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Summaries of Papers in this Issue

Critical Evaluation of a Multi-element Scheme Using Plasma Emission and Hydride Evolution Atomic-absorption Spectrometry for the Analysis of Plant and Animal Tissues

An analytical scheme that uses inductively coupled argon plasma emission spectroscopy (ICAP) and hydride evolution atomic-absorption spectrometry (HEAA) for the determination of trace elements in plant and animal tissues has been evaluated. The scheme incorporates the ion-exchange procedure of Kingston *et al.*, which uses Chelex 100 resin to concentrate trace elements and remove potentially interfering alkali and alkaline earth metals. The separation procedure is included in a scheme designed to maximise the number of analyte metals that can be determined from a single digestion of a biological matrix. Acid-digested samples are divided into two fractions. One fraction (5% of the total) is measured directly by ICAP for alkali and alkaline earth metals and phosphorus and for transition metals such as iron and manganese, which are not well behaved on the resin. The other fraction (95% of the total) is subjected to the separation procedure whereby a number of biologically important trace elements, including cadmium, copper, molybdenum, nickel, vanadium and zinc, are initially sequestered by the resin, and then stripped into a small volume of dilute nitric acid for ICAP measurement of the "matrix-free" analytes. Arsenic, selenium and antimony, which are not retained by the resin, are collected with the initial column effluent, acidified and determined by HEAA. The reliability of the scheme is influenced by the nature of the acid digestion procedure used to oxidise the organic matrix. The scheme was tested by analysis of ten National Bureau of Standards biological reference materials.

Keywords: *Plasma emission*; *hydride evolution atomic-absorption spectrometry*; *Chelex* 100 *separation scheme*; *NBS reference materials*; *plant and animal tissues*

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Analyst, 1982, **107**, 353–377.

Determination of Mercury in Pharmaceutical Products by Atomic-absorption Spectrophotometry Using a Carbon Rod Atomiser

An electrothermal atomisation procedure using a carbon rod atomiser is described for the determination of mercury after its extraction with dithizone (diphenylthiocarbazone) into chloroform. The increased stability of mercury after extraction allows drying and ashing to be carried out adequately without loss prior to atomisation. The sensitivity to mercury in the carbon rod atomiser is 1.1×10^{-10} g to give 1% absorption at 253.7 nm. Calibration graphs are linear over the range 0.2–2.0 $\mu g\ ml^{-1}$ of mercury. The method can be applied directly, without a preliminary digestion procedure, to solutions containing organic mercurial preservatives or bactericides [phenylmercury(II) acetate or nitrate or thiomersal (sodium ethylmercurithiosalicylate)], and to trace determination in basic and some neutral and acidic compounds, soluble in water or in dilute acids. A standard additions procedure is recommended to overcome possible matrix effects, and to allow the detection of contamination errors. The results compare favourably with those obtained by the conventional cold vapour technique.

Keywords: *Mercury determination*; *dithizonate*; *pharmaceuticals*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

PAMELA GIRGIS TAKLA and VICTOR VALIJANIAN
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Analyst, 1982, **107**, 378–384.

Gravimetric Determination of Nickel with Thiosemicarbazones

A systematic study of the use of thiosemicarbazones in the gravimetric determination of nickel is reported. The compounds tested were furfural thiosemicarbazone (FAT), thiophen-2-aldehyde thiosemicarbazone (TAT) and furfural 4-phenyl-3-thiosemicarbazone (FAPT). FAPT is the most appropriate reagent owing to its sensitivity and selectivity. FAPT has been applied to the gravimetric determination of nickel in diverse standard samples. The reagents tested in this work were compared with classical *vic*-dioximes used in the gravimetric determination of nickel.

Keywords: *Furfural thiosemicarbazone*; *thiophen-2-aldehyde thiosemicarbazone*; *furfural 4-phenyl-3-thiosemicarbazone*; *nickel determination*; *gravimetry*

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Analyst, 1982, **107**, 385–391.

Precipitation of Selenium and Tellurium from Homogeneous Solutions by Dimethyl Sulphite

Elemental selenium and tellurium are precipitated from homogeneous solutions by employing dimethyl sulphite for the *in situ* production of sulphur dioxide. Results obtained show that both selenites and selenates as well as tellurites and tellurates are quantitatively reduced by this reagent. The advantages of this precipitation over the conventional precipitation procedure are outlined. Based on the different conditions under which the two elements are precipitated, a procedure for the separation of selenium and tellurium from one another is reported.

Keywords: *Selenium determination*; *tellurium determination*; *homogeneous precipitation*; *dimethyl sulphite*; *separation*

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Analyst, 1982, **107**, 392–397.

Automatic Potentiometric Titration of Thiocyanate - Cyanide Mixtures in Hydrometallurgical Effluents

Mixtures of cyanide and thiocyanate in hydrometallurgical effluents heavily clouded with particulates are titrated quickly and successfully with silver nitrate solution by using a potentiometric automatic titrator fitted with a silver working electrode and a glass reference electrode. When thiocyanate is to be determined, cyanide is masked with formalin. Titrations over a wide range of concentration and ratio of the two species require minimum pre-treatment of the samples and give sharp end-points and good replication.

Keywords: *Thiocyanate - cyanide mixtures*; *silver - glass electrode pair*; *hydrometallurgical effluents*

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Analyst, 1982, **107**, 398–402.

Dead-stop Determination of EDTA and NTA in Commercially Available Detergents

A rapid and selective method for the determination of ethylenediaminetetraacetic acid (EDTA) and/or nitrilotriacetic acid (NTA) in commercially available detergents has been developed. It is based on a titration with a standard solution of copper(II) in acetate buffer, where the end-point is revealed by means of a "dead-stop" system with two polarised copper electrodes. Furthermore, it is possible to determine in the course of the same titration the relative amounts of both chelating agents by using 4-(2-pyridylazo)-resorcinol (PAR), which changes colour at the end-point of the first reaction (copper - EDTA). Most detergent constituents, including polyphosphates, have been observed to have no effect on the determination; interference from some constituents (perborates and zeolites) can easily be removed. The method has been shown to give good results in the analyses of different commercially available products.

Keywords: *EDTA and NTA determination*; *copper(II) sulphate titrant*; *dead-stop indicator*; *PAR indicator*; *detergents*

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Analyst, 1982, **107**, 403–407.

Comparison of Laser-excited Fluorescence and Photoacoustic Limits of Detection of Polynuclear Aromatic Hydrocarbons

An extremely simple and sensitive system that employs both fluorescence and photoacoustic detection modes simultaneously has been used to obtain limits of detection rapidly for 30 polynuclear aromatic hydrocarbons. The sensitivities of the two techniques are compared with both fixed and tunable laser excitation and a "levelling" effect is demonstrated by the photoacoustic results. High-energy pulse excitation demonstrates that the photoacoustic signal does not increase indefinitely with incident pulse energy. Application of the simultaneous detection scheme to the measurement of fluorophor quantum efficiencies in solutions and to antifluorochrome stains is suggested.

Keywords: *Photoacoustic*; *fluorescence*; *polynuclear aromatic hydrocarbons*; *laser detection*

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Analyst, 1982, **107**, 408–413.

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The Analyst

Critical Evaluation of a Multi-element Scheme Using Plasma Emission and Hydride Evolution Atomic-absorption Spectrometry for the Analysis of Plant and Animal Tissues

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An analytical scheme that uses inductively coupled argon plasma emission spectroscopy (ICAP) and hydride evolution atomic-absorption spectrometry (HEAA) for the determination of trace elements in plant and animal tissues has been evaluated. The scheme incorporates the ion-exchange procedure of Kingston *et al.*, which uses Chelex 100 resin to concentrate trace elements and remove potentially interfering alkali and alkaline earth metals. The separation procedure is included in a scheme designed to maximise the number of analyte metals that can be determined from a single digestion of a biological matrix. Acid-digested samples are divided into two fractions. One fraction (5% of the total) is measured directly by ICAP for alkali and alkaline earth metals and phosphorus and for transition metals such as iron and manganese, which are not well behaved on the resin. The other fraction (95% of the total) is subjected to the separation procedure whereby a number of biologically important trace elements, including cadmium, copper, molybdenum, nickel, vanadium and zinc, are initially sequestered by the resin, and then stripped into a small volume of dilute nitric acid for ICAP measurement of the "matrix-free" analytes. Arsenic, selenium and antimony, which are not retained by the resin, are collected with the initial column effluent, acidified and determined by HEAA. The reliability of the scheme is influenced by the nature of the acid digestion procedure used to oxidise the organic matrix. The scheme was tested by analysis of ten National Bureau of Standards biological reference materials.

Keywords: *Plasma emission*; *hydride evolution atomic-absorption spectrometry*; *Chelex* 100 *separation scheme*; *NBS reference materials*; *plant and animal tissues*

Acquisition of data on the trace element content of plant and animal tissues has become a major analytical effort for many laboratories. Despite recent instrumental advances such as electrothermal atomic-absorption spectrometry (EAAS) and argon plasma emission spectroscopy [inductively coupled (ICAP) or d.c. (DCP) plasmas], formidable problems remain for the determination of many trace elements. We have encountered both chemical and instrumental limitations in attempting to apply ICAP spectroscopy to the determination of low concentrations of toxic and nutritive elements in a wide variety of foods and related biological materials.

The detection powers of ICAP are usually in the microgram per litre range. Similarly, many biologically important elements are present in organic materials at microgram per kilogram levels. It is seldom possible to mineralise a tissue sample by conventional wet or dry ashing procedures and to dissolve the resulting ash in an appropriate solvent without diluting the original analyte concentration to some extent. Attempts to minimise this dilution factor by

ashing a relatively large sample and diluting it to a small volume (*e.g.*, 1 g dry mass in 10 ml of solution) are usually frustrated by the limited solubility of the inorganic matrix elements,[1,2] *e.g.*, alkali or alkaline earth metal pyrophosphates for dry ashed samples, or potassium perchlorate for perchloric acid digestions. Further, for samples that contain high concentrations of alkali and alkaline earth metals, dissolving the ash from large samples and concentrating the solution to small volumes results in high solution concentrations of these elements. This can be particularly troublesome for ICAP measurements because of the adverse effect on nebulisation efficiency[3,4] and the contribution to subtle but significant changes in spectral background due either to true spectral structure or to the introduction of stray light into the spectrometer system.[5,6]

Recently, chelating ion-exchange resins have been shown to be an effective means of concentrating and separating trace elements from complex matrices prior to AAS or ICAP measurement.[7–9] Kingston *et al.*[10] demonstrated the applicability of Chelex 100 cation-exchange resin to the isolation of eight transition metals from a seawater matrix. They were able to concentrate the trace elements from the seawater matrix 100-fold and eliminate the high concentrations of alkali and alkaline earth metals before EAAS measurement of the transition elements. Sturgeon *et al.*[11] used a similar approach to determine trace metals in seawater. The qualitative metal composition of seawater is similar to that of mineralised biological materials; sodium, potassium, calcium and magnesium predominate in both matrices. Because of this similarity, we have examined the possible utility of the Chelex 100 method of Kingston *et al.* to separate and concentrate several toxic and nutritive elements from acid digests of plant and animal tissues.

We have incorporated the separation procedure into a scheme that uses ICAP spectroscopy and hydride evolution atomic-absorption spectrometry (HEAA) to determine up to 18 elements in samples of plant and animal tissue. The behaviour of ten US National Bureau of Standards (NBS) Biological Standard Reference Materials (SRMs) was used to judge the effectiveness of the analytical scheme for a variety of complex matrices, and to identify those trace elements which could be determined successfully from a single digestion.

Experimental

Apparatus

Inductively coupled plasma

ICAP measurements were performed with a Jarrell-Ash, Model 975, Plasma Atom Comp 0.75-m direct-reading spectrometer operated at 1.1 kW source power at 27.12 MHz. Potassium measurements were performed on an attached Jarrell-Ash 0.5-m scanning monochromator ("N + 1"). A limited number of cobalt measurements were obtained on a second 0.75-m Jarrell-Ash plasma spectrometer. Analytical lines and standardisation concentrations used for all measurements are listed in Table I. Emission measurements were performed at

TABLE I

ICAP AND HEAA ANALYTICAL LINES AND MEASUREMENT CONDITIONS

Element	Line/nm*	Standardisation concentration/μg ml^{-1}	Background correction	Element	Line/nm*	Standardisation concentration/μg ml^{-1}	Background correction
Cd	226.5	1.00	Yes	Al	308.2	10.0	No
Co†	228.6	1.00	Yes	Fe	259.9	10.0	No
Cr	357.9	1.00	Yes				
Mo	203.8	1.00	Yes	Mg	279.5	25.0	No
Ni	231.6	1.00	Yes				
V	292.4	1.00	Yes	P	214.9	100	No
Ca	393.3	5.00	No	Ca	370.6	200	No
Cu	324.7	5.00	Yes	K‡	766.5	200	No
Mn	257.6	5.00	No				
Pb	220.3	5.00	Yes	As§	193.7	—	Yes
Zn	206.2	5.00	Yes	Se§	196.0	—	Yes
				Sb§	217.6	—	Yes

* ICAP measurements were made at 16 mm above the coil.
† Co measurements were performed at 228.6 nm using an independent ICAP polychromator system.
‡ K measurements were obtained on an "N + 1" 0.5-m scanning monochromator.
§ Determined by HEAA using calibration graphs prepared from 25–500 ng of each element.[12]

16 mm above the induction coil. The argon coolant - plasma flow was 18 l min^{-1}. The Jarrell-Ash crossflow nebuliser system was slightly modified by bubbling the nebuliser argon (0.5 l min^{-1}) through a fritted 20 × 5 cm cylindrical column of de-ionised water located at the gas inlet of the nebuliser. The resulting water-saturated nebuliser argon reduced crusting of salts at the crossflow nebuliser needle orifices. Solutions were delivered to the nebuliser by a peristaltic pump (Gilson Minipuls 2) at 1.1 ml min^{-1}. Wavelength modulation ("spectrum-shifter") background correction was used for some elements, as indicated in Table I, to compensate for minor base-line drift during the analyses.

Hydride evolution atomic-absorption spectrometer

HEAA measurements were performed on a Perkin-Elmer, Model 403, atomic-absorption spectrometer using a semi-automatic hydride generator as previously described.[12]

Glassware

Borosilicate volumetric ware was first hand-washed with hot tap water and rinsed with distilled water, then soaked in nitric acid (20% *V/V*) for 48 h, and rinsed with distilled, de-ionised water (DIW) before use. Borosilicate 100-ml Kjeldahl digestion flasks were cleaned by boiling a mixture of concentrated nitric, perchloric and sulphuric acids in them and rinsing with copious amounts of DIW before use.

Resin columns

Resin columns consisted of 200 × 8 mm polypropylene columns with a polyethylene frit resin support (Kontes Co., No. K-420160). Sample and reagent reservoirs were 125-ml FEP Teflon separating funnels (Nalgene 4301-0125). The drain stems of the separating funnels were removed and replaced with polypropylene micropipette tips cut to fit securely into the Teflon drain stopcock of the funnel. Reservoirs and columns were cleaned before the resin was loaded by soaking in warm 20% nitric acid for 2–3 h, followed by rinsing with copious volumes of DIW.

Reagents

Concentrated nitric and sulphuric acids. Distilled by sub-boiling procedure from quartz.

Concentrated perchloric acid, redistilled from Vycor. G. F. Smith Chemical Co., Columbus, Ohio.

Concentrated hydrofluoric acid. Analytical-reagent grade.

Concentrated ammonia solution. Analytical-reagent grade.

Chelex 100, *iminodiacetate chelating ion-exchange resin,* 200–400 *mesh,* Na^+ *form.* Bio-Rad Laboratories, Richmond, CA.

Metal standard solutions, 1000 p.p.m. Commercial or prepared from high-purity metals or metal salts.

Water, distilled and de-ionised (DIW). Milli-Q (Millipore Corp.).

Ammonium acetate solution, 1 M. Prepared from analytical-reagent grade crystals. As prepared, the solution was contaminated with unacceptable levels of copper, zinc and lead; it was cleaned before use by adjusting the pH to 5.3 with nitric acid or ammonia solution and passing the solution through columns of Chelex 100 resin (NH_4^+ form). Although the resulting reagent was clean enough for this study, a better preparation is to mix high-purity acetic acid and ammonia solution as described by Kingston *et al.*[10]

Sodium tetrahydroborate(III). High purity pellets (Ventron Corp., Beverley, MA).

Sodium iodide crystals. Analytical-reagent grade.

Concentrated hydrochloric acid. Analytical-reagent grade.

Digestion

Samples were digested by either of two procedures using a heating rack and perchloric acid fume trap. Binary nitric - perchloric acid digestions were performed by adding 25 ml of concentrated nitric acid and 5 ml of concentrated perchloric acid to 1–3 g of dry sample in a 100-ml Kjeldahl flask. The digestion was continued through the vigorous perchloric acid

reaction, and halted after an additional 5 min of boiling. Ternary nitric - perchloric - sulphuric acid digestions were conducted in a similar manner, except that 2 ml of concentrated sulphuric acid were added at the beginning of the digestion. These digestions were continued until the perchloric acid reaction subsided. At this point, the heater temperature was increased and the digestion was allowed to proceed until all of the residual perchloric acid was distilled from the flask and copious fumes of sulphur trioxide were evolved. The digestion was discontinued at this point. For all digestions, sample charring was avoided in order to minimise losses of volatile elements (*e.g.*, selenium). Digests were diluted to 100 ml with DIW to dissolve the acid-soluble portion of the samples.

Caution—As with all uses of perchloric acid, these procedures should be followed only by experienced personnel familiar with their potentially hazardous properties.

Post-digestion treatment of insoluble silicates with hydrofluoric acid was performed for some samples, and is described later.

Separation

For this study, we followed as closely as possible the separation conditions used by Kingston *et al.*,[10] except for the column reservoir system, which was inconvenient for our application. Instead, we used the simpler gravity flow system described under Apparatus. A solution of pH 5.0–5.5 containing trace transition elements was passed through a bed of Chelex 100 resin (NH_4^+ form).[10] A number of metals were strongly retained by the resin, whereas alkali and alkaline earth elements at pH 5 were minimally retained. Ammonium acetate was then used to elute any residual alkali and alkaline earth elements from the resin. Trace elements were removed from the resin as a group with 7–10 ml of dilute nitric acid. This approach is similar to that reported by Baetz and Kenner,[7] who used elution with ammonium sulphate to remove residual sodium (and probably other alkali and alkaline earth metals) from Chelex 100 before sulphuric acid elution of cadmium sequestered from acid digestions of foods.

Resin columns were prepared by pipetting 10.0 ml of a magnetically stirred slurry of 30 g of resin in the Na^+ form + 150 ml of DIW into the column. The approximately 2 g of water-saturated resin was then treated successively with two 15-ml portions of DIW, two 15-ml portions of 15% *V*/*V* nitric acid, two 15-ml volumes of DIW to rinse excess of acid from the resin, 10 ml of 15% *V*/*V* ammonia solution to convert the resin into the functional NH_4^+ form, and finally, two 15-ml volumes of DIW to remove excess of ammonia. A 2-cm layer of water was left above the resin bed. For this study the resin was discarded after a single use, although it can be regenerated by the above procedure.

The 100 ml of diluted digest solution was divided into two fractions. A 5-ml volume of this solution was transferred into a 25-ml calibrated flask and diluted to volume with 20% *V*/*V* perchloric acid (the non-Chelexed fraction). The remaining 95 ml were transferred into the Teflon separating funnel. The pH was adjusted to 5.3 ± 0.2 with concentrated ammonia solution followed by dilute ammonia solution and dilute nitric acid as required. A 1-ml volume of 1 M ammonium acetate solution (pH 5.3) was added to buffer the solution as in the reference method. At this pH, some NBS reference materials developed a slight flocculent white precipitate, which did not interfere with the separation other than to slow the flow-rate of sample through the resin. Sample solution was passed through the gravity-flow column by repetitive manual filling from the separating funnel. The effluent was collected in a Teflon bottle, capped and stored for the determination of arsenic, selenium and antimony (the HEAA fraction).

The column was washed with 40 ml of 1 M ammonium acetate solution (pH 5.3) to remove sequestered alkali and alkaline earth metals. Excess of ammonium acetate was rinsed from the resin with 10 ml of DIW. Next, 10 ml of 15% *V*/*V* nitric acid were added to the column to strip the sequestered trace elements from the resin. The nitric acid eluent was collected in calibrated 25-ml stoppered, graduated cylinders. Residual nitric acid was rinsed from the resin with a single 4-ml volume of DIW, which was collected in the cylinders. The eluents were diluted to 15 ml with water. This 10% *V*/*V* nitric acid solution of the trace elements was called the Chelexed fraction.

The shrinking and swelling of the Chelex 100 resin with changes in sorbed ions[13] did not drastically affect the separation time (usually 4–8 h), although different eluent flow-rates were observed for different cationic forms of the resin. We investigated only the 200–400-mesh resin. Larger resin particles may reduce the separation time.

ICAP Measurement

Concentrations of all solutions were determined under the conditions in Table I. Two-point calibrations using a standard blank and the standard concentrations shown in Table I were performed. Calibrations were based on the average of two sequential 21-s exposures of multi-element standards, *i.e.*, 14 s on line, 7 s on background. All sample intensities were measured by averaging two back-to-back 21-s integration periods. These intensity integrations were corrected for inter-element interferences and/or background fluctuations as required and output by the ICAP minicomputer as micrograms per millilitre. Raw concentration data were transferred from the spectrometer via magnetic tape to a larger computer for manipulation.[14]

Each non-Chelexed fraction and the corresponding Chelexed fraction were measured three times: first, against one calibration using multi-element standards in 20% *V*/*V* perchloric acid or 10% *V*/*V* nitric acid for non-Chelexed and Chelexed fractions, respectively, and then by two subsequent calibrations and measurements, for a total of six ICAP measurements of each digested sample. These data provided sufficient information to establish an estimate of the ICAP contribution to the over-all analytical variability. The non-Chelexed fractions were measured directly by ICAP against multi-element standards in 20% *V*/*V* perchloric acid for alkali and alkaline earth elements and for trace elements such as iron, manganese and zinc, which were usually present at readily measurable concentrations. The purpose of the relatively concentrated perchloric acid was to provide a sufficiently viscous solution matrix such that minor differences in residual acid concentrations between sample digests would be "swamped" by the perchloric acid, and therefore would not contribute to variability in aerosol transport to the plasma.[2] This diluent was used for all non-Chelexed fractions from all digestion procedures and for ICAP calibration standards, as the viscosity of this diluent was sufficient to overcome any aerosol transport variability associated with residual perchloric, sulphuric or hydrofluoric acid from the original digestions.

HEAA Measurement

HEAA measurements for arsenic, selenium and antimony were conducted as previously described.[12] The initial column effluents of the neutralised sample solution were collected in Teflon bottles, treated with 48 ml of concentrated hydrochloric acid and diluted to 160 ml with DIW. Aliquots of this solution ranging from 1 to 20 ml (depending on the analyte concentration) were transferred into test-tube reaction cells and diluted to 20 ml as necessary with 30% *V*/*V* hydrochloric acid. Calibration standards covering the range 25–500 ng of each analyte were prepared in 30% *V*/*V* hydrochloric acid. Samples for arsenic and antimony measurement were treated with sodium iodide pre-reductant to convert the pentavalant species into trivalent states. Hydrides of arsenic, selenium and antimony were generated by adding a basic 4% *m*/*V* solution of sodium tetrahydroborate(III) from a semi-automatic hydride generator. Analyte gases were passed directly into a hydrogen (nitrogen-diluted) flame for measurement of the transient absorption signals.

Results and Discussion

Preliminary Experiments

Before evaluation of the Chelex 100 procedure using NBS SRMs, preliminary separations were conducted on biological matrix-simulating standard solutions and on trace element-fortified food samples digested with the binary acids. In these experiments nanogram to microgram amounts of Al(III), As(III), Be(II), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Mn(II), Mo(VI), Ni(II), Pb(II), Se(IV), Sb(III), Sn(II), Te(IV), Ti(III), Tl(I), V(III) and Zn(II) were added to the acidified standard solutions of alkali and alkaline earth elements, and phosphorus (milligram amounts of calcium, magnesium, sodium, phosphorus and potassium), before pH adjustment and separation. Similar trace element spikes were added to food samples before binary acid digestion and subsequent separation.

These trial separations indicated that cadmium, copper, molybdenum, nickel and zinc could be consistently recovered quantitatively in the Chelexed fraction from most sample types examined. Cobalt, chromium, iron, manganese, lead and vanadium were occasionally recovered at about 100% for several (dissimilar) matrices, but frequently exhibited low (less than 80%) recovery. Aluminium and titanium were recovered quantitatively from undigested

standards, but yielded variable recoveries (60–120%) at low spike levels for acid-digested foods, presumably owing to leaching of aluminium and titanium from the borosilicate digestion flasks. Recoveries of these elements in the Chelexed fraction at spikes greater than about 10 μg were quantitative. Beryllium and tin recoveries in the Chelexed fractions were generally less than 60%. No arsenic, selenium, antimony, thallium or tellurium was detectable in the Chelexed fraction of any of the test samples. However, HEAA measurement of the initial column effluent indicated that arsenic and selenium, added as spikes, passed through the column quantitatively in most instances.

Matrix elements (calcium, magnesium and potassium) were present at very low concentrations in the Chelexed fraction, usually less than 1 μg ml^{-1}. Sodium was not measured by ICAP because no direct-reading channel was available, but no visible 589-nm emission was observed. The amount of phosphorus in the Chelexed fraction was usually less than 20 μg ml^{-1} and appeared to be directly associated with the amount of aluminium and iron in the final 10% *V/V* nitric acid eluent. All of the matrix elements (except sodium) were readily measurable in the non-Chelexed fraction.

Evaluation Using NBS Standard Reference Materials

The analytical scheme employing ICAP measurement of the Chelexed and non-Chelexed fractions and HEAA measurement of the initial column effluents was evaluated for several of the above elements using variations of the binary and ternary acid digestions. NBS SRMs were used for this evaluation in order to avoid differences in chemical behaviour of "synthetic spikes" *versus* the behaviour of "naturally occurring" trace elements.

