapter 4 (G. A. Uriano and J. P. Cali, "Role of Reference Materials and Reference Methods in the Measurement Process," 22pp.) the authors discuss the important role of reference materials and methods in schemes to achieve compatibility of results from laboratories on a national scale. The general discussion is illustrated by detailed consideration of the U.S. National Bureau of Standards' work on reference materials and methods for calcium in serum, nitrogen dioxide in

air, mercury in water and chromium in biological materials. The authors emphasise that although reference materials and methods are necessary, they are not sufficient alone to achieve compatibility; a continuing programme of analytical quality control is also essential.

In Chapter 5 (S. N. Deming, "Optimization of Experimental Parameters in Chemical Analysis," 14pp.) the author stresses the importance of selecting the experimental conditions of analytical methods so that results are not unduly sensitive to variations in those conditions. For this purpose a particular statistical experimental design is described and illustrated by results obtained during investigation of a spectrophotometric method for the determination of formaldehyde by the addition of chromotropic acid and sulphuric acid.

Finally, in Chapter 6 (R. C. Rhodes, "Components of Variation in Chemical Analysis," 23pp.) the author describes the application of analysis of variance techniques in order to assess the relative importance of the many factors that may affect the variability of results. Estimation of the variability caused by each factor helps in indicating those factors most requiring control. A detailed example is given of the application of such procedures in assessing the variability to be expected of results when a number of laboratories each analyse portions of a standard sample.

In conclusion, this book can be recommended for perusal by analytical chemists whether they are currently practising analytical quality control or not. A. L. WILSON

Erratum

APRIL (1976) ISSUE, p. 304, Table IV. Several values in Table IV were incorrectly calculated. A corrected version is printed below, in which the values concerned are shown in italic type.

TABLE IV

SELENIUM CONTENT OF MILK PRODUCTS AND ALBUMIN

Material	Mass of sample taken/g	Selenium(VI)*/ $\mu g\ g^{-1}$	Total selenium†/ $\mu g\ g^{-1}$	Selenium(VI), %
Dried skim milk ..	0.5	0.058	0.101 ± 0.002	57
Milk powder (baby food)	0.2–0.4	0.064	0.227 ± 0.017	28
Instant cream powder	0.1–0.2	N.D.	0.235 ± 0.010	0
Cheese	0.1	0.008	0.050 ± 0.002	16
Cheese	0.1	0.034	0.087 ± 0.008	39
Albumin, human (fraction V)	0.05–0.1	*0.348*	*0.477 ± 0.021*	73
Albumin, egg	0.05	*0.437*	*0.585 ± 0.024*	75
Albumin, egg, crystalline	0.04	*0.684*	*0.864 ± 0.037*	79
Albumin, bovine (fraction V)	0.2	0.106	0.226 ± 0.007	47
L-Cystine	0.05	N.D.	*0.587 ± 0.026*	0
L-Methionine	0.5	0.010	0.010 ± 0.001	100

* Results are the means of two determinations.
† Results are the means of five determinations.
N.D. denotes not detected.

Spectrophotometric Determination of Dequalinium Chloride in Pharmaceutical Preparations

Short Paper

Keywords: *Dequalinium chloride determination*; *spectrophotometry*; *picric acid*

C. P. LEUNG and S. Y. KWAN
Government Laboratory, Oil Street, North Point, Hong Kong.

Analyst, 1979, **104**, 143–146.

Spectrophotometric Determination of Isoprenaline Sulphate and Methyldopa Using Chloranil

Short Paper

Keywords: *Isoprenaline sulphate determination*; *methyldopa determination*; *chloranil reagent*; *spectrophotometry*; *charge-transfer complex*

MOHAMED A. KORANY and ABDEL-AZIZ M. WAHBI
Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

Analyst, 1979, **104**, 146–148.

Spectrophotometric Determination of Microgram Amounts of Hydroquinone, Pyrogallol and Resorcinol

Short Paper

Keywords: *Hydroquinone determination*; *pyrogallol determination*; *resorcinol determination*; *spectrophotometric microdetermination*; *sodium carbonate*

Q. S. USMANI, M. M. BEG and I. C. SHUKLA
Department of Chemistry, University of Allahabad, Allahabad, India.

Analyst, 1979, **104**, 148–151.

Simple Procedure for the Determination of Total Carbon and its Radioactivity in Soils and Plant Materials

Short Paper

Keywords: *Soil organic carbon determination*; *plant carbon determination*; *^{14}C radioactivity measurement*; *chromic acid digestion*

R. C. DALAL
University of New England, Armidale, N.S.W. 2351, Australia.

Analyst, 1979, **104**, 151–154.

Determination of Lead in Columbite Concentrates by Atomic-absorption Spectrometry After Sulphide Separation

Short Paper

Keywords: *Lead determination*; *columbite concentrates*; *atomic-absorption spectrometry*; *sulphide separation*

C. CHOW
Geological Survey Laboratory, P.O. Box 1015, Ipoh, West Malaysia.

Analyst, 1979, **104**, 154–156.

Direct Determination of Calcium, Magnesium and Zinc in Lubricating Oils and Additives by Atomic-absorption Spectrometry Using a Mixed Solvent System

Short Paper

Keywords: *Lubricating oil analysis*; *calcium determination*; *magnesium determination*; *zinc determination*; *atomic-absorption spectrometry*

ZSUZSA WITTMANN
Hungarian Oil and Gas Research Institute, Veszprém, Hungary.

Analyst, 1979, **104**, 156–160.

Spectrophotometric Determination of Iron by Synergistic Extraction with Isonitrosobenzoylacetone and Pyridine

Short Paper

Keywords: *Synergistic iron extraction*; *iron determination*; *spectrophotometry*; *alloy analysis*; *isonitrosobenzoylacetone*

B. J. DESAI and V. M. SHINDE
Department of Chemistry, Shivaji University, Kolhapur 416 004, India.

Analyst, 1979, **104**, 160–163.

Potentiometric Method for the Rapid Determination of Sulphate in the Presence of Chromium(VI)

Short Paper

Keywords: *Sulphate determination*; *barium ion-selective electrode*; *chromium(VI)*; *potentiometry*

R. PRASAD
Inco Europe Limited, European Research and Development Centre, Wiggin Street, Birmingham, B16 0AJ.

Analyst, 1979, **104**, 164–167.

Determination of Thiocyanates by Thermal Decomposition of Silver Thiocyanate

Short Paper

Keywords: *Thiocyanate determination*; *thermal decomposition of silver thiocyanate*; *halide and thiocyanate determination*

A. CYGAŃSKI and T. MAJEWSKI
Institute of General Chemistry, Technical University, ul. Zwirki 36, 90–924 Lódź, Poland.

Analyst, 1979, **104**, 167–171.

Selective Determination of Arsenic(III) and Arsenic(V) by Atomic-absorption Spectrophotometry Following Arsine Generation

Communication

Keywords: *Arsenic(III) and arsenic(V) selective determination*; *atomic-absorption spectrophotometry*; *hydride generation*

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Analyst, 1979, **104**, 172–173.

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