Sample solubility

These SRMs may be grouped into two general categories: totally acid-soluble and partially acid-soluble. The term "soluble" in this context refers to the absence of visible insoluble materials after digestion with the binary or ternary acids and dilution to volume. SRM bovine liver, wheat flour, rice flour and tuna are totally soluble, whereas orchard leaves, spinach, tomato leaves, pine needles and brewers' yeast (and, to a much lesser extent, oyster tissue) contain siliceous materials that are not readily dissolved by the acid digestion. Brewers' yeast contains the greatest relative amount of the insoluble materials; pine needles and oyster tissue contain the least. NBS certification for trace elements in the reference samples is based on total solubilisation of the matrix. We therefore subjected the Chelex 100 procedure to four commonly used digestion procedures, binary, binary plus hydrofluoric acid, ternary and ternary plus hydrofluoric acid. The digestion procedures are similar to those used by NBS to characterise biological reference materials.[15]

To dissolve high-silicate SRMs, acid digests were initially diluted to 90 ml with DIW, mixed thoroughly and filtered through nitric acid-cleaned polyethylene frits to collect the residues. The silicate residues collected on the frits were then dissolved by adding 0.5–0.9 ml of hot 47% *m/V* hydrofluoric acid directly to the polyethylene filter. This hydrofluoric acid solution was then returned to the original 90 ml of digest solution. The solutions were diluted to 100 ml in polypropylene calibrated flasks, sampled for the non-Chelexed fraction and carried through the Chelex procedure. For this experiment, solutions were initially made basic to pH paper with ammonia solution in order to avoid damage to the glass pH electrodes by hydrofluoric acid. No hydrofluoric acid treatments were performed for bovine liver, tuna, wheat flour, rice flour or oyster tissue.

The high-silicate SRMs required hydrofluoric acid treatment of the insoluble residues to obtain non-Chelexed results that agreed with NBS certified or informational values. This was particularly true for aluminium, iron and manganese. The presence of hydrofluoric acid during the separation step, however, caused precipitation and/or competitive complexation, which inhibited the reaction of Chelex 100 with aluminium, iron, manganese, chromium and lead. No useful Chelexed results were obtained for these elements when hydrofluoric acid was present. Other elements such as cadmium, copper, nickel, molybdenum and zinc were adequately recovered from the resin in the presence of hydrofluoric acid.

Effects of digestion procedure on removal of matrix

Table II gives separation efficiencies for calcium, magnesium, phosphorus and potassium in the reference materials. These results were obtained by comparing masses of matrix elements

TABLE II

EFFICIENCY OF REMOVAL OF MATRIX ELEMENTS FROM SRMS

Digestion	No. of samples	Proportion of original mass removed, %*			
		Ca	Mg	K	P
HNO_3 - $HClO_4$	40	99.3 ± 0.4	99.4 ± 0.5	97 ± 2	91 ± 6
HNO_3 - $HClO_4$ - HF	15	94 ± 2	91 ± 3	95 ± 3	98 ± 1
HNO_3 - $HClO_4$ - H_2SO_4	24	98 ± 2	97 ± 3	98 ± 1	90 ± 5
HNO_3 - $HClO_4$ - H_2SO_4 - HF	15	92.7 ± 0.6	94 ± 3	96 ± 2	99.0 ± 0.4

* Variability expressed as ±1 standard deviation.

in the Chelexed and non-Chelexed fractions. All four elements were removed with at least 90% efficiency from the resin before the final nitric acid eluate containing the transition metals. Both the binary and the ternary acid digests yielded essentially quantitative removal of calcium, magnesium and potassium from all samples. Phosphorus was removed less efficiently because of the aforementioned association of phosphorus with aluminium and iron. For the hydrofluoric acid-treated samples, removal of phosphorus was quantitative. Conversely, hydrofluoric acid-treated samples gave poorer separation for calcium and magnesium, apparently because of precipitates (probably Ca^{2+}, Mg^{2+}, SiO_x^- and F^- systems) that formed at pH 5.3 and partially redissolved during the nitric acid elution.

Procedural versus *measurement precision*

A minimum of three replicates were digested for each SRM for each digestion procedure, except for the ternary acid digestion of the high-silicate SRMs (orchard leaves, tomato leaves, spinach, pine needles and brewers' yeast), for which a single sample was digested, and for the binary acid digestion of tomato leaves and pine needles, for which duplicate samples were examined. For the purpose of method evaluation, each ICAP measurement was treated as a separate "result." Therefore, nine "results" per element (triplicate ICAP results from triplicate samples) were available from which to estimate the accuracy and precision of the chemical method and of the ICAP measurement. For the "soluble" SRMs, 4–6 samples per SRM were digested by each of the binary and ternary acid procedures, representing up to 18 individual "results" per element.

Table III summarises the elemental results for the non-Chelexed and Chelexed fractions of each SRM for each digestion procedure. Values are given as apparent concentration in micrograms per gram or mass per cent. dry mass as calculated from the measured solution concentrations. The over-all mean and accompanying uncertainties for each element in each SRM are shown in Table III. The non-parenthetical error estimates, expressed as ±1 standard deviation, represent the over-all method error including the variability incurred during sample preparation and during the ICAP measurement step. In addition, the uncertainty of the ICAP measurement step (ICAP error) is included in parentheses. These values are the "within" standard deviations, which were obtained by an analysis of variance on the triplicate ICAP measurements of each set of replicate sample solutions. They represent the uncertainty associated with the ICAP calibration - measurement step at a given solution concentration.

The data in Table III can be used to differentiate between chemical method failure and ICAP error. For example, when the ICAP error approaches or exceeds the over-all method error, instrumental uncertainty limits the precision of the analysis. Conversely, when the over-all method error is much larger than the ICAP error, method imprecision arising from chemical or manipulative variability controls the precision. Poor over-all method precision in the presence of good measurement precision is indicative of method failure. This is illustrated in Table III for the determination of manganese in binary acid digests of orchard leaves. For the Chelexed fraction, the over-all method mean and standard deviation for three independent digests were $51 \pm 11\ \mu g\ g^{-1}$. The ICAP measurement precision obtained for triplicate measurements was $\pm 1\ \mu g\ g^{-1}$, indicating that method failure was responsible for the over-all poor precision and, consequently, poor accuracy. On the other hand, when ICAP measurement precision is poor, *i.e.*, near the detection limit, the chemical method performance cannot be satisfactorily evaluated. Using these criteria, the data in Table III were evaluated to judge the effectiveness of the proposed analytical scheme.

Individual elements

In Table III the symbol ‡ indicates the experimental result that appears to be closest to the "true" analyte concentration. This symbol is used primarily to distinguish those samples which yield variable results due to silicate occlusions and only one of them is used for all samples, although two or more procedures may have yielded mean values with overlapping errors. For poorly behaved elements, such as chromium or lead, the symbol is omitted, as none of the digestions gave Chelexed values that agreed consistently with NBS or literature values.

Aluminium. Comparisons between Chelexed and non-Chelexed values for aluminium determined in digests that were *not* treated with hydrofluoric acid show that soluble aluminium can be recovered semi-quantitatively from solution for either the binary acid (90 $\pm$ 6% recovery) or the ternary acid (87 $\pm$ 12% recovery) digestion by the Chelex procedure. This compares well with recoveries obtained for the diet samples fortified with aluminium at concentrations above contamination levels leached from the borosilicate glass. Precipitation at pH 5.3 and resin saturation may have contributed to aluminium losses. The accuracy of Chelex determination of aluminium appears to be limited primarily by sample insolubility (silicates). Total dissolution using hydrofluoric acid was required in order to obtain non-Chelexed results that agreed with NBS or literature values. Blank contamination leached by the non-hydrofluoric acid digestions from the digestion flasks prevented evaluation for aluminium in bovine liver, tuna, wheat flour, rice flour and oyster tissue. Inadvertent contact of hydrofluoric acid with glass volumetric ware prevented aluminium determination for the ternary acid digestions of high-silicate SRMs.

Cadmium and nickel. Results for cadmium and nickel obtained for several of the SRMs showed generally good agreement with NBS certified values; agreement with NBS informational values for the high-silicate SRMs were not as good. However, the Chelexed results agreed well with literature values, particularly for nickel in spinach and pine needles. Similarly, good agreement with literature values was obtained for cadmium in tomato leaves. For cadmium in spinach, eight of a total of ten samples gave values very near the 1.5 $\mu g\ g^{-1}$ NBS informational value, but the other two results were drastically higher (one each in binary and ternary acids), causing apparently poor results for the non-hydrofluoric acid digestions. The source of the two spurious values was unknown. For the other samples, the cadmium method performance was primarily controlled by ICAP precision, particularly at the lower concentrations. In addition, nickel appeared to be associated with the silicate materials.

Molybdenum. Molybdenum behaved well through the Chelex separation for most of the digestion procedures. Some procedural imprecision occurred for the low-molybdenum concentrations in the high-silicate SRMs, but ICAP measurement imprecision appeared to be the more limiting factor. Silicate occlusion of molybdenum did not appear to be a major problem, although the brewers' yeast in particular did release more molybdenum after hydrofluoric acid treatment.

Lead. Results for lead for the high-silicate SRMs were consistently lower than NBS values. Only for the orchard leaves, at 45 $\mu g\ g^{-1}$, did the Chelex procedure agree well with the NBS value. Lead was lost from the resin during ammonium acetate elution of the samples, as seen in our initial attempts to remove lead contamination from the 1 M ammonium acetate solution by passing the reagent through Chelex 100 (NH_4^+). Lead elution (breakthrough) was observed with continued passage of ammonium acetate solution through the resin. In addition, a sample of NBS pine needles (binary acid) subjected to the Chelex procedure without the ammonium acetate elution gave a lead value of 10.5 $\pm$ 0.3 $\mu g\ g^{-1}$, which is in good agreement with the NBS value of 10.8 $\pm$ 0.5 $\mu g\ g^{-1}$. Therefore, it appears that the ammonium acetate elution step may have to be modified in order to ensure quantitative retention of lead on the resin before nitric acid elution. Additionally, measurement precision was poor for lead determinations below about 1 $\mu g\ g^{-1}$ for the SRMs (less than 0.06 $\mu g\ ml^{-1}$ of lead for a 1-g sample), preventing evaluation of the separation procedure for lead below this level.

Chromium and manganese. Chromium and manganese exhibited erratic behaviour on the resin in the SRM analyses, like that observed in preliminary standard and food digest separations. It is unclear from this work whether losses of chromium and manganese are associated with loss from the resin before the nitric acid elution[10,11] or are due to retention by the resin during the acid elution.[27] However, substantial loss of manganese from the resin during the

ammonium acetate elution is suspected because, as with lead, a sample of pine needles digested with binary acid and subjected to the separation procedure without ammonium acetate elution gave a manganese value of 620 $\mu g\ g^{-1}$. This is lower than the NBS value of 675 $\pm$ 15 $\mu g\ g^{-1}$ but significantly higher than the 475 $\pm$ 43 $\mu g\ g^{-1}$ obtained for the binary acid digestions with ammonium acetate elution.

The ternary acid digestions gave a consistently poorer Chelexed recovery for manganese than did the binary acid digestion. This suggests that in addition to losses associated with ammonium acetate elution, the digestion procedure significantly affects manganese (and possibly chromium) behaviour on the resin. Ternary acid digestion also resulted in apparently poorer results for chromium in pine needles and tomato leaves, relative to the binary acid digestion of the same samples. For spinach and orchard leaves, however, ternary acid yielded values in good agreement with the NBS values. Such inconsistent behaviour suggests the possibility that mixed oxidation states of chromium and manganese may be responsible for the variable results. The characteristic pink colour of MnO_4^- was observed in the 2 ml of residual sulphuric acid from ternary acid digests of pine needles but was absent from the residual perchloric acid from the binary acid digests.

Chromium(III) - chromium(VI) mixtures have been reported to exhibit different behaviour on the resin.[28] Other possible causes of variable chromium recovery include volatilisation or silicate occlusions.[29]

Iron. Except for tomato leaves, the recovery of *soluble* iron by Chelex 100 from the binary or ternary acid digestion (comparison of Chelexed *versus* non-Chelexed values) was generally greater than 90%. This was particularly true for ternary acid digestion of the low-silicate SRMs, where good agreement with NBS values occurred. Resin saturation may have contributed to loss of iron from the tomato leaves, which have the highest soluble iron concentration of the ten SRMs examined. Kingston *et al.*[10] recovered an average 90–93% of added iron from sea-water. Our results for much higher concentrations in digested biological matrices generally agree well with their findings. However, because iron may be measured directly in the non-Chelexed fraction, the latter procedure is preferred.

Vanadium. Vanadium exhibited a distinct difference in behaviour between binary and ternary acid digestion. Much lower vanadium results were obtained for samples prepared by the binary digestion than for those digested with ternary acid. This effect was not attributable solely to a more complete solubilisation of the silicate matrix by the ternary acid mixture, as the hydrofluoric acid-solubilised samples exhibited similar behaviour, *i.e.*, nitric - perchloric - hydrofluoric acid yielded lower vanadium results than nitric - perchloric - sulphuric - hydrofluoric acid. With the exception of the brewers' yeast, all of the SRMs that contained ICAP-measurable vanadium gave higher results for the ternary acid digestion than for binary acid digestion. Vanadium may be present in the neutralised binary acid digest solution as a mixture of two or more species that exhibit different affinities for the resin under the separation conditions employed. Addition of sulphuric acid to the digestion apparently shifts any equilibrium towards the species readily amenable to the separation procedure. No NBS certified or informational values, except an uncertified value of 2.8 $\mu g\ g^{-1}$ in oyster tissue, are available for vanadium in these SRMs. Our results using ternary acid or ternary plus hydrofluoric acid agree well with other values reported in the literature. With the exception of the high-silicate brewers' yeast, most vanadium in the SRMs appears to have been released by the ternary acid digestion without the addition of hydrofluoric acid.

Zinc. Chelexed and non-Chelexed zinc results were in general agreement with NBS values for all digestion procedures. There appeared to be silicate occlusion of zinc in some sample types, *e.g.*, pine needles and brewers' yeast, but it was a small fraction of the total zinc present. Chelexed and non-Chelexed results agreed very well, indicating that except for zinc levels below about 5 $\mu g\ g^{-1}$ dry mass the Chelex separation offers no advantage over direct ICAP measurement.

Cobalt. No cobalt values are given in Table III. A few results for SRMs were obtained on Chelexed samples of hydrofluoric acid-treated SRMs. Values for triplicate digestions of tomato leaves were 0.57 $\pm$ 0.07 and 0.57 $\pm$ 0.04 $\mu g\ g^{-1}$ of cobalt for the binary plus hydrofluoric acid and ternary plus hydrofluoric acid digestions, respectively. The NBS uncertified value for cobalt is 0.6 $\mu g\ g^{-1}$. For spinach, the results were 1.25 $\pm$ 0.06 and 1.15 $\pm$ 0.03 $\mu g\ g^{-1}$ of cobalt for binary plus hydrofluoric acid and ternary acid digestions, respectively; the NBS

TABLE III

EFFECT OF DIGESTION PROCEDURE ON THE BEHAVIOUR OF THE ANALYTICAL SCHEME TOWARDS NBS SRMs

SRM		Ca, % *m/m* HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	~0.004	~0.19	~0.006	~0.16
	Non-Chelexed	1.98 ± 0.04 (±0.03)	2.03 ± 0.02 (±0.02)	1.97 (±0.03)	2.03 ± 0.04 (±0.02)‡
	NBS or lit.†	2.09 ± 0.03			
Tomato leaves, SRM 1573	Chelexed	~0.008	~0.19	~0.01	~0.16
	Non-Chelexed	3.04 ± 0.05 (±0.03)	3.08 ± 0.05 (±0.01)	3.10 (±0.03)	2.99 ± 0.05 (±0.05)‡
	NBS or lit.	3.00 ± 0.03			
Spinach, SRM 1570	Chelexed	~0.02	~0.2	~0.02	~0.17
	Non-Chelexed	1.24 ± 0.08 (±0.02)	1.36 ± 0.04 (±0.005)	1.25 (±0.01)	1.39 ± 0.03 (±0.02)‡
	NBS or lit.	1.35 ± 0.03			
Pine needles, SRM 1575	Chelexed	~0.04	~0.17	~0.05	~0.05
	Non-Chelexed	0.40 ± 0.01 (±0.004)	0.410 ± 0.003 (±0.003)	0.409 (±0.002)	0.411 ± 0.003 (±0.003)‡
	NBS or lit.	0.41 ± 0.02			
Brewers' yeast, SRM 1569	Chelexed	~0.005	~0.05	~0.007	~0.08
	Non-Chelexed	0.227 ± 0.007 (±0.005)	0.242 ± 0.004 (±0.003)	0.229 (±0.001)	0.249 ± 0.003 (±0.003)‡
	NBS or lit.	—			
Oyster tissue, SRM 1566	Chelexed	~0.002	—	~0.0005	—
	Non-Chelexed	0.153 ± 0.003 (±0.001)	—	0.151 ± 0.002 (±0.001)‡	—
	NBS or lit.	0.15 ± 0.02			
Bovine liver, SRM 1577	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.0121 ± 0.0003 (±0.0002)	—	0.0120 ± 0.0002 (±0.0002)‡	—
	NBS or lit.	0.0124 ± 0.002			
Albacore tuna, RM 50	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.042 ± 0.004 (±0.0009)	—	0.041 ± 0.002 (±0.0004)‡	—
	NBS or lit.	0.045 ± 0.006[16]			
Wheat flour, SRM 1567	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.0194 ± 0.0006 (±0.0006)	—	0.0199 ± 0.0004 (±0.0002)‡	—
	NBS or lit.	0.019 ± 0.001			
Rice flour, SRM 1568	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.0148 ± 0.0005 (±0.0004)	—	0.0148 ± 0.0003 (±0.0002)‡	—
	NBS or lit.	0.014 ± 0.002			
		Mg, % *m/m* HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	~0.0002	~0.07	~0.0005	~0.03
	Non-Chelexed	0.60 ± 0.02 (±0.01)	0.62 ± 0.01 (±0.01)	0.598 (±0.007)	0.61 ± 0.02 (±0.02)‡
	NBS or lit.	0.62 ± 0.02			
Tomato leaves, SRM 1573	Chelexed	~0.0003	~0.07	~0.006	~0.04
	Non-Chelexed	0.67 ± 0.02 (±0.02)	0.70 ± 0.02 (±0.02)	0.69 (± 0.02)	0.69 ± 0.02 (±0.02)‡
	NBS or lit.	(0.7), 0.60 ± 0.06,[17] 0.73[18]			
Spinach, SRM 1570	Chelexed	~0.003	~0.1	~0.002	~0.06
	Non-Chelexed	0.86 ± 0.04 (±0.02)	0.89 ± 0.03 (±0.03)	0.88 (±0.01)	0.90 ± 0.02 (±0.03)‡
	NBS or lit.	0.73 ± 0.05,[17] 0.90,[18] 0.89 ± 0.01[19]			

TABLE III—continued

SRM		Mg, % *m/m*			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Pine needles, SRM 1575	Chelexed	~0.003	~0.02	~0.009	~0.002
	Non-Chelexed	0.118 ± 0.003 (±0.001)	0.119 ± 0.002 (±0.002)	0.120 (±0.002)	0.120 ± 0.003 (±0.003)‡
	NBS or lit.	0.12 ± 0.02,[17] 0.15[18]			
Brewers' yeast, SRM 1569	Chelexed	~0.0008	~0.03	~0.001	~0.002
	Non-Chelexed	0.173 ± 0.007 (±0.004)	0.190 ± 0.006 (±0.006)	0.187 (±0.005)	0.198 ± 0.006 (±0.001)‡
	NBS or lit.	0.178 ± 0.010[20]			
Oyster tissue, SRM 1566	Chelexed	~0.0008	—	~0.0002	—
	Non-Chelexed	0.143 ± 0.004 (±0.004)	—	0.141 ± 0.002 (±0.002)‡	—
	NBS or lit.	0.128 ± 0.009			
Bovine liver, SRM 1577	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.066 ± 0.002 (±0.001)	—	0.0657 ± 0.0009 (±0.0008)‡	—
	NBS or lit.	0.0604 ± 0.0005			
Albacore tuna, RM 50	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.120 ± 0.003 (±0.003)	—	0.119 ± 0.003 (±0.002)‡	—
	NBS or lit.	0.11 ± 0.01[16]			
Wheat flour, SRM 1567	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.042 ± 0.001 (±0.001)	—	0.0429 ± 0.0009 (±0.0005)‡	—
	NBS or lit.	0.0427 ± 0.0011[19]			
Rice flour, SRM 1568	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.051 ± 0.002 (±0.001)	—	0.051 ± 0.001 (±0.001)‡	—
	NBS or lit.	0.0517 ± 0.0007[19]			

SRM		P, % *m/m*			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	~0.02	~0.001	~0.07	~0.002
	Non-Chelexed	0.197 ± 0.007 (±0.006)	0.206 ± 0.004 (±0.005)	0.198 (±0.004)	0.209 ± 0.006 (±0.006)‡
	NBS or lit.	0.21 ± 0.01			
Tomato leaves, SRM 1573	Chelexed	~0.05	~0.001	~0.06	~0.003
	Non-Chelexed	0.34 ± 0.01 (±0.01)	0.35 ± 0.01 (±0.01)	0.35 (±0.01)	0.35 ± 0.01 (±0.01)‡
	NBS or lit.	0.34 ± 0.02			
Spinach, SRM 1570	Chelexed	~0.05	~0.002	~0.07	~0.004
	Non-Chelexed	0.52 ± 0.02 (±0.01)	0.55 ± 0.02 (±0.02)	0.53 (±0.01)	0.57 ± 0.02 (±0.02)‡
	NBS or lit.	0.55 ± 0.02			
Pine needles, SRM 1575	Chelexed	~0.04	~0.004	~0.04	~0.0006
	Non-Chelexed	0.119 ± 0.002 (±0.001)	0.117 ± 0.004 (±0.004)	0.119 (±0.005)	0.126 ± 0.002 (±0.003)‡
	NBS or lit.	0.12 ± 0.02			
Brewers' yeast, SRM 1569	Chelexed	~0.04	~0.0007	~0.07	~0.016
	Non-Chelexed	1.00 ± 0.04 (±0.04)	1.02 ± 0.03 (±0.03)	1.04 (±0.05)	1.08 ± 0.04 (±0.04)‡
	NBS or lit.	—			
Oyster tissue, SRM 1566	Chelexed	~0.02	—	~0.02	—
	Non-Chelexed	0.78 ± 0.02 (±0.02)	—	0.79 ± 0.01 (±0.01)‡	—
	NBS or lit.	(0.81)			

Table III—continued

SRM		P, % *m/m* HNO_3 - $HClO_4$	P, % *m/m* HNO_3 - $HClO_4$ - HF	P, % *m/m* HNO_3 - $HClO_4$ - H_2SO_4*	P, % *m/m* HNO_3 - $HClO_4$ - H_2SO_4 - HF
Bovine liver, SRM 1577	Chelexed	~0.01	—	~0.01	—
	Non-Chelexed	1.18 ± 0.03 (±0.02)	—	1.21 ± 0.01 (±0.01)‡	—
	NBS or lit.	(1.1), 1.21 ± 0.04[19]			
Albacore tuna, RM 50	Chelexed	~0.002	—	~0.003	—
	Non-Chelexed	0.82 ± 0.03 (±0.03)	—	0.85 ± 0.03 (±0.03)‡	—
	NBS or lit.	—			
Wheat flour, SRM 1567	Chelexed	~0.0008	—	~0.0009	—
	Non-Chelexed	0.137 ± 0.005 (±0.005)	—	0.142 ± 0.003 (±0.003)‡	—
	NBS or lit.	0.1239 ± 0.0033[19]			
Rice flour, SRM 1568	Chelexed	~0.0003	—	~0.0004	—
	Non-Chelexed	0.160 ± 0.006 (±0.006)	—	0.163 ± 0.003 (±0.004)‡	—
	NBS or lit.	0.1443 ± 0.0035[19]			

SRM		K, % *m/m* HNO_3 - $HClO_4$	K, % *m/m* HNO_3 - $HClO_4$ - HF	K, % *m/m* HNO_3 - $HClO_4$ - H_2SO_4*	K, % *m/m* HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	~0.004	~0.06	~0.003	~0.05
	Non-Chelexed	1.47 ± 0.07 (±0.05)	1.43 ± 0.07 (±0.08)	1.47 (±0.02)	1.56 ± 0.05 (±0.05)‡
	NBS or lit.	1.47 ± 0.03			
Tomato leaves, SRM 1573	Chelexed	~0.009	~0.13	~0.009	~0.08
	Non-Chelexed	4.3 ± 0.2 (±0.2)	4.4 ± 0.1 (±0.1)	4.4 (±0.2)	4.6 ± 0.2 (±0.2)‡
	NBS or lit.	4.46 ± 0.03			
Spinach, SRM 1570	Chelexed	~0.01	~0.1	~0.02	~0.09
	Non-Chelexed	3.6 ± 0.2 (±0.1)	3.7 ± 0.1 (±0.1)	3.7 (±0.04)	3.9 ± 0.1 (±0.1)‡
	NBS or lit.	3.56 ± 0.03			
Pine needles, SRM 1575	Chelexed	~0.009	~0.05	~0.01	~0.04
	Non-Chelexed	0.353 ± 0.008 (±0.004)	0.34 ± 0.02 (±0.02)	0.362 (±0.004)	0.385 ± 0.008 (±0.009)‡
	NBS or lit.	0.37 ± 0.02			
Brewers' yeast, SRM 1569	Chelexed	~0.005	~0.07	~0.009	~0.07
	Non-Chelexed	1.4 ± 0.1 (±0.06)	1.45 ± 0.05 (±0.04)	1.450 (±0.007)	1.59 ± 0.04 (±0.04)‡
	NBS or lit.	1.55 ± 0.05[20]			
Oyster tissue, SRM 1566	Chelexed	~0.006	—	~0.002	—
	Non-Chelexed	0.98 ± 0.04 (±0.04)	—	0.98 ± 0.02 (±0.02)‡	—
	NBS or lit.	0.969 ± 0.005			
Bovine liver, SRM 1577	Chelexed	~0.002	—	~0.003	—
	Non-Chelexed	0.97 ± 0.05 (±0.02)	—	0.99 ± 0.02 (±0.01)‡	—
	NBS or lit.	0.97 ± 0.06			
Albacore tuna, RM 50	Chelexed	~0.006	—	~0.006	—
	Non-Chelexed	1.23 ± 0.09 (±0.08)	—	1.20 ± 0.03 (±0.02)‡	—
	NBS or lit.	(1.22)			
Wheat flour, SRM 1567	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.130 ± 0.005 (±0.006)	—	0.131 ± 0.004 (±0.004)‡	—
	NBS or lit.	0.136 ± 0.004			
Rice flour, SRM 1568	Chelexed	<0.0001	—	<0.0001	—
	Non-Chelexed	0.115 ± 0.008 (±0.008)	—	0.114 ± 0.003 (±0.003)‡	—
	NBS or lit.	0.112 ± 0.002			

TABLE III—continued

SRM		Al/μg g^{-1}: HNO_3 - $HClO_4$	Al/μg g^{-1}: HNO_3 - $HClO_4$ - HF	Al/μg g^{-1}: HNO_3 - $HClO_4$ - H_2SO_4*	Al/μg g^{-1}: HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	158 ± 23 (±3)	78 ± 18 (±1)	256 (±4)	—§
	Non-Chelexed	178 ± 28 (±23)	322 ± 18 (±20)‡	266 (±9)	—§
	NBS or lit.	347 ± 8,[21] 330[22]			
Tomato leaves, SRM 1573	Chelexed	446 ± 10 (±6)	202 ± 43 (±15)	596 (±8)	—§
	Non-Chelexed	473 ± 20 (±21)	1170 ± 60 (±40)‡	666 (±12)	—§
	NBS or lit.	(1200)			
Spinach, SRM 1570	Chelexed	443 ± 36 (±6)	178 ± 35 (±2)	392 (±5)	—§
	Non-Chelexed	463 ± 39 (±24)	782 ± 31 (±33)‡	592 (±13)	—§
	NBS or lit.	870 ± 50			
Pine needles, SRM 1575	Chelexed	414 ± 19 (±6)	307 ± 24 (±13)	448 (±3)	—§
	Non-Chelexed	472 ± 12 (±10)	526 ± 17 (±19)‡	476 (±9)	—§
	NBS or lit.	545 ± 30			
Brewers' yeast, SRM 1569	Chelexed	476 ± 67 (±10)	465 ± 34 (±6)	1448 (±10)	—§
	Non-Chelexed	585 ± 150 (±35)	2000 ± 56 (±61)‡	1590 (±31)	—§
	NBS or lit.	2300 ± 10[20]			
Oyster tissue, SRM 1566	Chelexed	—¶	—	—¶	—
	Non-Chelexed	—	—	—¶	—
	NBS or lit.	—			
Bovine liver, SRM 1577	Chelexed	—¶	—	—¶	—
	Non-Chelexed	—¶	—	—¶	—
	NBS or lit.	—			
Albacore tuna, RM 50	Chelexed	—¶	—	—¶	—
	Non-Chelexed	—¶	—	—¶	—
	NBS or lit.	—			
Wheat flour, SRM 1567	Chelexed	—¶	—	—¶	—
	Non-Chelexed	—¶	—	—¶	—
	NBS or lit.	—			
Rice flour, SRM 1568	Chelexed	—¶	—	—¶	—
	Non-Chelexed	—¶	—	—¶	—
	NBS or lit.	—			
		Cd/μg g^{-1}: HNO_3 - $HClO_4$	Cd/μg g^{-1}: HNO_3 - $HClO_4$ - HF	Cd/μg g^{-1}: HNO_3 - $HClO_4$ - H_2SO_4*	Cd/μg g^{-1}: HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	0.17 ± 0.07 (±0.07)	0.12 ± 0.08 (±0.09)	0.2 (±0.1)	0.16 ± 0.07 (±0.08)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.11 ± 0.02			
Tomato leaves, SRM 1573	Chelexed	2.56 ± 0.06 (±0.05)	2.6 ± 0.1 (±0.1)	2.40 (±0.01)	2.55 ± 0.09 (±0.09)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	(3), 2.7 ± 0.4[17]			
Spinach, SRM 1570	Chelexed	2.2 ± 1 (±0.1)	1.40 ± 0.08 (±0.09)	2.8 (±0.1)	1.49 ± 0.08 (±0.09)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	(1.5)			

TABLE III—continued

SRM		Cd/µg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Pine needles, SRM 1575	Chelexed	0.14 ± 0.07 (±0.07)	0.16 ± 0.09 (±0.09)	0.3 (±0.1)	0.18 ± 0.09 (±0.10)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	(<0.5), 0.18 ± 0.03[17]			
Brewers' yeast, SRM 1569	Chelexed	0.08 ± 0.04 (±0.04)	0.18 ± 0.07 (±0.08)	0.12 (±0.07)	0.29 ± 0.06 (±0.05)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Oyster tissue, SRM 1566	Chelexed	3.61 ± 0.03 (±0.03)	—	3.54 ± 0.04 (±0.03)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	3.5 ± 0.4			
Bovine liver, SRM 1577	Chelexed	0.35 ± 0.05 (±0.06)‡	—	0.39 ± 0.07 (±0.06)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.27 ± 0.04			
Albacore tuna, RM 50	Chelexed	0.06 ± 0.03 (±0.04)‡	—	0.08 ± 0.07 (±0.07)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Wheat flour, SRM 1567	Chelexed	0.04 ± 0.01 (±0.01)‡	—	0.05 ± 0.03 (±0.02)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.032 ± 0.007			
Rice flour, SRM 1568	Chelexed	0.04 ± 0.02 (±0.01)‡	—	0.06 ± 0.03 (±0.03)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.029 ± 0.004			
		Cr/µg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	2.4 ± 0.1 (±0.1)	<0.05	2.6 (±0.3)	<0.05
	Non-Chelexed	—	—	—	—
	NBS or lit.	2.6 ± 0.3			
Tomato leaves, SRM 1573	Chelexed	3.8 ± 0.2 (±0.2)	<0.05	2.28 (±0.06)	<0.05
	Non-Chelexed	—	—	—	—
	NBS or lit.	4.5 ± 0.5			
Spinach, SRM 1570	Chelexed	3.6 ± 0.5 (±0.3)	<0.05	4.6 (±0.2)	<0.05
	Non-Chelexed	—	—	—	—
	NBS or lit.	4.6 ± 0.3			
Pine needles, SRM 1575	Chelexed	2.9 ± 0.2 (±0.2)	<0.05	1.3 (±0.2)	<0.05
	Non-Chelexed	—	—	—	—
	NBS or lit.	2.6 ± 0.2			
Brewers' yeast, SRM 1569	Chelexed	1.2 ± 0.6 (±0.1)	<0.05	0.7 (±0.1)	<0.05
	Non-Chelexed	—	—	—	—
	NBS or lit.	2.12 ± 0.05			
Oyster tissue, SRM 1566	Chelexed	0.34 ± 0.09 (±0.07)	—	0.6 ± 0.2 (±0.1)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.69 ± 0.27			

TABLE III—continued

SRM		Cr/μg g^{-1}: HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Bovine liver, SRM 1577	Chelexed	0.4 ± 0.5 (±0.5)	—	0.4 ± 0.5 (±0.5)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.088 ± 0.009			
Albacore tuna, RM 50	Chelexed	1.4 ± 0.3 (±0.1)	—	1.5 ± 0.4 (±0.2)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.75 ± 0.12[22]			
Wheat flour, SRM 1567	Chelexed	0.4 ± 0.2 (±0.07)	—	0.3 ± 0.1 (±0.08)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Rice flour, SBM 1568	Chelexed	0.2 ± 0.2 (±0.05)	—	0.08 ± 0.08 (±0.07)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—	—	—	—

SRM		Cu/μg g^{-1}: HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	12.0 ± 0.4 (±0.3)	12.0 ± 0.8 (±0.4)	12.0 (±0.2)	11.7 ± 0.2 (±0.2)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	12 ± 1			
Tomato leaves, SRM 1573	Chelexed	10.4 ± 0.5 (±0.6)‡	9.8 ± 0.4 (±0.4)	8.2 (±0.4)	9.5 ± 0.2 (±0.2)
	Non-Chelexed	—	—	—	—
	NBS or lit.	11 ± 1			
Spinach, SRM 1570	Chelexed	11.1 ± 0.5 (±0.5)	11.2 ± 0.4 (±0.3)	10.9 (±0.3)	11.1 ± 0.2 (±0.2)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	12 ± 2			
Pine needles, SRM 1575	Chelexed	3.0 ± 0.5 (±0.6)‡	2.7 ± 0.2 (±0.2)	2.9 ± (0.2)	2.5 ± 0.3 (±0.4)
	Non-Chelexed	—	—	—	—
	NBS or lit.	3.0 ± 0.3			
Brewers' yeast, SRM 1569	Chelexed	13 ± 1 (±0.6)	18.1 ± 0.7 (±0.3)	17.7 (±0.2)	18.4 ± 0.3 (±0.3)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	11 ± 2[30]			
Oyster tissue, SRM 1566	Chelexed	62.9 ± 0.5 (±0.6)‡	—	61.8 ± 0.9 (±0.6)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	63.0 ± 3.5			
Bovine liver, SRM 1577	Chelexed	185 ± 9 (±8)	—	189 ± 4 (±2)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	193 ± 10			
Albacore tuna, RM 50	Chelexed	3.2 ± 0.3 (±0.3)	—	3.1 ± 0.4 (±0.4)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	2.38 ± 0.9,[16] 3.27[23]			
Wheat flour, SRM 1567	Chelexed	1.9 ± 0.2 (±0.2)	—	1.8 ± 0.2 (±0.2)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	2.0 ± 0.3			
Rice flour, SRM 1568	Chelexed	1.9 ± 0.2 (±0.2)	—	1.9 ± 0.2 (±0.2)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	2.2 ± 0.3			

TABLE III—continued

SRM		Fe/μg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	238 ± 20 (±5)	99 ± 9 (±3)	271 (±4)	201 ± 12 (±2)
	Non-Chelexed	256 ± 11 (±8)	290 ± 6 (±4)	283 (±3)	300 ± 17 (±9)‡
	NBS or lit.	300 ± 20			
Tomato leaves, SRM 1573	Chelexed	494 ± 10 (±11)	193 ± 34 (±7)	538 (±12)	386 ± 14 (±6)
	Non-Chelexed	531 ± 14 (±14)	642 ± 17 (± 13)	625 (±14)	658 ± 18 (±18)‡
	NBS or lit.	690 ± 25			
Spinach, SRM 1570	Chelexed	462 ± 33 (±9)	136 ± 22 (±2)	502 (±8)	388 ± 19 (±5)
	Non-Chelexed	491 ± 20 (±11)	541 ± 15 (±14)	518 (±8)	557 ± 19 (±22)‡
	NBS or lit.	550 ± 20			
Pine needles, SRM 1575	Chelexed	154 ± 2 (±3)	79 ± 13 (±3)	163 (±4)	181 ± 9 (±3)
	Non-Chelexed	175 ± 7 (±4)	194 ± 4 (±4)	177 (±4)	195 ± 10 (±7)‡
	NBS or lit.	200 ± 10			
Brewers' yeast, SRM 1569	Chelexed	217 ± 5 (±4)	344 ± 23 (±8)	440 (±8)	630 ± 15 (±6)
	Non-Chelexed	257 ± 34 (±9)	660 ± 15 (±17)	499 (±15)	693 ± 25 (±28)‡
	NBS or lit.	707 ± 16[20]			
Oyster tissue, SRM 1566	Chelexed	191 ± 5 (±3)	—	194 ± 9 (±3)	—
	Non-Chelexed	196 ± 6 (±6)‡	—	203 ± 8 (±5)	—
	NBS or lit.	195 ± 34			
Bovine liver, SRM 1577	Chelexed	209 ± 28 (±7)	—	258 ± 10 (±9)	—
	Non-Chelexed	264 ± 6 (±3)	—	264 ± 3 (±2)‡	—
	NBS or lit.	270 ± 20			
Albacore tuna, RM 50	Chelexed	50 ± 2 (±0.8)	—	54 ± 2 (±2)	—
	Non-Chelexed	52 ± 2 (±1)	—	55 ± 2 (±2)‡	—
	NBS or lit.	57 ± 2,[16] 58.1[23]			
Wheat flour, SRM 1567	Chelexed	17.1 ± 0.8 (±0.2)	—	18 ± 1 (±0.8)	—
	Non-Chelexed	17 ± 1 (±0.8)	—	17.9 ± 0.8 (±0.5)‡	—
	NBS or lit.	18.3 ± 1.0			
Rice flour, SRM 1568	Chelexed	7.1 ± 0.4 (±0.1)	—	7.6 ± 0.4 (±0.4)	—
	Non-Chelexed	7.3 ± 0.4 (±0.3)	—	7.8 ± 0.4 (±0.5)‡	—
	NBS or lit.	8.7 ± 0.6			

SRM		Mn/μg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	51 ± 11 (±1)	23 ± 2 (±0.6)	37 (±1)	23 ± 2 (±0.2)
	Non-Chelexed	86 ± 2 (±2)	89 ± 1 (±1)	84 (±1)	85 ± 2 (±2)‡
	NBS or lit.	91 ± 4			
Tomato leaves, SRM 1573	Chelexed	110 ± 8 (±2)	61 ± 2 (±2)	26.8 (±0.4)	67 ± 4 (±0.8)
	Non-Chelexed	217 ± 5 (±5)	230 ± 5 (±5)	222 (±5)	221 ± 5 (±6)‡
	NBS or lit.	238 ± 7			
Spinach, SRM 1570	Chelexed	108 ± 6 (±1)	40 ± 1 (±0.6)	36.4 (±0.6)	54 ± 6 (±0.5)
	Non-Chelexed	158 ± 7 (±3)	166 ± 5 (±4)	160 (±3)	167 ± 6 (±6)‡
	NBS or lit.	165 ± 6			

TABLE III—continued

SRM		Mn/µg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Pine needles, SRM 1575	Chelexed	475 ± 43 (±10)	127 ± 7 (±5)	167 (±4)	154 ± 6 (±2)
	Non-Chelexed	652 ± 15 (±6)	657 ± 7 (±8)	655 (±13)	652 ± 14 (±16)‡
	NBS or lit.	675 ± 15			
Brewers' yeast, SRM 1569	Chelexed	6.4 ± 0.2 (±0.1)	2.70 ± 0.08 (±0.09)	2.35 (±0.09)	4.9 ± 0.9 (±0.03)
	Non-Chelexed	9.1 ± 0.6 (±0.6)	10.4 ± 0.8 (±0.9)	9.6 (±0.6)	10.9 ± 0.7 (±0.3)‡
	NBS or lit.	7.0 ± 0.8[20]			
Oyster tissue, SRM 1566	Chelexed	17.2 ± 0.2 (±0.2)	—	6.2 ± 0.3 (±0.07)	—
	Non-Chelexed	17.8 ± 0.9 (±1)	—	17.4 ± 0.6 (±0.7)‡	—
	NBS or lit.	17.5 ± 1.2			
Bovine liver, SRM 1577	Chelexed	9.7 ± 0.8 (±0.3)	—	4 ± 1 (±0.16)	—
	Non-Chelexed	10.5 ± 0.3 (±0.4)	—	10.4 ± 0.4 (±0.3)‡	—
	NBS or lit.	10.3 ± 1.0			
Albacore tuna, RM 50	Chelexed	0.49 ± 0.04 (±0.07)	—	0.27 ± 0.07 (±0.03	—
	Non-Chelexed	0.7 ± 0.4 (±0.3)	—	0.6 ± 0.4 (±0.3)‡	—
	NBS or lit.	(1.3), 0.54 ± 0.02,[16] 0.51[23]			
Wheat flour, SRM 1567	Chelexed	8.0 ± 0.4 (±0.1)	—	3 ± 1 (±0.1)	—
	Non-Chelexed	8.2 ± 0.3 (±0.3)	—	8.3 ± 0.2 (±0.2)‡	—
	NBS or lit.	8.5 ± 0.5			
Rice flour, SRM 1568	Chelexed	19.1 ± 0.9 (±0.2)	—	8 ± 2 (±0.4)	—
	Non-Chelexed	20.2 ± 0.5 (±0.5)	—	20.1 ± 0.3 (±0.3)‡	—
	NBS or lit.	20.1 ± 0.4			

SRM		Mo/µg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	0.2 ± 0.1 (±0.1)‡	0.2 ± 0.1 (±0.1)	0.2 (±0.2)	0.11 ± 0.08 (±0.08)
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.3 ± 0.1			
Tomato leaves, SRM 1573	Chelexed	0.5 ± 0.1 (±0.1)	0.5 ± 0.1 (±0.1)‡	0.4 (±0.2)	0.5 ± 0.3 (±0.1)
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.65 ± 0.10,[17] 0.62 ± 0.04[18]			
Spinach, SRM 1570	Chelexed	0.2 ± 0.1 (±0.08)	0.4 ± 0.2 (±0.09)‡	0.3 (±0.1)	0.2 ± 0.1 (±0.1)
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.3 ± 0.1[17]			
Pine needles, SRM 1575	Chelexed	0.2 ± 0.1 (±0.1)	0.13 ± 0.06 (±0.04)‡	0.2 (±0.1)	0.1 ± 0.1 (±0.1)
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.10[17]			
Brewers' yeast, SRM 1569	Chelexed	3.3 ± 0.3 (±0.1)	3.9 ± 0.2 (±0.1)	3.4 (±0.1)	3.8 ± 0.2 (±0.2)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Oyster tissue, SRM 1566	Chelexed	0.1 ± 0.1 (±0.1)	—	<0.07	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	(<0.2)			
Bovine liver, SRM 1577	Chelexed	3.1 ± 0.5 (±0.1)	—	3.4 ± 0.1 (±0.1)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	3.4 ± 0.1			

TABLE III—*continued*

SRM		Mo/μg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Albacore tuna, RM 50	Chelexed	0.1 ± 0.1 (±0.1)	—	<0.07	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Wheat flour, SRM 1567	Chelexed	0.39 ± 0.09 (±0.09)	—	0.42 ± 0.07 (±0.09)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	(0.4)			
Rice flour, SRM 1568	Chelexed	1.59 ± 0.07 (±0.08)	—	1.59 ± 0.09 (±0.08)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	(1.6)			
		Ni/μg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	1.15 ± 0.09 (±0.08)	1.15 ± 0.07 (±0.08)	1.27 (±0.08)	1.24 ± 0.07 (±0.06)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.3 ± 0.2			
Tomato leaves, SRM 1573	Chelexed	1.12 ± 0.06 (±0.05)	1.12 ± 0.08 (±0.09)	1.10 (±0.08)	1.3 ± 0.2 (±0.05)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.2 ± 0.3[18]			
Spinach, SRM 1570	Chelexed	4.9 ± 0.2 (±0.1)	5.1 ± 0.1 (±0.1)	5.4 (±0.1)	5.4 ± 0.1 (±0.08)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	(6), 4.1 ± 0.5,[18] 5.4 ± 1.0,[34] 5.68 ± 0.28[19]			
Pine needles, SRM 1575	Chelexed	2.07 ± 0.07 (±0.06)	2.2 ± 0.1 (±0.1)	2.24 (±0.06)	2.39 ± 0.09 (±0.1)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	(3.5), 2.3 ± 0.2,[17] 2.2[18]			
Brewers' yeast, SRM 1569	Chelexed	4.6 ± 0.3 (±0.2)	5.9 ± 0.2 (±0.2)	4.8 (±0.1)	6.0 ± 0.2 (±0.08)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Oyster tissue, SRM 1566	Chelexed	0.97 ± 0.09 (±0.05)‡	—	0.92 ± 0.04 (±0.04)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.03 ± 0.19			
Bovine liver, SRM 1577	Chelexed	<0.06	—	<0.06	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Albacore tuna, RM 50	Chelexed	0.7 ± 0.2 (±0.04)	—	0.9 ± 0.1 (±0.1)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.02 ± 0.12[16]			
Wheat flour, SRM 1567	Chelexed	0.16 ± 0.04 (±0.04)	—	0.20 ± 0.04 (±0.04)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	(0.18)			
Rice flour, SRM 1568	Chelexed	0.15 ± 0.02 (±0.02)	—	0.16 ± 0.03 (±0.03)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	(0.16)			

TABLE III—continued

SRM		Pb/µg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	44 ± 2 (±0.08)	12 ± 2 (±0.4)	46 (±1)	18 ± 2 (±0.3)
	Non-Chelexed	—	—	—	—
	NBS or lit.	45 ± 3			
Tomato leaves, SRM 1573	Chelexed	5.0 ± 0.2 (±0.2)	1.1 ± 0.2 (±0.2)	4.3 (±0.2)	2.0 ± 0.3 (±0.3)
	Non-Chelexed	—	—	—	—
	NBS or lit.	6.3 ± 0.3			
Spinach, SRM 1570	Chelexed	0.8 ± 0.3 (±0.2)	0.2 ± 0.1 (±0.1)	0.8 (±0.1)	0.6 ± 0.4 (±0.4)
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.2 ± 0.2			
Pine needles, SRM 1575	Chelexed	9.6 ± 0.4 (±0.4)	8.4 ± 0.4 (±0.3)	9.8 (±0.3)	8.8 ± 0.5 (±0.4)
	Non-Chelexed	—	—	—	—
	NBS or lit.	10.8 ± 0.5			
Brewers' yeast, SRM 1569	Chelexed	0.5 ± 0.5 (±0.5)	0.5 ± 0.1 (±0.1)	0.2 (±0.2)	0.8 ± 0.2 (±0.2)
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Oyster tissue, SRM 1566	Chelexed	0.5 ± 0.2 (±0.3)	—	0.5 ± 0.3 (±0.3)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.48 ± 0.04			
Bovine liver, SRM 1577	Chelexed	0.3 ± 0.3 (±0.2)	—	0.4 ± 0.3 (±0.3)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.34 ± 0.08			
Albacore tuna, RM 50	Chelexed	0.5 ± 0.3 (±0.3)	—	0.4 ± 0.2 (±0.2)	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Wheat flour, SRM 1567	Chelexed	<0.1	—	<0.1	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Rice flour, SRM 1568	Chelexed	<0.1	—	<0.1	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			

SRM		V/µg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	0.3 ± 0.1 (±0.05)	0.25 ± 0.08 (±0.06)	0.54 (±0.02)	0.53 ± 0.05 (±0.04)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.60 ± 0.2[25], 0.61[16]			
Tomato leaves, SRM 1573	Chelexed	0.79 ± 0.07 (±0.03)	0.69 ± 0.09 (±0.08)	1.19 (±0.01)	1.42 ± 0.08 (± 0.08)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.3 ± 0.2[18]			
Spinach, SRM 1570	Chelexed	0.6 ± 0.1 (±0.06)	0.74 ± 0.06 (±0.07)	1.34 (±0.06)	1.28 ± 0.07 (±0.07)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	1.06 ± 0.17[18]			

TABLE III—continued

SRM		V/μg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Pine needles, SRM 1575	Chelexed	0.33 ± 0.05 (±0.06)	0.28 ± 0.08 (±0.09)	0.37 (±0.09)	0.41 ± 0.06 (±0.05)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.47 ± 0.08[18]			
Brewers' yeast, SRM 1569	Chelexed	1.31 ± 0.09 (±0.05)	4.27 ± 0.09 (±0.06)	1.46 (±0.05)	4.4 ± 0.1 (±0.08)‡
	Non-Chelexed	—	—	—	—
	NBS or lit.	4.1 ± 0.1[20]			
Oyster tissue, SRM 1566	Chelexed	0.68 ± 0.06 (±0.06)	—	2.44 ± 0.06 (±0.06)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	(2.8)			
Bovine liver, SRM 1577	Chelexed	<0.05	—	0.09 ± 0.06 (±0.07)‡	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	0.055 ± 0.002[26]			
Albacore tuna, RM 50	Chelexed	<0.05	—	<0.05	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Wheat flour, SRM 1567	Chelexed	<0.05	—	<0.05	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
Rice flour, SRM 1568	Chelexed	<0.05	—	<0.05	—
	Non-Chelexed	—	—	—	—
	NBS or lit.	—			
		Zn/μg g^{-1}			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_3$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Orchard leaves, SRM 1571	Chelexed	25 ± 1 (±0.9)	23.5 ± 0.9 (±1)	26 (±1)	24.3 ± 0.3 (±0.2)‡
	Non-Chelexed	25 ± 1 (±1)	26 ± 2 (±1)	26 (±1)	27 ± 1 (±1)
	NBS or lit.	25 ± 3			
Tomato leaves, SRM 1573	Chelexed	56 ± 2 (±2)	60 ± 3 (±3)	59 (±2)	57 ± 2 (±1)‡
	Non-Chelexed	59 ± 3 (±1)	64 ± 3 (±2)	52 (±1)	62 ± 3 (±3)
	NBS or lit.	62 ± 6			
Spinach, SRM 1570	Chelexed	46 ± 1 (±0.8)	46.2 ± 0.6 (±0.5)	48 (±2)	48 ± 1 (±0.8)‡
	Non-Chelexed	46 ± 2 (±2)	48 ± 3 (±2)	49 (±2)	53 ± 3 (±3)
	NBS or lit.	50 ± 2			
Pine needles, SRM 1575	Chelexed	60 ± 3 (±3)	71 ± 10 (±4)	61 (±4)	65 ± 4 (±1)‡
	Non-Chelexed	63 ± 3 (±3)	74 ± 9 (±2)	64 (±4)	68 ± 5 (±3)
	NBS or lit.	53.5 ± 2.0,[17] 52,[18] 61 ± 10[20]			
Brewers' yeast, SRM 1569	Chelexed	59 ± 6 (±3)	66 ± 2 (±2)	64 (±4)	65 ± 2 (±0.6)‡
	Non-Chelexed	63 ± 2 (±2)	65 ± 2 (±3)	64 (±5)	70 ± 4 (±5)
	NBS or lit.	70 ± 2[20]			
Oyster tissue, SRM 1566	Chelexed	859 ± 9 (±10)‡	—	843 ± 12 (±6)	—
	Non-Chelexed	878 ± 15 (±12)	—	869 ± 8 (±6)	—
	NBS or lit.	852 ± 14			

TABLE III—continued

SRM		Zn/μg g⁻¹			
		HNO_3 - $HClO_4$	HNO_3 - $HClO_4$ - HF	HNO_3 - $HClO_4$ - H_2SO_4*	HNO_3 - $HClO_4$ - H_2SO_4 - HF
Bovine liver, SRM 1577	Chelexed	134 ± 7 (±3)	—	134 ± 6 (±4)‡	—
	Non-Chelexed	135 ± 4 (±5)	—	135 ± 2 (±2)	—
	NBS or lit.	130 ± 10			
Albacore tuna, RM 50	Chelexed	14.1 ± 0.3 (±0.4)	—	14.2 ± 0.6 (±0.6)‡	—
	Non-Chelexed	14 ± 1 (±1)	—	14 ± 1 (±1)	—
	NBS or lit.	(13.6 ± 1), 14.7[23]			
Wheat flour, SRM 1567	Chelexed	10.6 ± 0.5 (±0.2)	—	10.6 ± 0.4 (±0.4)‡	—
	Non-Chelexed	10.6 ± 0.7 (±0.8)	—	10.5 ± 0.7 (±0.8)	—
	NBS or lit.	10.6 ± 1.0			
Rice flour, SRM 1568	Chelexed	19.3 ± 0.7 (±0.6)	—	19.8 ± 0.8 (±0.7)‡	—
	Non-Chelexed	20 ± 1 (±0.9)	—	20.2 ± 0.8 (±0.7)	—
	NBS or lit.	19.4 ± 1			

* HNO_3 - $HClO_4$ - H_2SO_4 digestion of NBS orchard leaves, tomato leaves, spinach, pine needles and brewers' yeast represented by single samples; only ICAP measurement error is shown.

† NBS certified values given as mean ±95% confidence interval. NBS informational values given in parentheses. Literature references given as superscripts. NBS and literature values are placed under the HNO_3 - $HClO_4$ column for convenience: no digestion description is intended.

‡ These values represent our "best estimate" of the concentration of the analyte in a given SRM.

§ Al contamination occurred as a result of contact of hydrofluoric acid with volumetric glassware subsequent to sample digestion.

¶ Al contamination occurred from glassware during sample digestion. No hydrofluoric acid was involved.

uncertified value is 1.5 $\mu g\ g^{-1}$. These results indicate that cobalt may be amenable to the Chelex 100 separation, although additional verification is required.

HEAA SRM results

Table IV summarises the results for arsenic and selenium obtained by the Chelex 100 procedure. No hydrofluoric acid data are given for arsenic because of arsenic contamination in samples treated with hydrofluoric acid. In general, the data indicate that the ternary acid digestion must be used if both arsenic and selenium are to be determined in the same sample solution using rapid HEAA with sodium tetrahydroborate(III) reduction.

TABLE IV

SUMMARY OF RESULTS OBTAINED BY HEAA FOLLOWING THE CHELEX PROCEDURE AND COMPARISON WITH NBS AND LITERATURE VALUES

SRM	Element	Found/$\mu g\ g^{-1}$		
		Binary acid*,†	Ternary acid*,†	NBS or literature value
Orchard leaves, SRM 1571	As	12.0 ± 0.6 (±0.4)	12.9 (±0.4)	10 ± 2
	Se	<0.1	<0.1	0.08 ± 0.01
Tomato leaves, SRM 1573	As	0.29 ± 0.02 (±0.02)	0.29 (±0.01)	0.27 ± 0.05
	Se	<0.1	<0.1	0.06 ± 0.02[20]
Spinach, SRM 1570	As	0.15 ± 0.01 (±0.01)	0.16 (±0.01)	0.15 ± 0.05
	Se	<0.1	<0.1	0.039 ± 0.015[20]
Pine needles, SRM 1575	As	0.19 ± 0.01 (±0.01)	0.19 (±0.03)	0.21 ± 0.04
	Se	<0.1	<0.1	0.049 ± 0.004[20]
Brewers' yeast, SRM 1569	As	0.56 ± 0.03 (±0.03)	0.53 (±0.08)	—
	Se	0.98 ± 0.05 (±0.08)	1.01 ± 0.06 (±0.07)	0.92 ± 0.09[20]
Oyster tissue, SRM 1566	As	2.32 ± 0.09 (±0.1)	15.5 ± 0.3 (±0.6)	13.4 ± 1.9
	Se	2.22 ± 0.03 (±0.1)	2.42 ± 0.08 (±0.1)	2.1 ± 0.5
Bovine liver, SRM 1577	As	<0.1	<0.1	0.055 ± 0.004
	Se	1.0 ± 0.1 (±0.2)	1.03 ± 0.04 (±0.03)	1.1 ± 0.1
Albacore tuna, RM 50	As	<0.1	3.3 ± 0.2 (±0.1)	3.3 ± 0.4
	Se	3.7 ± 0.2 (±0.2)	3.8 ± 0.1 (±0.1)	3.6 ± 0.4
Wheat flour, SRM 1567	As	<0.05	<0.05	(0.006)
	Se	0.76 ± 0.08 (±0.05)	0.91 ± 0.03 (±0.05)	1.1 ± 0.2
Rice flour, SRM 1568	As	<0.05	0.43 ± 0.01 (±0.02)	0.41 ± 0.05
	Se	0.28 ± 0.03 (±0.05)	0.32 ± 0.04 (±0.03)	0.4 ± 0.1

* All values obtained by HEAA following the Chelex procedure.

† Values obtained for the high-silicate SRMs based on 1–3 samples per digestion. Values for NBS oyster tissue, bovine liver, tuna, wheat flour and rice flour based on 3–6 samples per SRM per digestion.

For selenium, little difference was seen between samples treated with hydrofluoric acid and those not so treated. Selenium values were therefore pooled under the two principal digestion procedures, nitric - perchloric and nitric - perchloric - sulphuric acids.

It is worth repeating that preliminary recoveries of inorganic arsenic spikes added to food samples and carried through the binary acid - Chelex procedure were quantitative. Good recoveries of arsenic were also obtained for analyses of NBS orchard leaves, spinach, tomato leaves, pine needles and brewers' yeast using binary acid digestion. No significant differences were observed between Chelex - HEAA results for the binary or the ternary acid digestions for these SRMs. However, for NBS tuna, rice flour and oyster tissue, severe depression or elimination of arsenic HEAA signals was observed for samples subjected to the binary acid digestion. The presence of refractory organic arsenic in marine samples that is resistant to conventional wet ashing is well documented,[30,31] and is confirmed here by the inability to generate HEAA signals from the two marine sample types. The ternary digestion, or an equivalently rigorous digestion, is also required for HEAA measurement of arsenic in most samples such as the rice flour. Similarly, for antimony in NBS orchard leaves (NBS value 3.0 $\mu g\ g^{-1}$), we obtained values of 1.15 and 3.00 $\mu g\ g^{-1}$ for the binary and ternary acid digestions, respectively, indicating that the ternary acid digestion may also be required for successful measurement of stibine by HEAA. Antimony could not be measured quantitatively in any other SRM by either digestion procedure, *i.e.*, the level was less than 0.2 $\mu g\ g^{-1}$.

The principal advantage of collecting the pH 5 column effluent for HEAA measurement is conservation of the analytical solution. Using the proposed analytical scheme, 95% of the original solution is available for HEAA measurements and the Chelex ICAP measurement. Conversely, a single HEAA measurement for the three hydride-forming elements discussed here would require up to 60% of the original digest solution if the arsenic, selenium or antimony aliquots were withdrawn before the column step.

Non-Chelexed results

The non-Chelexed results shown in Table III illustrate that the alkali and alkaline earth metals and phosphorus are readily measured by direct ICAP after the various acid digestions. The effective dilution volume of these solutions was 500 ml, *i.e.*, 1–3 g of digested sample is diluted to 100 ml, then 5 ml is diluted to 25 ml. For this relatively large dilution factor, the total dissolved salt concentration was usually below 500 μg ml^{-1}. For these low salt concentrations, no adverse nebuliser effects associated with needle crusting were observed. Further, the 500-ml dilution did not inhibit ICAP measurement of soluble iron, manganese and zinc in the non-Chelexed fraction. The measurement precision for these elements was slightly poorer in the dilute non-Chelexed samples, but was generally not a serious limiting factor in the analysis. An exception was manganese in the Albacore Tuna Research Material, where the measurement precision was relatively poor for the very low manganese solution concentration (2–3 ng ml^{-1}). For iron, aluminium and manganese, silicate occlusion represents a major source of error for several SRMs if hydrofluoric acid is not used in the digestion.

Proposed Scheme

The analytical scheme presented in Fig. 1 outlines the approach among those examined which appears most reliable when using the separation procedure for analysis of biological samples. The ternary acid digestion is suggested because of the apparent requirement of complete digestion in fuming sulphuric acid for arsenic and vanadium determinations. This digestion may not be the optimum procedure for some elements in all biological matrices, *e.g.*, high-calcium samples. The binary acid digestion and subsequent hydrofluoric acid treatment of residual silicates from the two digestions can be used for some sample types.

Iron and manganese must be measured in the non-Chelexed fraction because these elements are not consistently well behaved on the resin. Chromium is not included in the scheme. The dependence of the separation procedure on oxidation state and pH must be further investigated to determine if these three elements can be isolated quantitatively in the Chelexed fraction.

Elements in parentheses are amenable to the scheme under favourable conditions. Aluminium can apparently be determined semi-quantitatively if insoluble silicates are absent, and if contamination is reduced, *e.g.*, by use of quartz digestion flasks. Semi-quantitative lead estimates can be obtained on most samples. Cobalt and antimony appear to be determinable by the scheme, but further study is required for these elements.

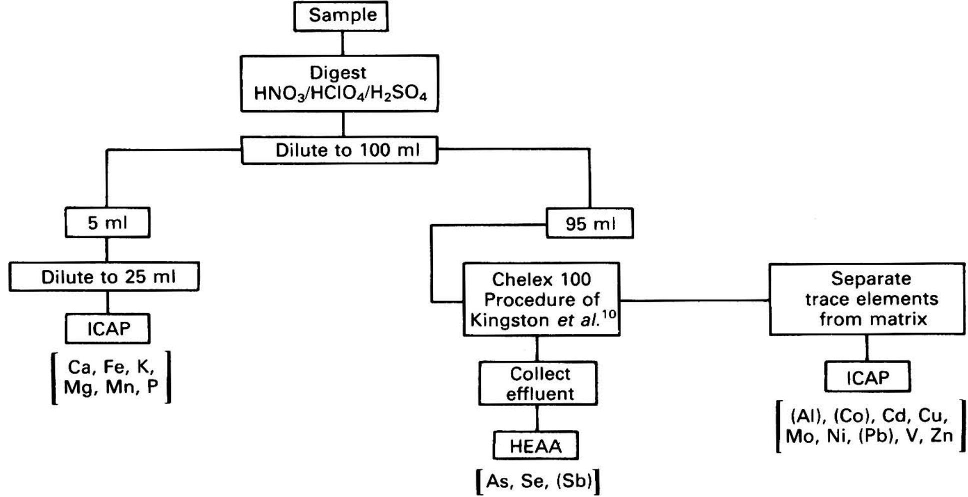

Fig. 1. Proposed analysis scheme.

Quantitation Limits

Table V lists approximate quantitation limits in micrograms. These values were obtained from estimates of the solution detection limits (micrograms per millilitre) in the Chelexed, non-Chelexed and HEAA fractions. Detection limits were multiplied by 5 to obtain solution quantitation limits, which were then multiplied by the appropriate dilution factors, *i.e.*, 15, 500 and 160 ml for the Chelexed, non-Chelexed and HEAA fractions, respectively.

TABLE V

APPROXIMATE MINIMUM ANALYTE MASSES REQUIRED FOR ACCURATE ANALYSIS BY PROPOSED MULTI-ELEMENT SCHEME

Element	Minimum required mass/μg	Measurement step*	Element	Minimum required mass/μg	Measurement step*
Al†	2.0	ICAP-C	Mn	2.5	ICAP-NC
As	0.20	HEAA	Mo	0.90	ICAP-C
Ca	2.5	ICAP-NC	Ni	0.15	ICAP-C
Cd	0.15	ICAP-C	P	250	ICAP-NC
Co	0.30	ICAP-C	Pb	1.5	ICAP-C
Cu	0.15	ICAP-C	Se	0.20	HEAA
Fe	5.0	ICAP-NC	Sb	0.20	HEAA
K	250	ICAP-NC	V	0.15	ICAP-C
Mg	5.0	ICAP-NC	Zn	0.15	ICAP-C

* C = Chelexed fraction; NC = non-Chelexed fraction.
† Al appears to be amenable to the scheme provided that glassware contamination can be avoided.

Conclusions

The Chelex 100 separation procedure of Kingston *et al.*[10] can be used for the separation and concentration of several elements from the inorganic matrices of digested tissue samples. Several elements, including cadmium, copper, molybdenum, nickel and zinc, react quantitatively with the resin under most of the conditions examined, and can be successfully isolated and determined by ICAP in several diverse matrices. Other elements, such as chromium, manganese and iron, are subject to losses from the resin, possibly because of ammonium acetate elution or mixed oxidation states.

The analytical scheme using the Chelex 100 separation followed by ICAP and HEAA measurement is influenced by the type of acid digestion procedure used to destroy the organic matrix. A thorough digestion using the ternary acid appears necessary to ensure quantitative recoveries of arsenic, vanadium and perhaps other elements.

Other factors, including the presence of insoluble silicates, which occlude several elements, and ICAP measurement imprecision near detection limits, can drastically influence the accuracy of the scheme.

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Determination of Mercury in Pharmaceutical Products by Atomic-absorption Spectrophotometry Using a Carbon Rod Atomiser

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An electrothermal atomisation procedure using a carbon rod atomiser is described for the determination of mercury after its extraction with dithizone (diphenylthiocarbazone) into chloroform. The increased stability of mercury after extraction allows drying and ashing to be carried out adequately without loss prior to atomisation. The sensitivity to mercury in the carbon rod atomiser is 1.1×10^{-10} g to give 1% absorption at 253.7 nm. Calibration graphs are linear over the range 0.2–2.0 μg ml^{-1} of mercury. The method can be applied directly, without a preliminary digestion procedure, to solutions containing organic mercurial preservatives or bactericides [phenylmercury(II) acetate or nitrate or thiomersal (sodium ethylmercurithiosalicylate)], and to trace determination in basic and some neutral and acidic compounds, soluble in water or in dilute acids. A standard additions procedure is recommended to overcome possible matrix effects, and to allow the detection of contamination errors. The results compare favourably with those obtained by the conventional cold vapour technique.

Keywords: *Mercury determination*; *dithizonate*; *pharmaceuticals*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

A rapid and specific method is needed to determine low mercury levels that may be present in drug substances or formulations, either from the use of mercury compounds as additives or due to contamination. The British Pharmacopoeia gives no procedure for the control of the organomercurial compounds phenylmercury(II) acetate or nitrate or thiomersal (sodium ethylmercurithiosalicylate) in injections, eye drops or eye lotions, although their use as bactericides in such preparations is official.[1] Little information is available concerning the likely extent of mercury contamination, but the only general limit test provided by the British Pharmacopoeia is the non-specific thioacetamide test for heavy metals,[1] which is considerably less sensitive for mercury than for lead, which is used as the standard for comparison.

The determination of mercury by atomic-absorption spectrophotometry has been widely used since the introduction of the cold vapour technique,[2] which involves the reduction of ionic mercury with tin(II) chloride, and is sensitive and specific. Matrix effects can interfere with the release of mercury from the test solution, and the use of a standard additions method is recommended.[3] The main disadvantage of the procedure is that a preliminary digestion step is generally required for organically combined mercury,[3,4] although a combined tin(II) chloride - cadmium chloride reagent[5] has been found to release mercury from phenylmercury. Reliable wet-digestion procedures[3,6] that prevent losses of the volatile mercury are tedious and potentially hazardous, and reviews[7,8] of the copious literature on mercury analysis show that the numerous more rapid procedures that have been developed are usually of limited applicability. Thus, various partial digestion procedures recommended[9–11] for organomercurial preservatives have been used only for certain specified medicinal products, and not all will produce cleavage of the mercury - sulphur bond of thiomersal.[12]

For a procedure to be generally applicable, yet avoid a digestion stage, it seemed advisable to separate the mercury as far as possible from the product being tested. For this purpose, dithizone (diphenylthiocarbazone) extraction was seen to be a well established method,[6] as mercury(II) ions in acidic or neutral solution will react[13] with excess of dithizone (H_2Dz) in an organic solvent to give a 1:2 complex $Hg(HDz)_2$. Alkyl- or aryl-organomercury(II) salts will react[14] to form 1:1 complexes RHg(HDz), which in some instances are decomposed at acidic pH values to give mercury(II) dithizonate. Of the other metals, only copper and

the noble metals will react appreciably with dithizone from 0.1 N acid solution. As the basis of a spectrophotometric procedure, the dithizone method requires a preliminary wet digestion to ensure that only mercury(II) dithizonate is obtained, as different organic complexes can vary in their absorption spectra.[14,15] Also the colour reaction can suffer reversible photochromic changes.[16]

In this work, it was found that, although direct electrothermal atomisation procedures for mercury are usually hampered by mercury losses even during drying,[17] the mercury content of extracted dithizone complexes can readily be determined using a carbon rod atomiser. Pre-atomisation temperatures of up to 250 °C can be used without loss of mercury. Background absorbances are low and no digestion is needed, even for thiomersal, as recoveries of mercury from organic and inorganic combinations are in agreement. Measurements also are unaffected by photochromic effects.

Experimental

Reagents

Mercury(II) nitrate stock standard solution, 1.00 mg ml^{-1}. A solution of mercury as mercury(II) nitrate in approximately 1 M nitric acid. This solution is used to prepare dilute standard solutions of mercury using 0.1 M hydrochloric acid as diluent.

Phenylmercury(II) acetate. Containing 100 ± 0.5% *m/m* of Hg calculated as $C_8H_8HgO_2$.

Phenylmercury(II) nitrate. Containing 97.6% *m/m* of $C_{12}H_{11}Hg_2NO_4$ determined by the procedure of the British Pharmacopoeia.[1]

Thiomersal. Minimum content 97% *m/m* of $C_9H_9HgNaO_2S$.

Dithizone. Use analytical-reagent grade material or purify if necessary.[6]

Dithizone stock solution, 0.05% *m/V in chloroform.* Store in a dark-glass bottle in a refrigerator.[6]

Dilute dithizone solution, 0.005% *m/V in chloroform.* Prepare by dilution of the stock solution. This solution may be kept in a dark-glass bottle at room temperature for at least 1 week.

Solutions of mercury in dilute dithizone solution. Dilute 1.0 ml of a suitable dilution in absolute ethanol of the mercury(II) nitrate stock standard solution to 10.0 ml using dilute dithizone solution as diluent.

Hydrochloric acid, 0.1 M. Prepare from acid supplied as "low in lead."

Chloroform.

Absolute ethanol.

Water. De-mineralise using an Elgastat or other suitable de-mineraliser.

Apparatus

A Varian Techtron CRA-90 carbon rod atomiser was used in conjunction with a Varian Techtron, Model AA-175, atomic-absorption spectrophotometer. The CRA-90 was kept cool with water flowing at a rate of 2 l min^{-1}, and set up using standard-size cup atomisers and with nitrogen as the inert gas at a pressure of 10 lb in^{-2}. A single-element mercury hollow-cathode lamp (Varian Techtron) was used as a line source with a lamp current of 3 mA to determine mercury at 253.7 nm. A spectral band-width setting of 0.5 nm was used for total absorbance measurements, and of 1.0 nm for corrected absorbance measurements. Atomisation peak absorbance signals were registered on a digital readout with the spectrophotometer set in the peak readout mode. Peak signals obtained during the temperature programme were recorded with an Oxford 3000 chart recorder, which was connected to the test socket of the spectrophotometer. Simultaneous measurements of corrected absorbance were made using a hydrogen lamp (Varian Techtron) in conjunction with the mercury lamp. Liquid samples were injected into the cup of the carbon rod using a 5-μl Autopette injection syringe (Excalibur Laboratories Ltd.) fitted with disposable polypropylene tips. Precautions against mercury contamination and loss were observed at all times. After washing, glassware was soaked overnight in 50% nitric acid, and rinsed well with water prior to use.[18]

Procedures

Extraction of mercury using dithizone

Procedure A: for aqueous solutions containing organic mercurial compounds as preservatives

or for sterilisation. Accurately dilute a suitable volume of the solution with 0.1 M hydrochloric acid to obtain 100 ml of test dilution containing the equivalent of about 0.05 μg ml^{-1} of mercury. Carry out the method of standard additions as follows. Pipette 20.0-ml aliquots of the test dilution into each of four separators to which have been added 0, 2.0, 3.0 and 4.0 ml of a dilute standard solution containing 0.5 μg ml^{-1} of mercury, and dilute to 25 ml with 0.1 M hydrochloric acid. Determine also a normal calibration graph by pipetting 2.0, 3.0 and 4.0 ml of the dilute standard solution containing 0.5 μg ml^{-1} of mercury into individual separators, and dilute each solution to 25 ml with 0.1 M hydrochloric acid. Measure 25 ml of 0.1 M hydrochloric acid into another separator for a blank determination.

Extract the solution in each separator by shaking once for 3 min with 2.0 ml of dilute dithizone solution. Allow the layers to separate, and run the lower layer into a glass-stoppered tube.

NOTE—The dilute dithizone solution should normally show no change from its original colour (blue-green) after extraction has been carried out. The appearance of a mixed colour or the characteristic orange colour of mercury(II) dithizonate indicates that the concentration or the volume of the test dilution used should be reduced. Interference from copper is not usual, but the presence of relatively large amounts of copper could cause some red copper(II) dithizonate to form after the reaction with mercury is complete.[6] This should not invalidate the determination.

Procedure B: for solid samples liable to contain mercury as an impurity. Accurately weigh a suitable amount of the solid, and dissolve it in 0.1 M hydrochloric acid to obtain a test dilution containing 0.003–0.005 μg ml^{-1} of mercury. As a preliminary trial, 10.0 g of solid can be made up to 100 ml of solution, but the mass needed will depend on the mercury content, and may be limited by the solubility of the sample. Carry out the method of standard additions as described for procedure A, using aliquots of 100 ml of the test dilution and adding 0, 2.0, 3.0 and 4.0 ml of a dilute standard solution containing 0.2 μg ml^{-1} of mercury. Pipette also 2.0-, 3.0- and 4.0-ml volumes of the dilute standard solution containing 0.2 μg ml^{-1} of mercury into individual separators for the determination of a normal calibration graph, and dilute each volume to 100 ml with 0.1 M hydrochloric acid. Measure 100 ml of 0.1 M hydrochloric acid into another separator for a blank determination.

Extract the solutions as described in the second paragraph in procedure A.

Selection of carbon rod atomiser control unit settings

Adjust the CRA-90 control unit settings to the following: dry, 80 °C for 45 s; ash, 220 °C for 15 s; and atomise, 1600 °C with a hold time of 3 s and a ramp rate of 600 °C s^{-1}. The sensitivity to mercury under these conditions is about 1.1×10^{-10} g to give 1% absorption at 253.7 nm. Table I shows that with higher atomisation temperatures or ramp rates there is no improvement in sensitivity. At lower atomisation temperatures and ramp rates there is some loss of sensitivity, although for some of the work described here an atomisation temperature of 1200 °C was used to prolong the life of the atomiser cup.

TABLE I

EFFECT OF ATOMISATION CONDITIONS ON MERCURY ABSORBANCE IN THE CRA-90 CARBON ROD ATOMISER

Injection: standard extract (2.0 μg ml^{-1} of Hg) in dilute dithizone solution (5 μl).

Atomisation temperature/°C (hold time 3 s)	1200			1600		1800	
Ramp rate/°C s^{-1}	300	600	800	600	800	600	800
Absorbance (corrected)	0.232	0.287	0.337	0.397	0.404	0.390	0.397

Preliminary trials are advisable for establishing suitable settings so that loss of mercury during drying or ashing is avoided. With the instrument used in this study, absorbance readings for mercury extracted with dithizone in chloroform remained constant with the dry setting at 80 °C maintained for 30 s up to at least 90 s. Table II shows that mercury(II) dithizonate in chloroform could be dried and ashed up to a temperature of 250 °C without loss of the metal, and possessed considerably greater thermal stability than mercury dissolved

TABLE II

EFFECT OF ASH TEMPERATURE ON MERCURY ABSORBANCE USING THE CRA-90 CARBON ROD ATOMISER FOR SOLUTIONS OF MERCURY CONTAINING 2 μg ml^{-1} OF Hg IN DILUTE DITHIZONE SOLUTION, 0.1 M NITRIC ACID AND 0.01 M HYDROCHLORIC ACID

Atomisation temperature, 1200 °C for 3 s; ramp rate, 300 °C s^{-1}.

Diluent	Dry settings Temperature/°C	Dry settings Time/s	Ash settings Temperature/°C	Ash settings Time/s	Mean Hg absorbance (uncorrected)	Standard deviation (9 degrees of freedom)	Blank absorbance
Dilute dithizone solution	80	60	150	15	0.255	0.011	0.015
			200	30	0.244	0.005	0.015
			200	60	0.254	0.012	0.015
			250	30	0.247	0.008	0.015
			300	30	0.058	0.013	0.010
0.1 M HNO_3	130	90	150	30	0.093	0.018	0.020
			200	30	0.149	0.024	0.000
			250	30	0.088	0.012	0.002
0.01 M HCl	130	90	150	30	0.020	—	0.000
			200	30	0.051	—	0.000
			250	30	0.017	—	0.000

in acidified aqueous solution. A further advantage is that high ashing temperatures are unnecessary for the dithizonate or for excess of dithizone, as the background absorption produced during atomisation is low, even though ashing conditions are unlikely to have been efficient for the destruction of organic matter. The ash temperature setting can, however, affect the release of mercury during the atomisation stage. Thus, with an ash setting of 200 °C for 15 s, lower absorbance readings were obtained for mercury extracted with dithizone from phenylmercury(II) acetate or phenylmercury(II) nitrate than for equivalent amounts of mercury extracted from mercury(II) nitrate or thiomersal, and this effect was independent of the atomisation temperature used. The responses only became equal for each substance when the ash temperature setting was increased to 220 °C for 15 s.

Determination of mercury

Measure the corrected absorbance of the dithizone extracts, adjusting the sensitivity (scale expansion) so that the readings lie within the range 0.2–0.8 absorbance unit. In this work, procedure A was carried out with a scale expansion of about $\times 2$ and procedure B with a scale expansion of $\times 4$. Determine the mean response for at least five absorbance measurements for each extract. The response for the blank dithizone extract should be zero.

Plot the standard-additions calibration graph of corrected absorbance against concentration of mercury added. The absorbance reading at the intercept of the plot on the absorbance axis should equal the corrected absorbance reading obtained for the test dilution extract (with no added mercury). The concentration of mercury in the test dilution can be read from the graph in the usual way, taking the intercept on the concentration axis as zero concentration. Where the slope of the standard-additions graph is seen to be the same as that of the normal calibration graph, it becomes unnecessary to use a standard-additions graph, and the concentration of mercury in the test dilution can be determined by reference to a normal calibration graph.

Results and Discussion

Calibration graphs (Fig. 1) of corrected absorbance *versus* concentration obeyed Beer's law over the range 0.2–2.0 μg ml^{-1} of mercury both for extracts and for direct solutions of mercury made with dithizone in chloroform. At higher concentrations, a pronounced negative deviation was observed, which could not be prevented either by changing the atomisation temperature over the range 1000–1800 °C, or by altering the dithizone concentration over the range 0.005–0.02% *m*/*V*. The use of 0.005% *m*/*V* dithizone solution in the recommended procedure for the extraction of mercury from 0.1 M acid solution was found to be convenient, as any change from the normal blue - green colour of the extract to purple - green or eventually to orange served to indicate not only that the extraction of mercury might be incomplete, but also that the mercury concentration was too high for measurements to be within the linear range of the calibration graph. To determine its

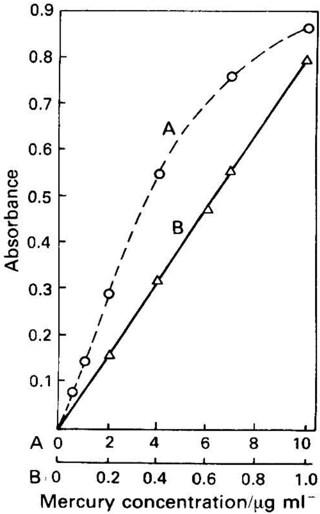

Fig. 1. Calibration graphs for mercury in (A) 0.02% *m/V* and (B) 0.005% *m/V* dithizone in chloroform determined with a CRA-90 carbon rod atomiser. Absorbance scale expansion: (A) ×1 and (B) ×4. Atomisation temperature: 1 200 °C.

precision, the dithizone extraction procedure was carried out in triplicate, and absorbance measurements made as shown in Table III. The mean relative deviation from the mean of the absorbances determined for the three extractions is 2%.

The recovery of mercury from organic mercurial preservatives was tested using freshly prepared solutions containing accurately weighed amounts of each of the compounds thiomersal, phenylmercury(II) acetate and nitrate, which were analysed for their mercury content after dilution and extraction according to procedure A. A dithizone extract of mercury(II) chloride containing 0.5 $\mu g\ ml^{-1}$ of mercury was used as reference standard. The results (Table IV) show a good recovery of mercury from each of the compounds tested, and signify that a preliminary wet-digestion stage can be avoided in the determination. A relative standard deviation of 3.9% was calculated for the procedure using five extracts of separate aliquots of the same dilution containing thiomersal. The results are shown in Table V.

Table III

Precision of dithizone extraction and mercury absorbance measurements using the CRA-90 carbon rod atomiser

Extraction of 4.0 μg of mercury from 100 ml of 0.1 M hydrochloric acid into 2.0 ml of dilute dithizone solution. Atomisation temperature, 1 600 °C.

Extraction No.	Mean total absorbance	Degrees of freedom	Standard deviation	Deviation from mean of 3 extractions
1	0.396	9	0.006	0.012
2	0.382	9	0.004	0.002
3	0.374	9	0.008	0.010
Mean ..	0.384		Mean ..	0.008

TABLE IV

DETERMINATION OF MERCURY IN DITHIZONE EXTRACTS OF STANDARD SOLUTIONS OF ORGANIC MERCURIAL PRESERVATIVES WITH THE CRA-90 CARBON ROD ATOMISER USING PROCEDURE A

Compound	Sample mass/ mg	Sample mass extracted/μg	Mercury content of extract/μg Found	Calculated	Recovery, %	Mean recovery, %
Thiomersal..	202.3	2.023	0.988	1.002	98.6	102
	198.1	1.981	1.034	0.982	105.3	
Phenylmercury(II) acetate	167.4	1.674	0.953	0.997	95.6	98
	168.5	1.685	0.998	1.004	99.4	
Phenylmercury(II) nitrate (97.6% pure)	83.50	1.670	0.988	1.031	101.3	99
	87.35	1.747	1.092	1.078	95.8	

TABLE V

PRECISION OF DETERMINATION OF MERCURY IN DITHIZONE EXTRACTS OF A SOLUTION OF THIOMERSAL USING PROCEDURE A

Test dilution: thiomersal sample mass of 0.2018 g made up to 100 ml in water and an aliquot of the solution diluted in 0.1 M hydrochloric acid to contain 2.018 μg in the volume extracted (20.0 ml) into 2.0 ml of dilute dithizone solution.

Extraction	Corrected absorbance ($\times$ 2)* Mean of 5 readings	Range	Standard deviation (9 degrees of freedom)	Mercury recovery,† %
1	0.195	0.176–0.201	0.016	101.0
	0.196	0.173–0.210		101.6
2	0.190	0.170–0.212	0.010	98.4
	0.189	0.186–0.192		97.9
3	0.184	0.175–0.195	0.009	95.3
	0.179	0.162–0.182		92.7
4	0.197	0.174–0.222	0.021	102.1
	0.188	0.166–0.219		97.4
5	0.201	0.191–0.207	0.006	104.1
	0.203	0.196–0.210		105.2
			Mean:	99.6
			Relative standard deviation:	3.9%

* Absorbance scale expansion $\times$ 2.

† Mean corrected absorbance determined for three extracts containing 0.5 μg ml^{-1}. of mercury from a dilute standard solution of mercury(II) chloride = 0.193.

Various compounds were tested for the presence of mercury as an impurity, as well as for their effect on the recovery of a standard addition of mercury. Recoveries of mercury were quantitative from samples of lactic acid, citric acid, sodium chloride, potassium chloride, potassium citrate, zinc sulphate, iron(II) sulphate, nicotinamide, sulphacetamide and glycerol, using 100 ml of a 10% *m*/*V* solution of each compound in 0.1 M hydrochloric acid (sulphacetamide was dissolved with the aid of an extra equivalent volume of hydrochloric acid). Recoveries of mercury were also quantitative from a 2% *m*/*V* solution of nicotinic acid and a 5% *m*/*V* solution of calcium lactate. However, recovery was inhibited by potassium bromide and potassium iodide, both of which will also interfere in the cold vapour technique.[3] Mercury was detected as an impurity only in samples of potassium chloride, sulphacetamide, nicotinamide and citric acid. The background (molecular) absorbances due to these compounds in the extracts were 0, 0.9, 0.6 and 0.03, respectively.

The compounds were then assayed for mercury by procedure B, taking 10-g amounts of sample per 100 ml of test dilution, and making three standard additions of mercury. The results (Table VI) were compared with those obtained by the cold vapour method,[3,19] which, with the exception of potassium chloride, was carried out after a preliminary wet digestion[6] of the sample with nitric acid and sulphuric acids. Reasonable agreement of results was obtained by the two procedures for potassium chloride and sulphacetamide. With the nicotinamide and citric acid samples, repeated attempts to digest the samples, and subsequently to determine the mercury by the cold vapour technique or by using dithizone

TABLE VI

COMPARISON OF THE CRA-90 (PROCEDURE B) AND COLD VAPOUR METHODS FOR THE DETERMINATION OF MERCURY IN VARIOUS CHEMICALS

Sample	Mercury found, p.p.m.	
	CRA-90 method	Cold vapour method
Potassium chloride	0.052	0.058
Sulphacetamide	0.072	0.066
Nicotinamide	0.030	No recovery
Citric acid	0.044	No recovery

extraction, were unsuccessful. The standard-additions calibration graph obtained by the electrothermal atomisation procedure B had the same slope as the normal calibration graph for each of the four compounds. The determination of a standard-additions calibration graph does, even in the absence of matrix effects, help to prevent errors due to contamination of solutions, which is a problem that requires constant attention in mercury analysis. For the determination of mercury in some water-soluble compounds it is not always convenient to adjust the test dilution to an acidic pH. In testing for mercury in sodium hydrogen carbonate, for example, an aqueous 10% *m/V* solution was extracted directly with dilute dithizone solution. The omission of acid made other metals also liable to extraction, and the sodium hydrogen carbonate caused the extract to show a colour change to red. With the sample examined, no mercury could be found in the extract, but standard additions of mercury were recovered. In such instances, more than one extraction with dithizone should be made, until complete extraction of any mercury is assured.

Work is in progress to evaluate further applications for the determination of mercury by this procedure.

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Gravimetric Determination of Nickel with Thiosemicarbazones

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A systematic study of the use of thiosemicarbazones in the gravimetric determination of nickel is reported. The compounds tested were furfural thiosemicarbazone (FAT), thiophen-2-aldehyde thiosemicarbazone (TAT) and furfural 4-phenyl-3-thiosemicarbazone (FAPT). FAPT is the most appropriate reagent owing to its sensitivity and selectivity. FAPT has been applied to the gravimetric determination of nickel in diverse standard samples. The reagents tested in this work were compared with classical *vic*-dioximes used in the gravimetric determination of nickel.

Keywords: *Furfural thiosemicarbazone; thiophen-2-aldehyde thiosemicarbazone; furfural 4-phenyl-3-thiosemicarbazone; nickel determination; gravimetry*

Thiosemicarbazones have been used as gravimetric reagents for some metal ions: nickel with β-resorcylaldehyde thiosemicarbazone[1]; copper with dihydroxybenzaldehyde, *p*-dimethylaminobenzaldehyde and cinnamaldehyde thiosemicarbazones[2]; cadmium with salicylaldehyde thiosemicarbazone[3] and β-resorcylaldehyde thiosemicarbazone[4]; mercury with *p*-ethylsulphonylbenzaldehyde thiosemicarbazone[5]; and palladium with *p*-ethylsulphonylbenzaldehyde thiosemicarbazone,[6] furfural thiosemicarbazone,[7] thiophen-2-aldehyde and benzaldehyde thiosemicarbazones[8] and furfural 4-phenyl-3-thiosemicarbazone.[9] With palladium, the precipitates formed (pH 3–5) are easy to filter off and stable, and can be dried at 170 °C; their compositions are PdL_2 (HL being the undissociated thiosemicarbazones, which are weak acids). The complexes are slightly soluble and their high relative molecular masses give appreciably higher sensitivities than classical reagents such as dimethylglyoxime, furfuraldoxime and thiophenaldoxime.

In this work, a systematic study was made of the use of thiosemicarbazones in the gravimetric determination of nickel. The reagents tested were furfural thiosemicarbazone (FAT), thiophen-2-aldehyde thiosemicarbazone (TAT) and furfural-4-phenyl-3-thiosemicarbazone (FAPT). Interferences of foreign ions were examined. FAPT reagent was applied to the determination of nickel in real samples. The results are compared with those obtained by the use of *vic*-dioximes.

Experimental

Reagents

All chemicals were of analytical-reagent grade. Thiosemicarbazones were obtained by the methods previously described.[7–9] Stock solutions of the reagents were prepared in ethanol at concentrations of 0.9% *m*/*V* for FAT, 0.5% *m*/*V* for TAT and 0.2% *m*/*V* for FAPT. The solutions of nickel were prepared from the nitrate salt and were standardised with dimethylglyoxime before use. Acetate buffer of pH 3.7 was prepared by mixing 450 ml of 2.0 M acetic acid with 50 ml of 2.0 M sodium acetate and diluting to 1 l with water.

Apparatus

pH measurements were made using a Philips PW 9408 potentiometer with a combined glass - calomel electrode. Thermogravimetric curves were obtained with an Adamel 93 thermobalance. Infrared spectra were recorded with a Beckman Acculab-2 spectrophotometer (in potassium bromide discs). Nuclear magnetic resonance (NMR) spectra were obtained with a Perkin-Elmer R-12B spectrometer. Magnetic susceptibilities were measured with a Gouy balance.

Procedures

Determination of nickel with FAT

Take a sample solution containing 10–50 mg of nickel, adjust the pH to 8.0–11.0 with an ammonia buffer solution and dilute to 100–150 ml. Add 10–50 ml of 0.9% FAT solution with constant stirring and heat the mixture in a water-bath for 30 min. After 1 h, collect the precipitate on a weighed G-4 sintered-glass crucible, wash with 200–300 ml of 10% ammonia solution in 1+5 ethanol - water, and then with 100–200 ml of 1+5 ethanol - water. Dry at 70–90 °C and weigh. The conversion factor for $Ni(C_6H_6N_3SO)_2$ to Ni is 0.1486.

Determination of nickel with TAT

Place an aliquot of the nickel solution (10–50 mg of nickel) in a beaker, adjust the pH to 5.0–11.0 with acetate or ammonia buffer solution, dilute to 100–150 ml and add 20–100 ml of 0.5% TAT solution. Heat the mixture in a water-bath for 30 min. After 1 h collect the precipitate on a weighed G-4 sintered-glass crucible, wash with 200–300 ml of 10% ammonia solution in 1+4 ethanol - water, and then with 100–200 ml of 1+4 ethanol - water. Dry at 70–90 °C and weigh. The conversion factor for $Ni(C_6H_6N_3S_2)_2$ to Ni is 0.1374.

Determination of nickel with FAPT

Take a solution containing 5–50 mg of nickel, adjust the pH to 3.3–11.0 with acetate or ammonia buffer solution and dilute to about 100 ml. Add 30–300 ml of 0.2% FAPT solution with constant stirring and heat the mixture in a water-bath for 30 min. After 30–60 min collect the precipitate and wash it with 300–400 ml of a 0.1 M sodium hydroxide solution, and then with 200–300 ml of 2+3 ethanol - water. Dry at 70–90 °C and weigh. The conversion factor for $Ni(C_{12}H_{10}N_3SO)_2$ to Ni is 0.1073.

Analysis of Real Samples

Determination of nickel in Raney nickel

A 1-g amount of sample was dissolved in 50 ml of dilute hydrochloric acid (1+1), the solution was evaporated to dryness, the residue was dissolved in water and the solution was diluted to 100 ml with water. Aliquots of 10 ml were used in the gravimetric determination of nickel with dimethylglyoxime in accordance with the literature.[10] A similar procedure was used with FAPT, with 25 ml of acetate buffer at pH 3.7.

Determination of nickel in alloy cast iron

A 10-g amount of sample was treated with 100 ml of a mixture of sulphuric and orthophosphoric acids (20 ml of 96% sulphuric acid plus 10 ml of 85% orthophosphoric acid and 70 ml of water), and boiled for 1 h nearly to dryness. It was then treated with 200–300 ml of distilled water and acidified, drop by drop, with concentrated sulphuric acid to dissolve the precipitate. The silicic acid that was precipitated was filtered off and the solution was diluted to 500 ml with water. Aliquots of 50 ml were treated with 50 ml of dilute ammonia solution (1+1) and then filtered on Albert 238 filter-paper (equivalent to Whatman 41). The precipitate was dissolved by adding hot hydrochloric acid dropwise and the solution was collected in a beaker, reprecipitated with 50 ml of ammonia (1+1) and filtered on the same filter-paper. The solution was concentrated to 300 ml and nickel was determined with FAPT at pH 3.7 with 25 ml of acetate buffer. Alloy Cast Iron 33b had the following certificate composition: iron 91.72%, carbon 2.24%, silicon 2.00%, sulphur 0.035%, phosphorus 0.11%, manganese 0.64%, nickel 2.24%, chromium 0.61% and molybdenum 0.40%.

Determination of nickel in chromium nickel steel

A 2-g amount of sample was dissolved in 80 ml of aqua regia and a small amount of hydrogen peroxide. The solution was evaporated to dryness, redissolved and diluted to 200 ml with water. Aliquots of 25 ml were then treated using the above procedure commencing with the addition of ammonia. Nickel was then determined using FAPT at pH 3.7 with 25 ml of acetate buffer. The standard composition of the steel was iron 68.15%, chromium 17.77%, nickel 11.84%, molybdenum 2.15% and carbon 0.09%.

Determination of nickel in nickel stone

A 1-g amount of sample was dissolved with 100 ml of aqua regia - bromine (3+1) and the solution was evaporated to dryness. The residue was dissolved in 25 ml of concentrated hydrochloric acid, the solution evaporated to dryness, the residue dissolved in water and the solution diluted to 100 ml with water. Silicic acid was filtered off. Aliquots of 5 ml were treated with hydrogen sulphide at pH 0–1 to remove copper. After filtration on Albert 240 filter-paper (equivalent to Whatman 44), nickel was determined using FAPT with 25 ml of acetate buffer at pH 3.7. The standard composition of the stone was nickel 55.89%, copper 30.03%, sulphur 8.53%, arsenic 0.04%, silica 0.53% and the sum of iron(III) and aluminium oxides 1.58%.

Determination of nickel in aluminium bronze

A 10-g amount of sample was dissolved in 100 ml of aqua regia and a small amount of hydrogen peroxide. The solution was evaporated to dryness, the residue was dissolved in 25 ml of concentrated hydrochloric acid, the solution again evaporated to dryness, the residue redissolved in water and the solution diluted to 100 ml with water. Aliquots of 25 ml were treated with hydrogen sulphide to remove copper, and nickel was determined at pH 3.7 using FAPT in the presence of 400 mg of tartrate and 25 ml of acetate buffer. Aluminium Bronze 32a had the following certificate composition: copper 85.9%, iron 2.67%, aluminium 8.8%, nickel 1.16%, manganese 0.27% and zinc 0.94%.

Determination of nickel in aluminium alloy

A 10-g amount of sample was treated in the same manner as the aluminium bronze. Aliquots of 25 ml were treated first with ammonia, then with hydrogen sulphide following the methods described above. Nickel was determined using FAPT with 25 ml of acetate buffer at pH 3.7. Aluminium Alloy 20b had the following certificate composition: aluminium 91.45%, copper 4.10%, nickel 1.93%, iron 0.43%, manganese 0.19%, silicon 0.29% and magnesium 1.61%.

Results and Discussion

Properties of the Reagents

The thiosemicarbazones studied are poorly soluble in water, but their solubilities increase in alkaline medium owing to the deprotonation of the thiol group, which possesses a weak acid character. Solubilities in ethanol are 11.5, 8.0 and 2.5 g l^{-1} for FAT, TAT and FAPT, respectively. Solutions in ethanol stored at room temperature were stable for at least 1 month. Values of the apparent ionisation constants are 2.0×10^{-11} for FAT and TAT[11] and 1.6×10^{-11} for FAPT.[9] Slow hydrolysis of the reagents to aldehyde and amine occurs in dilute solutions (10^{-4}–10^{-5} M) at pH 1.

The compounds tested appear to be bidentate ligands with a convenient steric arrangement of their donor group, and contain a conjugate system of π-electrons connected with the donor group. The chelates are uncharged.

Study of Nickel - Thiosemicarbazone Complexes

Elemental analysis of the nickel complexes confirmed the formula NiL_2. A study was made to elucidate the structures. The infrared spectra show an appreciable increase in the C=N stretching vibration bands in relation to the reagents at 1600–1620 cm^{-1}, and double bands appear; on the other hand, bands due to the C=S group disappear and C–S vibration bands show up.

NMR spectra in deuterated dimethyl sulphoxide show multiplets between 6.5 and 8.0 p.p.m. due to the aromatic and amine groups. The ultraviolet spectra show a broad band in each instance, with maximum absorption at 335, 340 and 386 nm for FAT, TAT and FAPT, respectively.

A study of the magnetic susceptibilities showed that the nickel - thiosemicarbazone complexes tested are diamagnetic at 293 K.

From these results it can be concluded that the complexes studied are square planar. In general, thiosemicarbazones usually react as chelating ligands with transition metals ions by bonding through the sulphur and hydrazine nitrogen atoms,[12] so that the structure of the

nickel complexes could be as shown in Fig. 1, or the alternative one with the two sulphur and the two nitrogen atoms occupying mutually *trans*-positions.

Fig. 1. Structure of the nickel complexes.

Thermal Analysis

Thermogravimetric curves for the nickel complexes were recorded (Fig. 2). The complexes are stable up to 180 °C; a sharp decrease occurs at 200 °C and also at 450 °C. Suitable drying conditions were investigated. In all subsequent work, drying of the precipitates was always carried out at 70–90 °C.

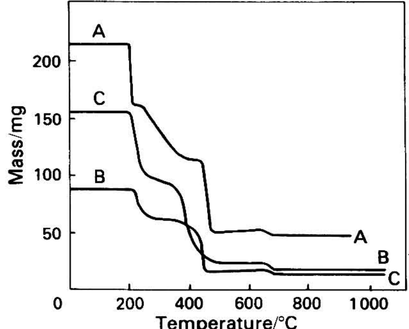

Fig. 2. Thermogravimetric curves for the nickel complexes: A, Ni - FAT; B, Ni - TAT; and C, Ni - FAPT.

Determination of the Optimum Experimental Conditions for Precipitation

The influence of various solvents for washing the precipitates was studied. Pure water is unsatisfactory and, in general, mixtures of ethanol and water are more suitable. The optimum percentage of ethanol is different in each instance (Fig. 3) and must therefore be controlled. The volume of washing solutions can be reduced if the precipitates are previously treated with

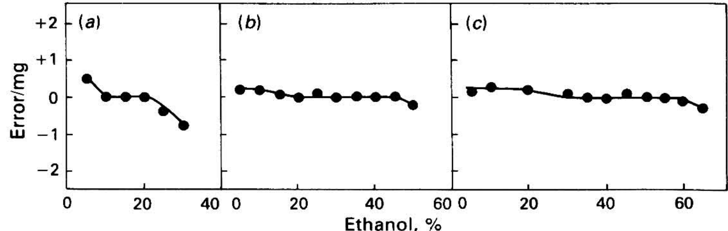

Fig. 3. Influence of ethanol used for washing. Amount of nickel, 25.9 mg. Reagents: (*a*) FAT; (*b*) TAT; and (*c*) FAPT.

an alkaline solution: ammonia-water (1+9) solution for FAT and TAT and 0.1 M sodium hydroxide solution for FAPT. Owing to the acidic character of thiosemicarbazones, small amounts of coprecipitated reagents can be easily removed.

The effect of increasing amounts of reagents was investigated in each instance. In general, excesses of 30–40% over the stoicheiometric amounts are adequate.

The influence of temperature on the precipitation of nickel complexes was investigated (Fig. 4). With FAPT, precipitation can be carried out at room temperature or by heating to 100 °C. With FAT and TAT, precipitation must be effected by heating to over 50 °C in a water-bath; incomplete precipitation occurs at room temperature with both of these reagents.

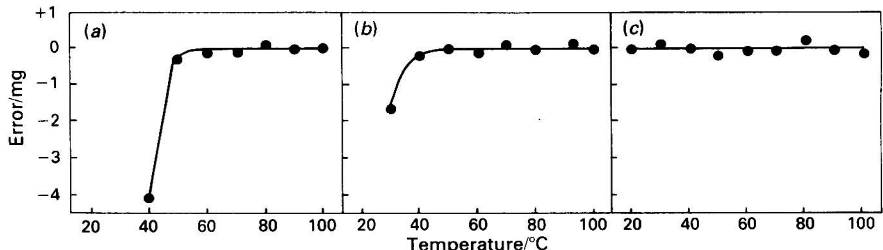

Fig. 4. Influence of temperature. Amount of nickel, 24.2 mg. Reagents: (*a*) FAT; (*b*) TAT; and (*c*) FAPT.

To determine the optimum pH, complexes were precipitated from solutions at different pH values, adjusted by means of chloroacetate, acetate, phosphate and ammonia buffers, using the methods previously outlined. For Ni - FAT the optimum pH is 8.4–11.0, for Ni - TAT 5.0–11.0 and for Ni - FAPT 3.4–11.0 (Fig. 5).

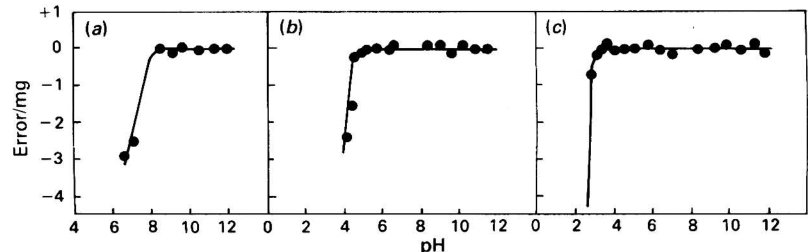

Fig. 5. Influence of pH. Amount of nickel, 25.9 mg. Reagents: (*a*) FAT; (*b*) TAT; and (*c*) FAPT.

Evaluation of the Relative Error of the Methods

Twelve determinations were made in each instance with the same amount of nickel. The results are shown in Table I.

TABLE I

RELATIVE ERROR OF THE METHODS

Reagent	Amount of Ni/mg	Standard deviation/mg	Standard deviation of the mean/mg	Relative error, % (P = 0.05)
FAT	48.5	0.091	0.026	±0.1
TAT	48.5	0.135	0.039	±0.2
FAPT	47.4	0.074	0.021	±0.1

Interferences

Interference by foreign ions was investigated. Amounts up to at least 0.1 g of the following ions do not interfere: alkali and alkaline earth metals, chromium(III), antimony(III), aluminium, bismuth, manganese(II), tin(II), lead(II), phosphate, fluoride, citrate, oxalate, tartrate, thiosulphate, thiocyanate, arsenate and arsenite. With FAT reagent, serious interferences were observed with iron(III), copper(II), cobalt(II), mercury(II), silver, zinc, cyanide and EDTA; 10 mg of cadmium does not interfere.

With TAT, iron(III), zinc and cadmium do not interfere in acetate buffer; nor does 10 mg of cobalt in the same medium at pH 5.0–5.2, but it is necessary to wash the precipitate with ethanol - water (2+3). Copper(II), mercury(II), silver, cyanide and EDTA interfered.

With FAPT, iron(III), zinc, cadmium and small amounts of mercury(II) or silver ($\leqslant$5 mg) do not interfere in acetate buffer medium. Cobalt up to 20 mg does not interfere at pH 3.4–3.6 if the precipitate is washed with ethanol - water (1+1 or 3+2). Copper interferes at low concentrations, but it can be previously precipitated as the sulphide. Serious interferences were observed with cyanide and EDTA.

Applications

The gravimetric determination of nickel with FAPT reagent was applied to standard samples of Raney nickel, alloy cast iron, chromium nickel steel, nickel stone, aluminium bronze and aluminium alloy. The results obtained are given in Table II.

TABLE II

DETERMINATION OF NICKEL WITH FAPT IN STANDARD SAMPLES

Sample	Nickel content, % *m/m* (standard value)	Nickel found, % *m/m**
Raney nickel	30.44†	30.41
Alloy cast iron	2.24	2.14
Chromium nickel steel	11.84	11.87
Nickel stone	55.89	55.85
Aluminium bronze	1.16	1.14
Aluminium alloy	1.93	1.95

* Each result is the mean of three determinations.
† Determined with dimethylglyoxime. Mean result of three determinations.

Conclusion

We have examined gravimetric procedures for the determination of nickel using three thiosemicarbazones. FAPT is the preferred reagent because precipitation occurs at a lower pH, which gives greater selectivity in its behaviour with metal ions. It also exhibits the most favourable gravimetric factor, and the Ni - FAPT complex is the least soluble.

The justification for a new gravimetric method for nickel is difficult because *vic*-dioximes behave as excellent reagents for this metal ion[13–15]; nevertheless, the results obtained with FAPT reagent are comparable to those obtained with the use of *vic*-dioximes, so FAPT can be used as an effective quantitative precipitant of nickel ion. Although FAPT reagent is less soluble in ethanol than *vic*-dioximes, it exhibits a most favourable gravimetric factor (Table III), which implies greater sensitivity.

The cost of FAPT is similar to that of dimethylglyoxime (Table III), but the operating time

TABLE III

GRAVIMETRIC FACTOR AND RELATIVE COST OF SOME REAGENTS FOR NICKEL

Reagent	Gravimetric factor for nickel	Relative cost
Dimethylglyoxime	0.2032	1
Nioxime	0.1722	32
Heptoxime	0.1590	160
FAT	0.1486	0.6
TAT	0.1374	1.4
α-Furyldioxime	0.1181	55.5
α-Benzyldioxime	0.1093	16
FAPT	0.1073	1.8

is shorter. A further advantage of the reagents tested includes the excellent mechanical properties of the precipitates, which are crystalline and very easy to filter.

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Precipitation of Selenium and Tellurium from Homogeneous Solutions by Dimethyl Sulphite

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Elemental selenium and tellurium are precipitated from homogeneous solutions by employing dimethyl sulphite for the *in situ* production of sulphur dioxide. Results obtained show that both selenites and selenates as well as tellurites and tellurates are quantitatively reduced by this reagent. The advantages of this precipitation over the conventional precipitation procedure are outlined. Based on the different conditions under which the two elements are precipitated, a procedure for the separation of selenium and tellurium from one another is reported.

Keywords: *Selenium determination; tellurium determination; homogeneous precipitation; dimethyl sulphite; separation*

Most of the gravimetric methods for determining selenium and tellurium are based on reduction to their elemental forms in hydrochloric acid. Various reducing agents[1–14] have been used for this purpose. Of these, sulphur dioxide was considered to be the most suitable reagent; it reduces selenium when it is in solutions greater than 3 N in hydrochloric acid and tellurium in 2–4 N solutions of the same acid. Lenher and Homberger,[15] however, reported that the reduction of tellurium with sulphur dioxide alone is too slow and that the precipitated tellurium is so very finely divided that it oxidises readily, in spite of washing with alcohol and diethyl ether. They therefore recommended the use of both sulphur dioxide and hydrazine hydrochloride for effecting the reduction. However, when tellurium is precipitated by this method using sulphur dioxide and hydrazine hydrochloride, the precipitated tellurium was found to stick to the walls of the beaker and to the bottom of the watch-glass, making its complete transfer to the filter very difficult.

As precipitation from homogeneous solution would produce purer precipitates with good filtration characteristics, and as there are not many methods for the homogeneous precipitation of selenium and tellurium, it was thought worthwhile to see whether sulphur dioxide generated under homogeneous conditions would give improved precipitates. We also investigated whether tellurium could be quantitatively reduced under these conditions without the necessity of a further reducing agent, as in the conventional method. Dimethyl sulphite,[16] which hydrolyses to give sulphur dioxide according to the reaction

$$CH_3O.SO.OCH_3 + H_2O \rightarrow SO_2 + 2CH_3OH$$

was employed for this purpose and the results obtained are presented in this paper.

Experimental

Reagents

Selenium(IV) solution. A 10.0-g sample of sodium selenite (Riedel, Germany) was dissolved in water, transferred into a 1-l calibrated flask and made up to the mark with water.

Selenium(VI) solution. A 4.7032-g sample of sodium selenate (BDH Chemicals Ltd.) was dissolved in water, transferred into a 250-ml calibrated flask and made up to the mark with distilled water.

The selenium contents of the two solutions were determined by independently analysing the respective salts by the standard method.[17]

Tellurium(IV) solution. A 1.9632-g sample of sodium tellurite (BDH Chemicals Ltd.) was dissolved in about 50 ml of concentrated hydrochloric acid by gently warming. The

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solution, after cooling, was transferred into a 1-l calibrated flask and diluted to the mark with water.

Tellurium(VI) solution. A 4.8893-g sample of sodium tellurate (BDH Chemicals Ltd.) was dissolved in 25 ml of concentrated hydrochloric acid and made up to the mark with water in a 250-ml calibrated flask.

The two solutions were analysed for their tellurium contents by the standard method.[17]

Dimethyl sulphite. E. Merck, Darmstadt, Germany, was used as supplied.

All the other chemicals used were of analytical-reagent grade.

Procedure

Determination of selenium

Known volumes of the standard selenium solutions were treated with concentrated hydrochloric acid, 80 ml for the selenium(IV) and 100 ml for the selenium(VI), followed by 40 ml of methanol. The contents were diluted to 150 ml with water and cooled to room temperature. A 2-ml aliquot of the dimethyl sulphite was then added, with stirring. The beaker containing these reagents was left for about 2 h, with occasional stirring. The red selenium precipitate was filtered through a previously weighed sintered-glass crucible (porosity 4), washed thoroughly with water and finally alcohol, dried at 100 °C for 1 h, cooled and weighed as elemental selenium.

Determination of tellurium

Concentrated hydrochloric acid [20 ml for tellurium(IV) and 30 ml for tellurium(VI) solutions] was added to known volumes of the tellurium standard solutions; 25 ml of methanol were then added to each mixture. The solutions were diluted to about 100 ml with water and treated with about 2 ml of dimethyl sulphite, with stirring. The beaker was then kept over a boiling water-bath for about 1.5–2 h, with occasional stirring. Losses of solution due to evaporation were not replaced. The precipitate was filtered through a weighed 1G4 sintered-glass crucible (porosity 4), washed with hot water and alcohol, dried at 105–110 °C for 1 h, cooled and weighed as elemental tellurium.

Results and Discussion

Determination of Selenium

Table I gives results for the determination of selenium in selenium(IV) and selenium(VI) compounds with reasonable accuracy. The results agree well with those obtained by the conventional method.[17] For a series of six determinations on 45.64 mg of selenium, the standard deviation and the relative mean error were 0.155 mg and 0.13%, respectively.

TABLE I

DETERMINATION OF SELENIUM

Selenites			Selenates		
Selenium(IV) taken/mg	Selenium(IV) found/mg	Error, %	Selenium(VI) taken/mg	Selenium(VI) found/mg	Error, %
22.82	22.70	−0.53	15.86	15.90	+0.25
45.64	45.60	−0.09	39.64	39.80	+0.40
68.46	68.30	−0.23	79.28	79.70	+0.53
91.28	91.40	+0.13	118.92	119.10	+0.15
114.10	114.40	+0.26			

Determination of Tellurium

Results for the determination of tellurium in tellurium(IV) and tellurium(VI) compounds are given in Table II. The standard deviation and the relative mean error of six determinations on 45.40 mg of tellurium were 0.061 mg and 0.46%, respectively.

Effect of Hydrochloric Acid on the Reduction of Selenium and Tellurium

Mellor and Thompson[18] reported that the concentrations of hydrochloric acid suitable for the reduction of selenium and tellurium were 30% and 15–16%, respectively. Experiments

TABLE II

DETERMINATION OF TELLURIUM

Tellurites			Tellurates		
Tellurium(IV) taken/mg	Tellurium(IV) found/mg	Error, %	Tellurium(VI) taken/mg	Tellurium(VI) found/mg	Error, %
18.16	18.20	+0.22	22.70	22.60	−0.44
36.32	36.50	+0.49	45.40	45.50	+0.22
45.40	45.50	+0.22	90.80	91.30	+0.55
90.80	91.30	+0.55	113.50	114.20	+0.62
108.96	108.60	−0.33			

were conducted on the effect of this acid on the reductions using the proposed method, by adding varying amounts of acid to known amounts of selenium and tellurium containing 25 ml of methanol and 5 ml of dimethyl sulphite in a total volume of 100 ml. The precipitates were filtered and weighed as described previously. The results are recorded in Tables III and IV.

From the Tables, it is clear that selenium(IV) is quantitatively reduced in solutions of greater than 5 N, while selenium(VI) is reduced in greater than 6 N acid solutions. The reduction of tellurium(IV) was found to be complete in 1–2 N hydrochloric acid solution while tellurium(VI) was quantitatively reduced only at a higher acid concentration of 3 N.

TABLE III

EFFECT OF HYDROCHLORIC ACID ON THE REDUCTION OF SELENITES AND SELENATES

Selenites*			Selenates†		
Acidity/N	Selenium(IV) found/mg	Selenium(IV) recovery, %	Acidity/N	Selenium(VI) found/mg	Selenium(VI) recovery, %
1.0	7.0	15.3	5.0	30.2	76.2
2.0	24.7	54.1	6.0	39.8	100.4
3.0	31.3	90.5	7.0	39.8	100.4
4.0	44.3	97.1	8.0	39.7	100.2
5.0	45.4	99.5	9.0	39.5	99.7
6.0	45.6	99.9			
7.0	45.5	99.7			
8.0	45.5	99.7			
9.0	45.6	99.9			

* Amount of selenium(IV) taken, 45.64 mg.
† Amount of selenium(VI) taken, 39.64 mg.

TABLE IV

EFFECT OF HYDROCHLORIC ACID ON THE REDUCTION OF TELLURITES AND TELLURATES

Tellurites*			Tellurates†		
Acidity/N	Tellurium(IV) found/mg	Tellurium(IV) recovery, %	Acidity/N	Tellurium(VI) found/mg	Tellurium recovery, %
0.5	44.9	98.9	1.0	10.5	23.1
1.0	45.4	100.0	2.0	42.3	93.1
1.5	45.5	100.2	3.0	45.6	100.4
2.0	45.5	100.2	4.0	44.9	98.9
2.5	45.2	99.5			
3.0	45.1	99.3			

* Amount of tellurium(IV) taken, 45.40 mg.
† Amount of tellurium(VI) taken, 45.40 mg.

Effect of Reagent Concentration

Although theoretical calculations showed that 0.1 ml of the dimethyl sulphite could bring about a quantitative reduction of 100 mg of both selenium and tellurium, it was found from experiments that a minimum of 1 ml of the reagent has to be used to obtain satisfactory results.

Effect of Methanol Concentration

As the reagent was not soluble in aqueous solutions, water-miscible organic solvents, such as methanol, were employed to bring about homogeneous conditions for the precipitation. It was found that the reagent was completely soluble when the methanol concentration was 25% *V/V* or greater. Hence a methanol concentration of 25% was employed in all the investigations.

Effect of Associated Metal Ions

Selenium and tellurium are usually found associated with metals such as copper, nickel, cobalt, iron, lead and antimony. Hence, the effect of these metals on the determination of both selenium and tellurium was studied and the results obtained are presented in Table V.

The results in Table V show that selenium could be conveniently determined in the presence of these metals without any interferences. The results also show that, except for copper, all the other metals did not interfere in the determination of tellurium. When lead was present as well as tellurium high results were obtained, owing to the formation of lead sulphate, thereby contaminating the tellurium precipitate. This interference could be overcome by washing the precipitate with hot ammonium acetate solution before washing with hot water and alcohol. Lead chloride precipitation will not take place owing to the formation of complex chloro compounds of lead.[19]

TABLE V

EFFECT OF ASSOCIATED METAL IONS ON THE DETERMINATION OF SELENIUM AND TELLURIUM

Amount of selenium taken was 45.64 mg and the amount of tellurium taken was 44.50 mg.

Foreign metal	Amount added/mg	Selenium found/mg	Selenium recovery, %	Tellurium found/mg	Tellurium recovery, %
Copper	100	45.3	99.3	40.5	91.0
Nickel	200	45.5	99.7	44.4	99.8
Iron	100	—	—	44.7	100.4
	200	45.3	99.3	—	—
Cobalt	200	45.6	99.9	44.6	100.2
Lead	100	45.9	100.6	44.7	100.4
Antimony	50	—	—	44.6	100.2
	100	45.9	100.6	—	—

Separation and Determination of Selenium and Tellurium

From the results, it is seen that selenium was quantitatively precipitated from hydrochloric acid solutions when the acid concentration was greater than 5 N, while tellurium was completely precipitated from acid solutions of below 3 N. Attempts were therefore made to separate selenium and tellurium at these acid concentrations. However, it was observed from experiments that at both of these acid concentrations, both of the metals were either wholly or partially precipitated. The extent of coprecipitation of one metal in the presence of the other is indicated in Tables VI and VII.

From the results, it is evident that tellurium was not precipitated when the acid concentration was 9 N with respect to hydrochloric acid, at which concentration selenium was completely precipitated. This is in accordance with results reported by Kolthoff and Elving.[20] Thus, to achieve a perfect separation of selenium and tellurium from one another, experiments were conducted by taking known amounts of selenium(IV) and tellurium(IV) solutions, adjusting the acid concentration to 9 N, when selenium was precipitated. The precipitate was filtered, washed, dried and weighed as described previously.

TABLE VI

EXTENT OF COPRECIPITATION OF SELENIUM WITH TELLURIUM

Tellurium taken/mg	Selenium added/mg	Acidity/N	Total mass of precipitate/ mg	Selenium precipitated with tellurium/mg	Amount of selenium coprecipitated, %
44.50	143.0	1.0	186.8	142.3	99.5
44.50	143.0	1.0	181.9	137.4	96.1
44.50	143.0	2.0	186.7	142.2	99.5
44.50	143.0	2.0	186.8	142.3	99.5

TABLE VII

EXTENT OF COPRECIPITATION OF TELLURIUM WITH SELENIUM

Selenium taken/mg	Tellurium added/mg	Acidity/N	Total mass of precipitate/ mg	Tellurium precipitated with selenium/mg	Amount of tellurium coprecipitated, %
45.64	100.0	6.0	128.8	83.16	83.2
45.64	150.0	6.0	170.6	124.96	83.3
45.64	100.0	7.0	84.0	38.36	38.4
45.64	150.0	7.0	103.1	57.46	38.3
45.64	100.0	8.0	48.7	3.06	3.1
45.64	150.0	8.0	50.0	4.36	2.9
45.64	100.0	9.0	45.4	—	—
45.64	200.0	9.0	45.6	—	—

To determine tellurium, the filtrate was quantitatively transferred into a separate beaker and diluted with water to reduce the acidity to 2 N. Precipitation of black tellurium was observed immediately on dilution. The determination of tellurium was then completed by the procedure described previously. The results are shown in Table VIII.

TABLE VIII

RESULTS FOR SEPARATION AND DETERMINATION OF SELENIUM AND TELLURIUM

Selenium taken/mg	Tellurium taken/mg	Selenium found*/mg	Tellurium found†/mg	Selenium recovery, %	Tellurium recovery, %
45.64	44.50	45.40	44.70	99.5	100.4
45.64	44.50	45.50	44.60	99.7	100.2

* At the initial acid concentration of 9 N with respect to hydrochloric acid.
† Determined after dilution of the filtrate to 2 N with respect to hydrochloric acid.

Conclusions

The advantages of the proposed procedure include production of a dense precipitate of selenium and tellurium with good filtration characteristics. The precipitates obtained did not show any tendency to stick to the beaker walls or watch-glass, thus making their transfer to the filter very easy. The results obtained clearly show that, under homogeneous conditions of precipitation, the reagent brings about a quantitative reduction of tellurium, thus obviating the necessity of using another reducing agent, as is required in the conventional method. Moreover, this reagent has the added advantage of bringing about a quantitative reduction of selenium(VI) and tellurium(VI), which was not possible with sulphur dioxide alone in the conventional precipitation procedure.

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Automatic Potentiometric Titration of Thiocyanate - Cyanide Mixtures in Hydrometallurgical Effluents

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Mixtures of cyanide and thiocyanate in hydrometallurgical effluents heavily clouded with particulates are titrated quickly and successfully with silver nitrate solution by using a potentiometric automatic titrator fitted with a silver working electrode and a glass reference electrode. When thiocyanate is to be determined, cyanide is masked with formalin. Titrations over a wide range of concentration and ratio of the two species require minimum pre-treatment of the samples and give sharp end-points and good replication.

Keywords: *Thiocyanate - cyanide mixtures; silver - glass electrode pair; hydrometallurgical effluents*

Recovery of gold and silver from sulphur-containing ores, tailings and residues by cyanidation results in the formation of thiocyanate. Although the conversion of cyanide into thiocyanate represents a significant reagent loss to the leaching process, the major concern is the disposal and detoxification of many thousands of cubic metres of cyanide - thiocyanate effluent generated in the course of precious metal extraction. Our research has been directed at electrochemical methods of treating cyanidation effluents. It is necessary to measure both thiocyanate and cyanide in solutions clouded with a suspension of fine particles. This suspension consists of entrained particles from leaching and additional eroded material from the electrode surfaces of the reactor. For successful reactor development a rapid and reliable analytical method capable of determining thiocyanate and cyanide in cloudy solutions is required.

Many methods exist for the determination of thiocyanate and cyanide separately. Thiocyanate may be determined gravimetrically as silver thiocyanate[1] or as copper thiocyanate.[2] Schulek[3] developed a method in which thiocyanate was oxidised to cyanogen bromide, then potassium iodide was added and the liberated iodine was titrated with thiosulphate. There are also numerous argentimetric methods such as the Volhard titration,[4] and adsorption indicator titrations with eosin.[5] Cyanide may be determined gravimetrically as silver cyanide,[6] by Schulek's method[3] or by titrations such as the Liebig - Deniges method[6] or direct titration with Rhodamine 6G as indicator.[7]

These methods suffer from severe drawbacks when the thiocyanate and cyanide are in solution together. In many instances, sample pre-treatment such as volatilisation of cyanide from an acidic medium is prohibitively time consuming, and even then difficult to carry to completion confidently.

The methods used originally in this laboratory were chosen principally to minimise sample pre-treatment times. Thiocyanate was titrated using a modified Volhard titration. A 25.0-ml aliquot of standard 0.01 or 0.1 M silver nitrate solution was pipetted into a 250-ml Erlenmeyer flask, treated with 5 ml of 6 M nitric acid and 1 ml of ammonium iron(III) sulphate indicator, and titrated using the thiocyanate sample as titrant to a faint brown end-point. Vigorous shaking is necessary throughout. Cyanide was titrated with silver nitrate solution using Rhodamine indicator.[7] The cyanide titration showed definite end-points even at concentrations below 10 p.p.m. However, the thiocyanate titration was less satisfactory for concentrations of thiocyanate below 500 p.p.m. in the clouded reactor samples because of indefinite visual end-points. In seeking to improve this determination, a more satisfactory method was developed, which is reported here.

The new procedure involves titrating the sample with silver nitrate solution while observing the potential difference between a glass electrode used as reference and a silver indicator electrode. When titrating thiocyanate, the cyanide was masked with formalin.[8]

Kolthoff and Lingane[9] investigated the accuracy of the potentiometric titration of thiocyanate with silver nitrate solution and reported it to be good (0.08%). A single titration at this accuracy took more than 1 h. Conrad[10] investigated the silver - silver sulphide ion-selective electrode for the potentiometric titration of cyanide. In general, such electrodes show strong mutual interferences of cyanide and thiocyanate as well as sulphide, and are thus inappropriate for use in the liquors under study.

Experimental

A Sargent-Welch recording potentiometric titrator, Model DG, was fitted with a Radiometer P401 NH silver electrode and a Sargent-Welch S-30050-15A glass electrode that functioned as the reference. Silver nitrate solutions (0.01 M and 0.001 M) were prepared as required by accurate dilution of Harleco Volumetric Concentrate. Other reagents were supplied by the J. T. Baker Chemical Company and used as received. Formalin solution (37%) was used to mask cyanide. Stock solutions of potassium thiocyanate and potassium cyanide were prepared from analytical-reagent grade salts and standardised by Schulek's bromimetric method.[3]

In order to determine thiocyanate in the thiocyanate - cyanide mixture, a suitable aliquot was placed in a 250-ml beaker, diluted to about 125 ml with de-ionised water and treated with 5 ml of formalin to mask the cyanide. The mixture was stirred and allowed to stand for 10 min. Nitric acid (6 M, 5 ml) was added to give a total volume of 135 ml and the sample was titrated with silver nitrate solution. For cyanide determination, a similar aliquot was taken and diluted to 135 ml with de-ionised water as before. The potentiometric titration was then carried out to the first sharp end-point. A second end-point was also observed if the titration was continued. The concentrations of thiocyanate and cyanide were calculated as follows:

$$[SCN^-]\ (mg\ l^{-1}) = \frac{AgNO_3\ molarity\ (mol\ l^{-1}) \times AgNO_3\ titration\ volume\ (l) \times 58.08 \times 1\,000\ (mg\ mol^{-1})}{Sample\ volume\ (l)}$$

$$[CN^-]\ (mg\ l^{-1}) = \frac{AgNO_3\ molarity\ (mol\ l^{-1}) \times AgNO_3\ titration\ volume\ (l) \times 2 \times 26.018 \times 1\,000\ (mg\ mol^{-1})}{Sample\ volume\ (l)}$$

The silver electrode was cleaned after approximately every five titrations by wiping it with a fine metal polishing cloth and rinsing it with de-ionised water.

Results and Discussion

The potentiometric titration technique was applied to a number of synthetic solution mixtures of cyanide and thiocyanate prepared from appropriate stock solutions. Standard addition experiments were performed on those synthetic solution mixtures that had undergone partial electrochemical oxidation and therefore contained considerable turbidity due to suspended electrode erosion material. Addition of chloride was also carried out in order to check for possible interference. All data presented are based on the average of three determinations using an initial volume for titration of 135 ml.

Table I gives selected data for the potentiometric determination of thiocyanate in the presence and absence of cyanide. The cyanide added appears to be without effect on the thiocyanate determination when it is masked by formalin. Fig. 1 (curve A) shows a representative potentiometric titration of 10 mg of thiocyanate. The sharp end-point represents the complete formation of silver thiocyanate. A trace superimposable on curve A is obtained when 10 mg of thiocyanate are titrated in the presence of 20 mg of cyanide masked with formalin. If no masking agent is present the thiocyanate end-point is indistinct and non-reproducible, with the problem increasing in severity as the cyanide level is raised. Fig. 2 (curve A) shows similar behaviour for 0.25 mg of thiocyanate. This curve is unchanged when the 0.25 mg of thiocyanate is titrated in the presence of 100 mg of cyanide masked with formalin.

TABLE I

POTENTIOMETRIC DETERMINATION OF THIOCYANATE IN THE PRESENCE AND ABSENCE OF CYANIDE

An initial titration volume of 135 ml was used.

Thiocyanate/mg		Cyanide present/mg	Absolute error/mg	Recovery, %
Taken	Found			
25.18	25.46	—	0.28	101.1
25.18	25.50	10.0	0.32	101.3
12.50	12.65	—	0.15	101.2
12.50	12.66	10 0	0 16	101.3
9.47	9.52	—	0.05	100.5
9 47	9 58	20.0	0.11	101 2
12.58	12 65	—	0.07	100.6
12.58	12.63	50.0	0.05	100.4
6.25	6.28	—	0.03	100 5
6.25	6.28	50.0	0.03	100.5
4.74	4.76	—	0.02	100.4
4.74	4.76	100.0	0 02	100 4
1.26	1.14	—	−0.12	90.5
1.26	1.13	50.0	−0 13	89.7
0.625	0.565	—	−0 06	90 4
0.625	0.578	50 0	−0.047	92 2
0 474	0.449	—	−0 025	94 7
0.474	0.459	100.0	−0.015	96.8
0.252	0.241	—	−0 011	95.6
0.252	0.249	100.0	−0 003	98.8
0 125	0.121	—	−0 004	96.8
0.125	0.109	100 0	−0 016	87 2

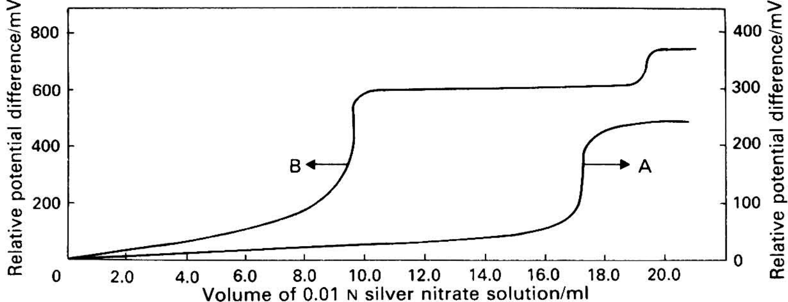

Fig. 1. Potentiometric titrations for high concentrations of thiocyanate and cyanide. A, 10 mg of thiocyanate and B, 5 mg of cyanide. Both initial volumes were 135 ml.

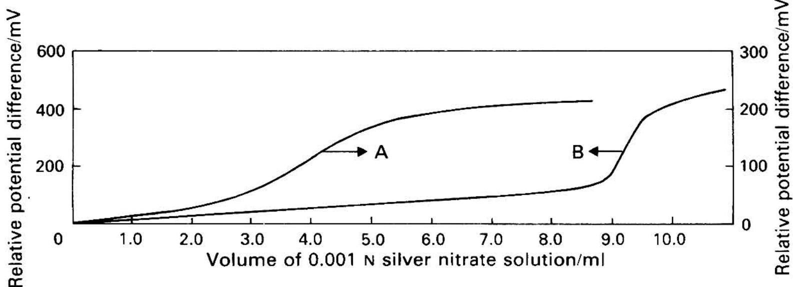

Fig. 2. Potentiometric titrations for low concentrations of thiocyanate and cyanide. A, 0.25 mg of thiocyanate and B, 0.50 mg of cyanide. Both initial volumes were 135 ml.

Table II presents data for the potentiometric determination of cyanide in the presence and absence of thiocyanate. The data show that cyanide may be titrated effectively in the presence of thiocyanate. Fig. 1 (curve B) shows a typical titration of 5.0 mg of cyanide. Two end-points are observed representing the formation of $Ag(CN)_2^-$ and that of silver cyanide. Additions of thiocyanate had no detectable effect on either end-point. Fig. 2 (curve B) shows the first end-point for a titration of 0.50 mg of cyanide. Even at this low level, addition of thiocyanate had no effect on the shape of the curve at the end-point or on its reproducibility.

TABLE II

POTENTIOMETRIC DETERMINATION OF CYANIDE IN THE PRESENCE AND ABSENCE OF THIOCYANATE

An initial titration volume of 135 ml was used.

Cyanide/mg		Thiocyanate present/mg	Absolute error/mg	Recovery, %
Taken	Found			
9.73	9.72	—	−0.01	99.9
9.73	9.70	12.5	−0.03	99.7
4.88	4.88	—	0.0	100.0
4.88	4.95	62.5	+0.07	101.4
0.488	0.433	—	−0.005	88 7
0.488	0.413	62.5	−0.075	84.6
0.098	0.125	—	+0.027	127.6
0.098	0.070	125.0	−0.028	71.4

The data in Table III represent the results of standard addition experiments performed on thiocyanate - cyanide partially oxidised effluents. These solutions were products of electrochemical oxidation and were very discoloured and turbid. The data indicate no difficulty in determining both cyanide and thiocyanate potentiometrically over a wide range of concentrations. Replicate experiments showed a typical precision of $\pm 1\%$.

TABLE III

STANDARD ADDITION DATA FOR THIOCYANATE - CYANIDE EFFLUENTS

Effluents were selected samples of electrochemically oxidised laboratary-simulated waste solutions,

Effluent composition/mg l^{-1}		Amount taken/mg		Standard addition/mg		Amount found/mg		Thiocyanate or cyanide recovery, %
Thiocyanate	Cyanide	Thiocyanate	Cyanide	Thiocyanate	Cyanide	Thiocyanate	Cyanide	
1482	6.1	7.41	0.031	4.735	—	12.23	—	100.7
990.8	99.5	24.27	2.49	—	9.73	—	12.12	99.2
762	95.6	3.81	0.478	4.735	—	8.49	—	99.4
213	79.2	5.33	1.98	4.735	—	10.08	—	100.1
8.2	19.4	0.205	0.485	—	9.73	—	9.835	96.3
2.09	1.4	0.052	0.038	4.735	—	4.78	—	99.9
2.09	1.5	0.052	0.038	—	9.73	—	9.679	99.1

The interference of chloride on both the titration of cyanide and of thiocyanate was examined. Table IV gives selected data for both titrations. Generally it appears that chloride causes no interference. Fig. 3 (curve A) depicts a titration of thiocyanate in the presence of chloride. Addition of cyanide masked with formalin has no effect. The second

TABLE IV

CHLORIDE NON-INTERFERENCE

Amount taken/mg		Chloride added/ mg	Amount found/mg		Absolute error/mg	Thiocyanate or cyanide recovery, %
Thiocyanate	Cyanide		Thiocyanate	Cyanide		
6.25	—	12.53	6.40	—	0.15	102.4
—	4.88	12.53	—	4.85	−0.03	99.4

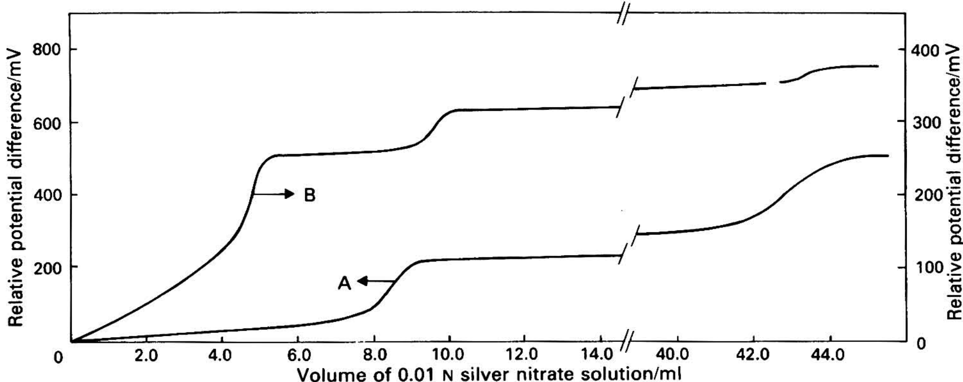

Fig. 3. Graphs showing non-interference of chloride. A, 5.0 mg of thiocyanate in the presence of 12.5 mg of chloride; B, 2.5 mg of cyanide in the presence of 12.5 mg of chloride. Both initial volumes were 135 ml.

end-point shows completion of silver chloride formation. Curve B shows the two characteristic cyanide end-points and a final chloride end-point. The determination of cyanide was found to be unaffected.

This new method is now used routinely in our laboratory. We believe it merits consideration by others dealing with turbid solutions of cyanide and thiocyanate.

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Dead-stop Determination of EDTA and NTA in Commercially Available Detergents*

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A rapid and selective method for the determination of ethylenediaminetetraacetic acid (EDTA) and/or nitrilotriacetic acid (NTA) in commercially available detergents has been developed. It is based on a titration with a standard solution of copper(II) in acetate buffer, where the end-point is revealed by means of a "dead-stop" system with two polarised copper electrodes. Furthermore, it is possible to determine in the course of the same titration the relative amounts of both chelating agents by using 4-(2-pyridylazo)-resorcinol (PAR), which changes colour at the end-point of the first reaction (copper - EDTA). Most detergent constituents, including polyphosphates, have been observed to have no effect on the determination; interference from some constituents (perborates and zeolites) can easily be removed. The method has been shown to give good results in the analyses of different commercially available products.

Keywords: *EDTA and NTA determination; copper(II) sulphate titrant; dead-stop indicator; PAR indicator; detergents*

Ethylenediaminetetraacetic acid (EDTA) and/or nitrilotriacetic acid (NTA) are frequently used in detergent formulations at low concentrations to support the activities of polyphosphates (*e.g.*, sodium tripolyphosphate) and to inhibit trace amounts of free metal ions from catalysing the decomposition of perborates and depressing the bleaching performance of optical brighteners.[1] In some countries, such as Canada and Finland, higher percentage contents of NTA (up to 10%) are allowed as polyphosphate substitutes in order to reduce the phosphorus content of waste water, which is a source of eutrophication. In other countries the environmental convenience of this substitution has been questioned for a long time and alternatives to polyphosphates have been sought in other directions, *e.g.*, zeolites. However, the environmental and health risks from the use of NTA in laundry detergents have recently been re-evaluated in the US by the Environmental Protection Agency and judged to be generally low.[2]

Thus, the use of NTA and other chelating agents in detergent formulations as well as the interest in establishing practical methods of determination will probably increase in the future. Present methods are founded on complexometric titrations with various metal ions and with both optical and instrumental end-point indicators.[3–8] One of the most recent methods consists of a titration with a standard iron(III) solution using a redox potentiometric end-point detector, but the procedure is complicated on account of the interference of polyphosphates, which have to be converted into orthophosphates and removed as ammonium magnesium phosphate.[9] Another recent method, based on ultraviolet spectrophotometric titration with copper(II) and on the difference in absorptivity between the NTA complex and the aquo complex, is without serious interferences, but the absorbance break is insufficiently sharp to afford precise results and may be obscured by other components in the formulation that absorb in the ultraviolet region.[10]

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This paper is concerned with the proposal of a dead-stop amperometric system with two polarised copper electrodes, suitable for the selective detection of the end-point of an EDTA plus NTA titration with a standard copper(II) solution in acetate buffer. Moreover, the addition of 4-(2-pyridylazo)resorcinol (PAR), which changes in colour at the end of the first reaction [copper(II) - EDTA], allows the relative amounts of both chelating agents to be determined in the course of the same titration. Hence the results are obtained very rapidly and the process can easily be automated.

Experimental

Reagents

All chemicals were of analytical-reagent grade. All of the reagent solutions were standardised complexometrically: a standard solution of zinc(II)[11] was used to standardise 0.1 M solutions of EDTA[11] and NTA,[12] as the disodium salts; then the EDTA standard solution was employed to standardise a 0.1 M solution of copper(II) sulphate.[11] PAR was used as a saturated methanolic solution. The acetate buffer of pH 4.4 $\pm$ 0.2 was prepared by mixing 33.6 g of acetic acid (sp. gr. 1.0499) and 7.0 g of sodium hydroxide and then diluting the mixture to 1 l.

Apparatus

Fig. 1 shows the apparatus necessary for the amperometric titration with two identical polarised electrodes. The polarising tension (50–200 mV) is taken from a common 1.5-V battery through a potential divider and is measured with a voltmeter; the circuit current and the volume of titrant are measured with a 25-μA full-scale microammeter and a 5-ml full-scale microburette, within 0.2 μA and 0.01 ml, respectively; the solution is stirred at a constant velocity by a synchronous motor. Both electrodes consist of wires of electrolytic copper (about 100 mm long and 1 mm diameter) dipped into a small glass tube (80 mm long) by sucking in melted paraffin and then cooling quickly under water. The copper wire emerges 10 mm from either side of the tube. Before each titration both electrodes must be dipped into 2.5 M nitric acid for a few seconds and then washed with distilled water. Different pairs of electrodes gave reproducible titration curves.

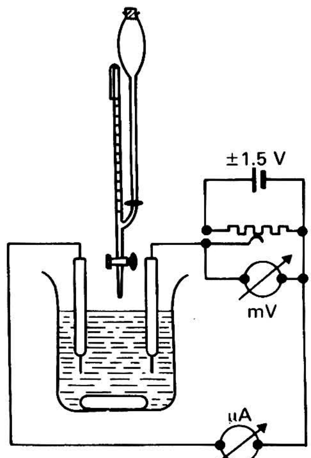

Fig. 1. Dead-stop device.

Titration Tests

Fig. 2 shows typical conditions and titration curves; only a residual current (less than 5 μA) passes on the addition of copper(II) ions as long as free EDTA and/or NTA are present in the solution. After the equivalence point has been reached, the current rises in proportion to the excess of added copper ions. The break is so sharp that its graphical location is

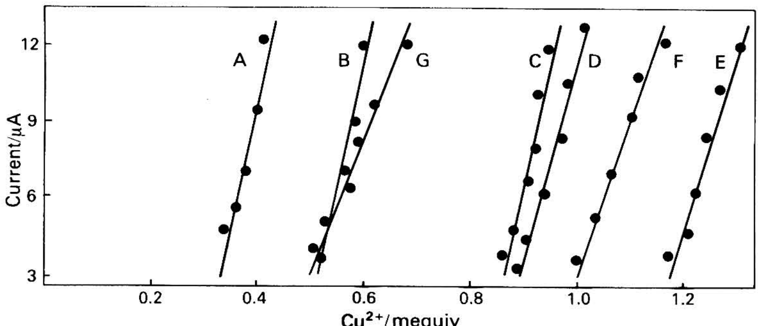

Fig. 2. Dead-stop titration graphs for: A, 0.34 mmol of EDTA; B, 0.53 mmol of NTA; C, 0.34 mmol of EDTA + 0.53 mmol of NTA; D, 0.68 mmol of EDTA + 0.21 mmol of NTA; E, 0.13 mmol of EDTA + 1.05 mmol of NTA; F, 1.00 mmol of NTA in the presence of 0.54 mmol of STP; and G, 0.50 mmol of EDTA in the presence of 1.63 mmol of STP.

superfluous. This enables the titration to be easily automated. Moreover, when a few drops of PAR are added to the solution, its colour changes from yellow to full red at the equivalence point of the copper(II) - EDTA complex formation only, without any interference from the successive NTA reaction or on the amperometric end-point. Although a larger pH range (4–8) is tolerated when observing the amperometric end-point, the optical end-point needs a buffered solution at pH 4.4 $\pm$ 0.2. This pH range was therefore selected.

Effects of Detergent Constituents

Titration tests have also been performed in the presence of the main constituents of detergent formulations. Surfactant concentrations of 0.5–15% *m/V*, for example, lauryl sulphate, sodium dodecylbenzenesulphonate and isooctylphenoxypolyethoxyethanol (approximately 10 mol of ethylene oxide) and 0.2–0.8 g of sodium tripolyphosphate have been found to have no effect on either equivalence point. No effects have also been observed from pyro- and orthophosphates, silicates, sulphates and carbonates.

Zeolites release aluminium ions in acidic solutions containing EDTA or NTA.[13] They should therefore be filtered before adding the acetate buffer.

Finally, perborates affect the amperometric titration. Nevertheless, a prior reduction with an excess of sodium sulphite in warm solution for 5 min is sufficient to eliminate their interference.

Procedure

The following procedure is proposed for commercially available detergents. About 2 g of liquid or powdered detergent are carefully weighed and dissolved in about 20 ml of distilled water in a beaker. If insoluble matter persists, even after slightly warming, the solution is centrifuged and the solid discarded. Then, 30 ml of acetate buffer and 4–5 drops of PAR solution are added to the solution, the stirrer is set in motion, the electrodes are dipped and polarised with a low tension (so as to maintain the current under 5 μA), and the titration with the standard copper(II) solution is commenced.

The colour change of the optical indicator may take place either immediately (at the beginning of titration), at a point when the same volume of titrant has been added as gives the amperometric end-point, or at an intermediate volume. These three events mean that only NTA, only EDTA or both are present, respectively. In the first two instances, the addition of PAR is superfluous. In the third instance, the optical equivalence point is relative to the amount of EDTA (in milliequivalents) while the amperometric end-point considers EDTA plus NTA milliequivalents; the milliequivalents of NTA will be obtained from the difference.

TABLE I

EDTA AND/OR NTA DETERMINATION IN COMMERCIALLY AVAILABLE DETERGENTS

Both chelating agents were added to all the detergent samples, except samples 3 and 8 (only EDTA) and samples 5 and 13 (only NTA).

Sample	Use of detergent (about 2 g)	EDTA			NTA			EDTA + NTA		
		Added/ mg	Found/ mg	Recovery, %*	Added/ mg	Found/ mg	Recovery, %†	Added/ mequiv	Found/ mequiv	Recovery, %‡
1	General purpose cleanser	98.9	94	95.0	100.5	103	102.5	0.864	0.860	99.5
2	General purpose cleanser	98.9	96	97.1	100.5	98	97.5	0.864	0.841	97.4
3	General purpose cleanser	98.9	98	99.1	—	—	—	0.338	0.335	99.2
4	Dishwashing, by hand	39.5	37	93.7	160.9	167	103.8	0.977	0.999	102.3
5	Dishwashing, by hand	—	—	—	100.5	103	102.5	0.526	0.539	102.5
6	Clothes washing, by hand	98.9	95	96.1	100.5	101	100.5	0.864	0.854	98.8
7	Clothes washing, by hand	158.2	154	97.3	40.2	43	106.9	0.751	0.746	99.3
8	Clothes washing, by hand	98.9	97	98.1	—	—	—	0.338	0.332	98.2
9	Clothes washing, by hand	59.3	58	97.8	60.3	64	106.1	0.518	0.533	103.0
10	Delicate clothes washing	98.9	93	94.0	100.5	100	99.5	0.864	0.843	97.6
11	Delicate clothes washing	118.6	112	94.4	120.6	129	106.8	1.037	1.058	102.0
12	Machine clothes washing	79.1	77	97.3	80.4	78	97.0	0.692	0.671	96.9
13	Machine clothes washing	—	—	—	160.9	159	98.8	0.842	0.832	98.8
14	Machine clothes washing	98.9	95	96.1	100.5	107	106.5	0.864	0.886	102.5
15	Machine clothes washing	197.7	193	97.6	201.1	196	97.5	1.728	1.686	97.6

* PAR end-point.
† Determined by difference.
‡ Dead-stop end-point.

Only if perborates are present, as in some types of European laundry detergents, should the procedure be modified as follows: about 2 g of detergent are carefully weighed, poured into a beaker and mixed with 1 g of sodium sulphite and 25–30 ml of distilled water. After 5 min of stirring and slight warming, the solution is centrifuged, 30 ml of acetate buffer are added and the solution is titrated with copper(II) solution as before.

Results and Discussion

Known amounts of EDTA and/or NTA were added to various commercially available detergents, which were known to be free of them, and were then determined according to the above procedure. The results are shown in Table I. The recovery of either or both chelating agents, as given by the amperometric equivalence point, is very good, being complete to within $\pm 3\%$. Although the optical equivalence point shows a higher error ($\pm 7\%$) due to a heavier matrix effect the determination of the relative amounts of both chelating agents is still largely satisfactory for technical analysis purposes.

Thus, with respect to other recent methods, this method produces comparatively precise analytical results with greater rapidity and selectivity.

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Comparison of Laser-excited Fluorescence and Photoacoustic Limits of Detection of Polynuclear Aromatic Hydrocarbons

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An extremely simple and sensitive system that employs both fluorescence and photoacoustic detection modes simultaneously has been used to obtain limits of detection rapidly for 30 polynuclear aromatic hydrocarbons. The sensitivities of the two techniques are compared with both fixed and tunable laser excitation and a "levelling" effect is demonstrated by the photoacoustic results. High-energy pulse excitation demonstrates that the photoacoustic signal does not increase indefinitely with incident pulse energy. Application of the simultaneous detection scheme to the measurement of fluorophor quantum efficiencies in solutions and to antifluorochrome stains is suggested.

Keywords: *Photoacoustic*; *fluorescence*; *polynuclear aromatic hydrocarbons*; *laser detection*

In a previous paper,[1] we discussed our development of a highly sensitive and almost ultimately simple photoacoustic detection system. We found that we could measure, with a signal to noise ratio of 3, a thermalised energy of about 6 nJ and achieved limiting detectable absorptivities of 4×10^{-6} cm^{-1} at 1.6 mJ incident laser pulse energy. The principal improvement we have made in the ordinary Suprasil fluorescence cuvette and "C-clamp" mounting fixture due to Tam and Patel[2] is in the elimination of the mounting fixture. This simplification results in greatly improved signal reproducibility, elimination of acoustic artifacts (generated when the mounting fixture is struck by stray light) and negligible artifact generated by light impinging on the piezoelectric transducer via the pyroelectric effect.[3] We have also found the use of light-baffling acoustic waveguides[4] to be unnecessary.

The cuvette and piezoelectric transducer coupling arrangement is shown in Fig. 1. It is immediately apparent that the photoacoustic detection scheme may be readily used with other spectroscopic detection techniques such as fluorescence. For example, Kato and Sugitani[5] have described the design of a simultaneous fluorescence and photoacoustic sample cell and have used it to obtain the fluorescence excitation and photoacoustic spectra of natural zircon

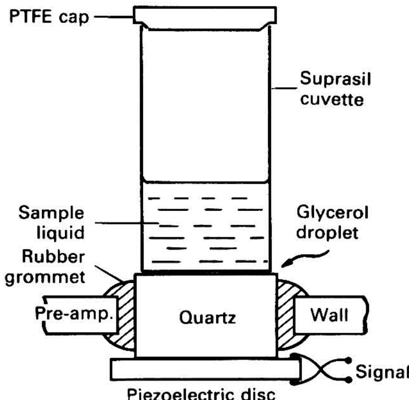

Fig. 1. Cuvette cell and piezoelectric transducer coupling scheme.

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($ZrSiO_4$). Their system employs conventional chopped xenon lamp excitation varied by monochromator and capacitance microphone and lock-in amplifier detection. The cell is intended for powdered specimens. In addition, Sugitani and Kato[6] have employed their sample cell to determine the quantum efficiencies of uranium - mica-type compounds by simultaneously measuring their fluorescence excitation and photoacoustic spectra.

As the photoacoustic effect is complementary to luminescence techniques, we have combined our photoacoustic detection scheme with nitrogen laser-excited (337.1 nm) fluorescence detection and both simultaneously and rapidly determined the limiting detectable concentrations (signal to noise ratio = 3) of 30 polynuclear aromatic hydrocarbons (PAHs).

We have also employed nitrogen laser-pumped tunable dye laser excitation and excimer laser (xenon chloride, 308 nm) excitation. The compounds chosen for examination, PAHs, are of considerable interest as environmental pollutants and are suspected of being carcinogens or precursors of carcinogenic metabolites. Although PAHs are generally excellent fluorophors and therefore are not optimal for photoacoustic detection, they illustrate that sample fluorescence does not produce an artifact inteference in the photoacoustic detection mode. They also demonstrate an interesting "levelling effect" in their limits of detection.

Experimental

The experimental arrangement is shown in Fig. 2. The piezoelectric transducer preamplifier was previously described in detail.[1] The n-JFET input pre-amplifier ($A_V = 37.5$, noise = 4 μV r.m.s., $f_{-3db} = 28$ kHz, 440 kHz) and PAR 211 amplifier (A_V = 1–1000, $f_{-3db} = 1$ Hz, 1 MHz) provided low-noise amplification of the transducer output. The signal was acquired with a PAR 160 boxcar averager (EG&G Princeton Applied Research, Princeton, NJ, USA) with a gate width of about 1 μs, a gate delay of about 12 μs and an observed time constant of 5 s. Excitation was provided by a Molectron UV-14 nitrogen laser and a Molectron DL-400 tunable dye laser. High-energy pulses were provided by a Lumonics TE 861S excimer laser. Pulse energies were measured with a Molectron J3-05 pyroelectric joulemeter. The joulemeter output exponential pulses ($\tau \approx 50$ μs) were acquired with the peak detector shown in Fig. 3.

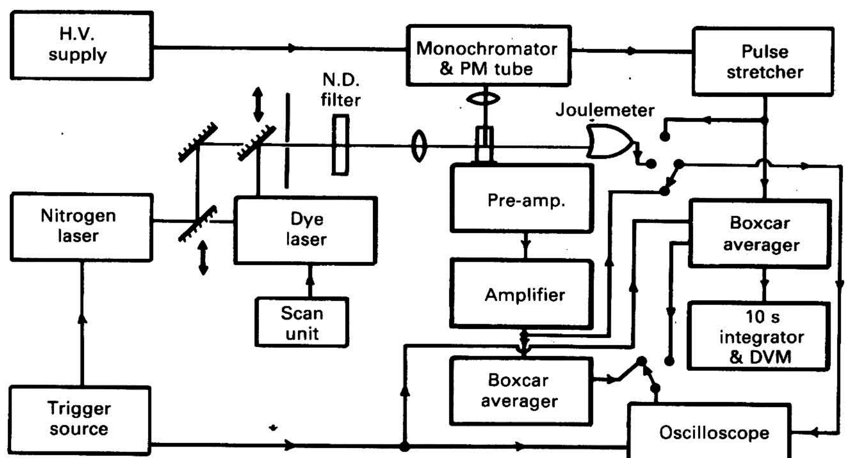

Fig. 2. Schematic diagram of the simultaneous fluorescence and photoacoustic detection system.

For fluorescence detection, a Jobin-Yvon H-10 monochromator with an 8-nm spectral band pass and an RCA 1P28 PM tube with fast base wiring modifications[7] was employed. The PM tube output was stretched with a pulse stretcher, which produces exponential pulses having $\tau = 300$ μs, and then acquired with a PAR 162/164 boxcar averager with gate delay of 10 μs and a gate width of 10 μs. The nitrogen laser, a Tektronix 454 oscilloscope and both

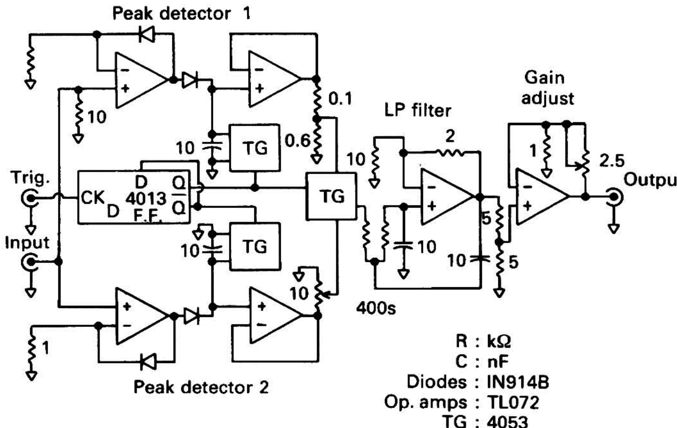

Fig. 3. Dual peak detector circuit.

boxcars were triggered by a Wavetek 802 pulse generator. The PAR 162 output was integrated for 10 s with a home-made integrator and read out with a digital voltmeter.

The effects of filtering the nitrogen laser excitation source with an interference filter having peak transmittance of 42% at 340 nm and 10-nm spectral band pass were examined. The emission side of the system was also filtered with a Corning CS3-144 sharp cut yellow glass filter to attenuate scattered light below 400 nm. The excitation source was focused in the centre of the solution (about volume 1 ml) in a standard 1-cm Suprasil cuvette. For photoacoustic detection, the beam was focused 1 cm in front of the cuvette. Neutral density filters (Corion Corp., Holliston, MA, USA) were used as necessary to avoid PM tube saturation. The fluorescence and photoacoustic detection limit experiments, with nitrogen laser excitation, were run simultaneously and required 15 min per PAH. The dye laser excited results were run consecutively and, as the dye laser photoacoustic results are relatively poor, not all of the PAHs were so run. To reduce scatter, the dye laser excited fluorescence results were obtained using solution (emission) filters. These filters were 5 g l^{-1} potassium biphthalate (about 308 nm cutoff),[8] 2 M potassium nitrate solution (about 340 nm cutoff) and 0.2 M potassium nitrate solution (about 325 nm cutoff).

Stock solutions of the PAHs were prepared in distilled-in-glass, ultraviolet spectrometric grade heptane (Burdick and Jackson Laboratories, Muskegon, MI, USA) at concentrations of the order of 100 μg ml^{-1}. These solutions were diluted immediately prior to the experiment in carefully cleaned calibrated flasks. Sixteen blank measurements were run followed by the dilutions (three measurements each) in ascending order of concentration. Blank standard deviations for photoacoustic detection were 2–4% and 2–3% for nitrogen laser excited fluorescence detection. The standard deviations were about 6% for dye laser excited detection in both modes.

The laser dyes used were supplied by Exciton (Exciton Chemical Co., Dayton, OH, USA). The PAHs were obtained from Aldrich Chemical Company, Eastman Organic Chemistry, City Chemical Corporation, K&K Laboratories, Chem Service, Nutritional Biochemicals and Fluka. Solvent purity was checked with an Aminco-Bowman spectrophotofluorimeter and with a Varian 634 ultraviolet - visible spectrophotometer.

Results and Discussion

Typical calibration graphs for both fluorescence modes, without excitation or emission filters, and the photoacoustic mode are shown in Fig. 4 for azulene in ethanol. Extrapolation of such graphs to those concentrations producing signals three times the standard deviations of 16 blanks yields the limit of detection data given in Table I. The nitrogen laser excited fluorescence limits of detection were determined using both excitation and emission filters,

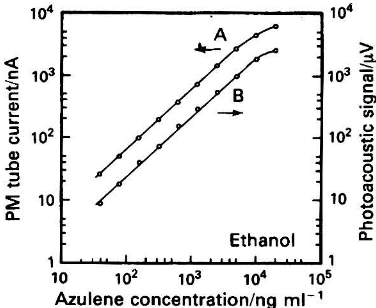

Fig. 4. Azulene calibration graphs. The log - log slopes and correlation coefficients, omitting the upper two points on each graph, are (A) 0.96, 1.000 (fluorescence) and (B) 0.96, 0.993 (photoacoustic).

although the effects of eliminating either filter or both filters were also examined. Without filters, the limits of detection were about five times higher and only slightly higher with either filter than with both filters. The implication is that the excitation source is not substantially "bright" at extraneous wavelengths and the cuvette system has only a small scattered light interference problem.

Examination of the nitrogen laser excited limits of detection in Table I demonstrates that

Table I

Laser excited fluorescence and photoacoustic limits of detection of PAHs in heptane at 298 K

Compound	Pulse energy/ µJ	$\lambda_{em.}$*/ nm	$\lambda_{ex.}$†/ nm	Fluorescence limit of detection‡/ng ml^{-1}		Pulse energy/ µJ	Photoacoustic limit of detection/ng ml^{-1}	
				At $\lambda_{ex.}$	At 337.1 nm§		At $\lambda_{ex.}$	At 337.1 nm§
Anthanthene	1	430	305	0.001	0.002	—	—	8
Anthracene	8	399	260	0.02	0.002	0.4	700	0.7
Azulene	5	374	268	0.005	2.0	—	—	0.7
1,2-Benzanthracene	5	385	290	0.005	0.08	5	1000	0.4
1,12-Benzoperylene	5	419	290	0.02	0.03	5	900	1
1,2-Benzopyrene	5	388	290	0.02	0.5	5	1000	0.8
3,4-Benzopyrene	5	403	290	0.007	0.004	5	1000	0.6
Chrysene¶	8	402	260	0.03	0.2	0.4	300	7
Coronene	5	444	290	0.02	0.003	5	1000	0.3
1,2:3,4-Dibenzanthracene	5	386	290	0.01	0.06	—	—	0.5
1,2:5,6-Dibenzanthracene	5	394	290	0.006	0.05	5	1000	0.4
1,2:7,8-Dibenzocarbazole	5	378	290	0.005	0.03	—	—	0.5
1,2:7,8-Dibenzophenanthrene	5	396	290	0.03	0.5	—	—	7
1,2:3,4-Dibenzopyrene	70	503	400	0.004	0.001	—	—	0.7
1,2:4,5-Dibenzopyrene	5	397	290	0.01	0.02	—	—	0.5
3,4:8,9-Dibenzopyrene	1	448	305	0.001	0.003	—	—	7
3,4:9,10-Dibenzopyrene	5	432	290	0.06	0.04	—	—	2
9,10-Dimethylanthracene	8	400	260	0.0004	0.04	—	—	2
5,7-Dimethyl-1,2-benzacridine	5	387	290	0.003	0.002	—	—	0.8
7,12-Dimethyl-1,2-benzanthracene	5	407	290	0.02	0.01	—	—	1
9,10-Diphenylanthracene	8	405	260	0.002	0.002	—	—	2
Fluoranthene	5	461	290	0.002	0.004	—	—	0.4
2-Methylanthracene	8	404	260	0.008	0.002	—	—	0.7
9-Methylanthracene	8	410	260	0.007	0.002	—	—	2
20-Methylcholanthrene	5	393	290	0.003	0.02	—	—	1
1-Methylpyrene	5	376	268	0.02	0.06	—	—	0.2
Naphthacene	5	471	290	0.01	0.01	5	7000	7
Perylene	80	438	406	0.002	0.002	60	30	4
Pyrene	5	383	268	0.04	0.2	0.8	800	0.2
Rubrene	5	549	290	0.001	0.0008	5	2000	3

* Wavelengths were determined using a 4-nm spectral band pass.
† Excitation wavelengths are the maximum for the dye.
‡ Measurements were made using an 8-nm spectral band pass. The limit of detection is that concentration giving a signal three times the standard deviation of 16 blanks.
§ Both the excitation interference filter and emission ultraviolet cut-off filters were used.
¶ Emission peak is not the maximum, but it gave a higher signal to noise ratio.

fluorescence is substantially better than photocoustic detection for the 30 PAHs examined. In each mode, blank noise was found to be the limiting factor. It should be noted that the photoacoustic detection scheme is electronic noise limited by the equivalent input voltage noise of the JFET and that this limit is about ten times the piezoelectric transducer Brownian motion noise, with the 2N5484 JFET employed. Another factor of 2 in noise reduction is possible with the 2N6550 (Teledyne Crystalonics CM 860 n-JFET, $E_n = 1.4$ nV $Hz^{-\frac{1}{2}}$). As these noise sources are white, signal averaging may be employed to recover weak signals buried in noise. This is essentially what is done in the minimally damped gravity wave detectors.[9]

Comparison of the fluorescence limits of detection of anthracene (4.4 pg g^{-1}), fluoranthene (1.0 pg g^{-1}) and pyrene (0.5 pg g^{-1}) obtained by Richardson and Ando[10] with our values indicates that, with the exception of the pyrene result, our fluorescence detection scheme is almost as sensitive. It should be noted that the use of a pulse stretcher and long (10 μs) boxcar gate necessarily degrades the signal to noise ratio. This is more than compensated for by the elimination of short boxcar gates and their attendent jitter and drift and it is unnecessary to employ optical triggering and low distortion delay lines to circumvent the inherent boxcar trigger delay (75 ns for the PAR 162/164 boxcar used). In fact, the boxcar averager may be replaced with a peak detector, such as that in Fig. 3, if a pulse stretcher is employed and if the signal to noise ratio is approximately 10 or more. This is also possible in the photoacoustic detection mode if the peak detector is preceded by a fast (about 500-kHz band width) full-wave rectifier (absolute value) circuit.[11] In each instance, the peak detector output would be integrated for 10 s. Neither of these simplified schemes is useful if optimal detection limits are necessary, although our fluorescence limits of detection are not greatly increased by using a pulse stretcher.

It is also apparent that the ranges of the limits of detection for fluorescence and photoacoustic detection with nitrogen laser excitation are very different. For fluorescence, the ratio of the worst limit of detection (azulene, 2 ng ml^{-1}) to the best limit of detection (rubrene, 0.8 pg ml^{-1}) is 2500. For photoacoustic detection, the worst result (anthanthrene, 8 ng ml^{-1}) is only 40 times the best value (pyrene, 0.2 ng ml^{-1}). This is readily explained by noting that the fluorescence signal is directly proportional to both fluorophore concentration and fluorescence quantum efficiency, Q. Hence the fluorescence limit of detection is inversely proportional to Q.

The situation is different with respect to photoacoustic detection as the photoacoustic signal, S_{PA}, is given by the following equation:

$$S_{PA} = R\epsilon cE_p(1 - \lambda_{ex.}\bar{\lambda}^{-1}Q) \quad \ldots \quad (1)$$

where R is the responsivity, ϵ is the molar absorptivity, c is the fluorophore concentration, E_p is the incident pulse energy at wavelength $\lambda_{ex.}$ and $\bar{\lambda}$ is the mean emission wavelength. This is a simple rearrangement of a result given by Adams *et al.*[12] Thus, for photoacoustic limits of detection (L) we have the following:

$$\frac{L_{Q=1}}{L_{Q=0}} = \left(1 - \lambda_{ex.}\bar{\lambda}^{-1}\right)^{-1} \quad \ldots \quad (2)$$

For azulene, with $\lambda_{ex.} = 337.1$ nm and $\bar{\lambda} \approx 374$ nm, this ratio is 10. For rubrene, with $\bar{\lambda} \approx 549$ nm, the ratio is 2.6. Thus, although photoacoustic detection is not optimal for highly fluorescent (high Q) species, it is much less sensitive to widely varying Q values.

One possible application of this result, which also incorporates simultaneous fluorescence and photoacoustic detection, would be to fluorochrome and antifluorochrome stains used in biological testing. As Hirschfeld[13] has pointed out, the increases in quantum efficiency that fluorochromes exhibit in the bound state are not due to substantial shifts in the absorption bands. Similarly, antifluorochromes, which fluoresce less strongly in the bound state, may be difficult to distinguish from excess of unbound dye or sample autofluorescence. With antifluorochromes, the photoacoustic signal would increase on binding. Even with fluorochromes, it would decrease only by a small factor. Our piezoelectric transducer may easily be attached to a modified microscope translation stage.

Examination of the dye laser excited limits of detection in Table I shows that the low incident pulse energies are highly detrimental to the photoacoustic limits of detection, but of little

consequence for the fluorescence limits of detection. It is clear that low incident pulse energies suffice for fluorescence excitation even when the excitation wavelengths are selected for optimal dye laser performance rather than for optimal matching to the molar absorptivities of the relevant PAHs.

These results suggest that little is to be gained from using higher incident pulse energies in the fluorescence detection mode, but they may possibly prove beneficial for the photoacoustic detection scheme. That the latter possibility is not so is shown in Table II. The fluorescence limits of detection are approximately equal whereas the photoacoustic limits of detection are far worse. The deterioration in the photoacoustic limits of detection is due to a greatly increased blank noise and a synchronous artifact produced by the Suprasil cuvette itself. Consequently, it is not possible to improve photoacoustic limits of detection by greatly increasing the incident laser pulse energy. It is at present unclear whether this applies to conventional gas photoacoustic cells and whether such cells might afford higher sensitivities.

TABLE II

COMPARISON OF EXCIMER AND NITROGEN LASER EXCITED FLUORESCENCE AND PHOTOACOUSTIC SPECTROSCOPY LIMITS OF DETECTION OF PAHs IN HEPTANE AT 289 K

Compound	$\lambda_{em.}$*/nm	Limit of detection/ng ml^{-1}			
		Fluorescence†	Photoacoustic†	Fluorescence‡	Photoacoustic‡
1,2-Benzanthracene ..	385	0.08	0.4	0.3	30
1,12-Benzoperylene ..	419	0.03	1	0.3	30
1,2-Benzopyrene	388	0.5	0.8	0.3	50
Chrysene	402	0.2	7	0.2	80
1,2:5,6-Dibenzanthracene ..	394	0.05	0.4	0.1	20

* Wavelengths were determined using a 4 nm spectral band pass.
† $\lambda_{ex.} = 337.1$ nm; $E_p = 1.6$ mJ.
‡ $\lambda_{ex.} = 308$ nm (XeCl); $E_p \approx 80$ mJ.

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Determination of Saccharin by Desorption of Ferroin from Silica Gel

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Ferroin is strongly adsorbed on silica gel but its ion-association complexes are easily desorbed. A simple, rapid and accurate procedure for the determination of saccharin has been devised utilising these properties. The procedure is based on the quantitative formation of the ion-association complex [(Fe phen$_3$)$^{2+}$(saccharin)$_2$$^-$] when saccharin dissolved in water, 60% aqueous ethanol, 70% aqueous acetone or 80% aqueous methanol is shaken with ferroin-impregnated silica gel. The absorbance of the characteristic orange colour of the ferroin ion-association complex is measured at 510 nm.

There are no interferences from glucose, sucrose and dulcin even when these constituents are present at 1 600 times the concentration of saccharin. Sodium cyclamate, sorbic acid, benzoic acid, citric acid and sodium chloride give positive errors. Although the presence of sodium hydrogen carbonate results in a low recovery of saccharin, this compound may be eliminated easily by the addition of dilute sulphuric acid.

A procedure for the routine determination of saccharin in saccharin tablets is proposed.

Keywords: *Saccharin determination*; *silica gel adsorption*; *tris*(1,10-*phenanthroline*)*iron*(*II*)

Methods for the determination of saccharin in foodstuffs are continually being developed to improve sensitivity, specificity, accuracy and reproducibility. Many techniques have been used, such as thin-layer chromatography,[1–5] gravimetry,[6] polarography,[7] colorimetry,[8,9] infrared spectroscopy,[10] gas chromatography,[11,12] ultraviolet spectrophotometry[13] and molecular emission cavity analysis.[14]

Spectrophotometric methods usually involve either solvent extraction of saccharin from an acidified solution into an organic solvent, the absorbance of which is measured in the ultraviolet range, or, alternatively, the saccharin is extracted with a reagent to form a coloured complex that is soluble in an organic solvent.

The desorption of ferroin from silica gel by potassium iodide solution has been studied by Vydra and Markova,[15] who developed a method for the determination of trace amounts of iron in nickel, chromium, molybdenum and tungsten. Desorption of iron occurs because of the formation of the ion-association complex [(Fe phen$_3$)$^{2+}$.2I$^-$].

Ferroin has been employed as a "clean up" reagent for the extraction of saccharin from food products and may be determined colorimetrically.[16] In this method, the ion-association complex is extracted into nitrobenzene.

In the sorption and desorption technique described here, ferroin - silica gel is used and an excess of the reagent in the final solution for spectrophotometric analysis is avoided. Another advantage is that the ferroin-impregnated silica gel may be used several times provided that new calibration graphs are made. Desorption is quantitative for saccharin in the four solvents water, 60% aqueous ethanol, 70% aqueous acetone and 80% aqueous methanol.

Experimental

Apparatus

Absorbance measurements were carried out using 1.0-cm silica or glass cells and a Pye Unicam SP6–400 spectrophotometer.

Reagents

Ammonium iron(II) sulphate solution, 3.3×10^{-2} M. Dissolve 3.2678 g of the hexahydrate salt (A. R., Fisons) in 50 ml of water containing 0.5 ml of concentrated sulphuric acid. Transfer into a calibrated flask and make up to 250 ml.

1,10-Phenanthroline solution, 1×10^{-1} M. Dissolve 4.9557 g of the reagent (AnalaR, BDH) in 250 ml of distilled water.

Tris(1,10-phenanthroline)iron(II) stock solution, 3.3×10^{-2} M. Prepare the ferroin reagent solution by adding 170 ml of ammonium iron(II) sulphate solution (3.3×10^{-2} M) to 170 ml of 1,10-phenanthroline solution (1×10^{-1} M).

Standard saccharin solution, 2.5×10^{-2} M. Dissolve 1.1449 g of saccharin (AnalaR, BDH) in 250 ml of distilled water. Dilute the saccharin solution ten-fold to give a 2.5×10^{-3} M solution for use in the subsequent experiments.

Sodium hydroxide solution, 0.1 M. Dissolve 4.0 g of sodium hydroxide (Cambrian Chemicals) in 1 l of distilled water.

Silica gel. Silica gel (Fisons laboratory reagent, chromatographic grade), 80–200 mesh, is used and should be conditioned before use.

Standard solutions

D-*Glucose*, 1 M. Dissolve 4.504 g of glucose (AnalaR, BDH) in 25 ml of distilled water.

Sucrose, 1 M. Dissolve 8.5575 g of sucrose (AnalaR, BDH) in 25 ml of distilled water.

Sodium chloride, 1 M. Dissolve 1.461 g of sodium chloride (AnalaR, BDH) in 25 ml of distilled water.

Sodium hydrogen carbonate, 0.1 M. Dissolve 0.2100 g of sodium hydrogen carbonate (AnalaR, BDH) in 25 ml of distilled water.

Citric acid, 0.1 M. Dissolve 0.5253 g of citric acid (AnalaR, BDH) in 25 ml of distilled water.

Dulcin [1-(4-ethoxyphenyl)urea]. Dissolve 0.4505 g of dulcin (Eastman Organic Chemicals) in 17.5 ml of acetone and dilute to 25 ml with distilled water.

Sorbic acid, 0.01 M. Dissolve 0.0280 g of sorbic acid (BDH laboratory-grade reagent) in 17.5 ml of acetone and dilute to 25 ml with distilled water.

Benzoic acid, 0.01 M. Dissolve 0.0305 g of benzoic acid (AnalaR, BDH) in 17.5 ml of acetone and dilute to 25 ml with distilled water.

Sodium cyclamate, 0.1 M. Dissolve 0.5030 g of sodium cyclamate (BDH laboratory-reagent grade) in 25 ml of distilled water.

Recommended Analytical Procedures

Conditioning of silica gel

Weigh 280 g of silica gel (80–200 mesh) into a beaker, wash with several amounts of 0.1 M sodium hydroxide solution using a total volume of 400 ml. Repeat the washing with successive amounts of distilled water using a total volume of about 400 ml. Remove any fine particles of silica gel or any turbidity by decantation. Activate by heating in an oven at 120 °C for 10–15 h.

Preparation and conditioning of ferroin-impregnated silica gel matrix

Transfer the activated silica into a 5-l beaker and add 400 ml of distilled water. From a burette add 6.6×10^{-3} M ferroin solution, *i.e.*, stock solution diluted five-fold. The silica gel adsorbs the ferroin, rapidly at first and then more slowly. Continue adding the ferroin solution until the supernatant liquid becomes orange - red in colour. Stir for about 10 min and allow the contents to settle. Discard the supernatant liquid. Wash the matrix several times with distilled water until the supernatant liquid is clear. Filter off the orange-coloured matrix under vacuum and wash it several times with dry ethanol. Finally, dry under vacuum and store in a desiccator.

Recommended procedure for standard calibration graphs

Weigh out 1.00-g amounts of the ferroin-impregnated silica gel matrix into 25-ml bottles.

Add saccharin solutions of the following concentrations:

(*i*) 1.25×10^{-4}–5.0×10^{-4} M when using distilled water;
(*ii*) 7.5×10^{-5}–5.0×10^{-4} M when using 60% aqueous ethanol or 80% aqueous methanol; or
(*iii*) 5.0×10^{-5}–5.0×10^{-4} M when using 70% aqueous acetone.

Make up the volume of the solutions to 10.0 ml using the chosen solvent. Shake the contents of the bottle for 5 min using a mechanical shaker. Measure the absorbance of the decanted and filtered supernatant liquid at 510 nm using 1.0-cm cells and a blank or distilled water as the reference.

Procedure for tablet saccharin "commercial samples"

Two methods can be used for determination of saccharin in tablets.

Direct method. Grind a sufficient number of the tablets to a powder. Weigh accurately a portion of the powdered tablets equivalent to approximately 12.5 mg of saccharin into a small beaker and add 0.80 ml of sulphuric acid (2% *V*/*V*) dropwise. The pH should be between 4.9 and 5.2 after complete dissolution of the tablet. Dilute the solution to 100 ml in a calibrated flask. Shake the contents for a few minutes and then filter through a No. 41 Whatman filter-paper. Transfer 2.0 ml of this solution into a 25-ml bottle containing 1.00 g of ferroin-impregnated silica gel matrix, followed by 1.0 ml of distilled water and 7.0 ml of acetone to make the total volume 10.0 ml. Shake for 5 min using a mechanical shaker. Filter through a No. 41 Whatman filter-paper. Measure the absorbance of desorbed ferroin at 510 nm using distilled water as a reference. From the calibration graph, calculate the saccharin content.

Standard additions method. Prepare the saccharin sample solution as in the method mentioned above (*i.e.*, to give 100.0 ml of solution in a calibrated flask) and transfer 1.0 ml of this solution into each of a number of 10-ml calibrated flasks. Dilute one of these solutions to the mark with 2.0 ml of distilled water and 7.0 ml of acetone. To the other flasks, add known amounts (0.4–1.4 ml) of standard saccharin solution (2.5×10^{-3} M) followed by 7.0 ml of acetone, and dilute to the mark with distilled water. Add the contents of each to 25-ml bottles containing 1.00 g of ferroin-impregnated silica gel. Shake for 5 min using a mechanical shaker. Filter through a filter-paper and measure the absorbances at 510 nm. Extrapolation of the plot thus obtained back to an absorbance of zero gives the saccharin concentration.

Results and Discussion

Preparation and Conditioning of the Silica Gel and Ferroin-impregnated Silica Gel Matrices

Preliminary studies were concerned with obtaining the best conditions for the adsorption of the ferroin on the activated silica and desorption by the saccharin solution. A silica gel with the required properties was obtained by treatment with aqueous sodium hydroxide solution followed by washing with water and finally by thermal activation at 120 °C as described under Recommended Analytical Procedures.

A ferroin-impregnated silica gel matrix having reproducible properties was conveniently made by the procedure given under Recommended Analytical Procedures. The sorption capacity of this ferroin matrix needs to be determined because, in the analytical determination, sufficient ferroin must be present on the silica gel to form the ion-association complex with the saccharin.

Determination of the Sorption Capacity of the Ferroin on Silica Gel

This parameter, defined as "the maximum amount of ferroin adsorbed for each gram of silica gel" was determined as follows.

Portions of washed and activated silica gel (1 g) were placed in 25-ml bottles. Varying amounts of 6.6×10^{-3} M ferroin solution were added ranging from 0.2 to 10.0 ml. The volume of the solution in each bottle was made up to 10.0 ml with distilled water and the contents were shaken for 20 min. The supernatant liquid was filtered through a No. 41 Whatman filter-paper and the absorbance of ferroin in the solution measured at 510 nm using

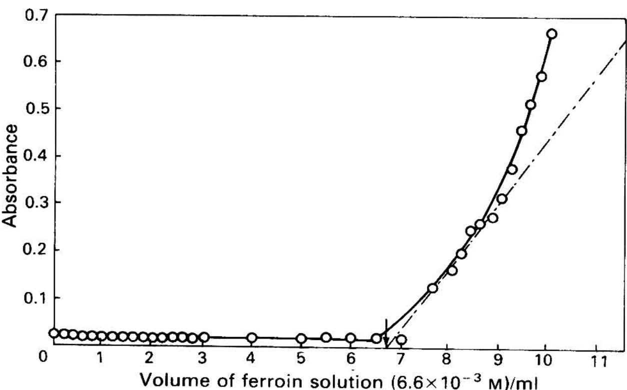

Fig. 1. Determination of sorption capacity of ferroin on silica gel. 1.00-g amounts of silica gel; 0.2–10.0 ml of 6.6 $\times$ 10^{-3} M ferroin solution were added and the volume was made up to 10.0 ml with distilled water. Shaking time, 20 min. Unadsorbed ferroin was measured at 510 nm using a 1.0-cm cell.

1.0-cm cells. Fig. 1 shows a typical graph. The absorbance of the unadsorbed ferroin in solution is plotted as a function of the total amount of ferroin originally added to the silica gel. From such graphs, the amount of ferroin sorbed per gram of silica gel was determined by extrapolation to zero absorbance. It was found that 6.7 ml of 6.6 $\times$ 10^{-3} M ferroin solution is adsorbed per gram of silica gel. It must be pointed out here that the value of the sorption capacity serves as a guide to the amount of ferroin solution that must be added to the silica gel to provide a matrix capable of giving reproducible results in the quantitative desorption step. It is recommended that about 5% in excess of the sorption capacity is added.

Absorption Maximum of the Ion-association Complex

The absorption spectra of the ion-association complex [(Fe phen$_3$)$^{2+}$ (saccharin)$_2$$^-$] shows a maximum at 510 nm irrespective of whether water, aqueous methanol, aqueous ethanol or aqueous acetone is used as solvent.

Solutions of aqueous acetone give the largest absorption for the same amount of saccharin but this does not mean that there is the same concentration of the ion-pair. There is an advantage in using a solvent that gives a high absorbance as this gives a higher sensitivity, and it is recommended that aqueous acetone be used.

Effect of Varying the Shaking Time for the Desportion Process

The effect of varying the shaking time of the batch desorption process from 0.5 to 20 min using a mechanical shaker is shown in Fig. 2. The results show that a constant absorbance was obtained after 5 min for each solvent.

Precision of the Method Using the Four Solvents

Six samples of saccharin solution, 2.5 $\times$ 10^{-4} M in concentration, were subjected to the desorption process using 1.00-g amounts of ferroin-impregnated silica gel matrix and the four solvents. All variables were kept constant and the mean value of the absorbance of the desorbed ferroin was determined (Table I). Standard deviation values are also given. The table shows that satisfactory results are obtained using the recommended procedures and the spectrophotometric method. This suggests that the ferroin is sorbed and homogeneously distributed over the surface of the silica gel matrix. The desorption process is quantitatively reproducible.

Solvent Effects on the Desorption Process

An investigation of the effect of different solvents on the desorption process of the ferroin - saccharin complex is shown in Fig. 3. For each of the aqueous solutions of the organic solvents the absorbance varies with the aqueous organic solvent content, maxima being obtained at a specific concentration for each solvent, *i.e.*, 60% for ethanol, 70% for acetone and 80% for methanol.

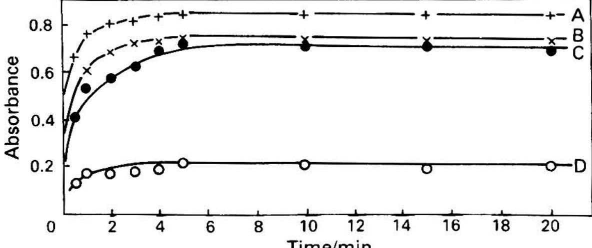

Fig. 2. Effect of varying shaking time on desorption process. 1.00 g of ferroin-impregnated silica gel matrix; 1.0 ml of saccharin solution (2.5 × 10^{-3} M), 6.0 ml of ethanol, or 8.0 ml methanol, or 7.0 ml of acetone, diluted to 10.0 ml with distilled water. Shaking time, 5 min. Desorbed ferroin was measured at 510 nm using a 1.0-cm cell. A, 70% acetone; B, 80% methanol; C, 60% ethanol; and D, water.

TABLE I

PRECISION OF THE METHOD

Sample No.	Absorbance, absorbance units: Distilled water	60% Ethanolic solution	80% Methanolic solution	70% Acetone solution
1	0.215	0.740	0.760	0.870
2	0.210	0.725	0.770	0.875
3	0.215	0.725	0.780	0.860
4	0.220	0.740	0.750	0.875
5	0.220	0.745	0.750	0.850
6	0.220	0.745	0.730	0.850
Mean absorbance	0.216	0.737	0.757	0.863
Standard deviation*	0.004	0.009	0.017	0.015
Relative standard deviation, % ..	1.85	1.22	2.25	1.74

* The values shown include the precision in the weighing, adsorption and desorption steps and the spectrophotometric measurements.

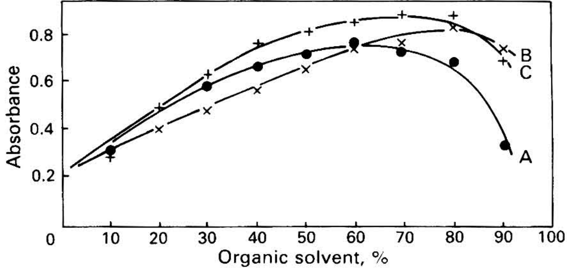

Fig. 3. Effect of solvent on desorption process. 1.00 g of ferroin-impregnated silica gel matrix; 1.0 ml of saccharin solution (2.5 × 10^{-3} M), 0–9.0 ml of organic solvent, diluted to 10.0 ml with distilled water. Shaking time, 5 min. Absorbance measured at 510 nm using a 1.0-cm cell. A, Ethanol; B, methanol; and C, acetone.

Re-usability of the Ferroin-impregnated Silica Gel Matrix

It was found that the matrix may be re-used for two or three subsequent determinations providing that a new calibration graph is prepared.

Calibration Graphs

Calibration graph were obtained by measuring the absorbance of the ferroin desorbed by saccharin solutions of various concentrations. The graphs (Fig. 4) were obtained using four different solvent media. Experiments were carried out as described under Recommended Analytical Procedures.

Linearity was found over the following ranges:

1.25×10^{-4}–5.0×10^{-4} M of saccharin for distilled water;

7.50×10^{-5}–5.0×10^{-4} M of saccharin for 60% aqueous ethanol solution and 80% aqueous methanol solution; and

5.0×10^{-5}–5.0×10^{-4} M of saccharin for 70% aqueous acetone solution.

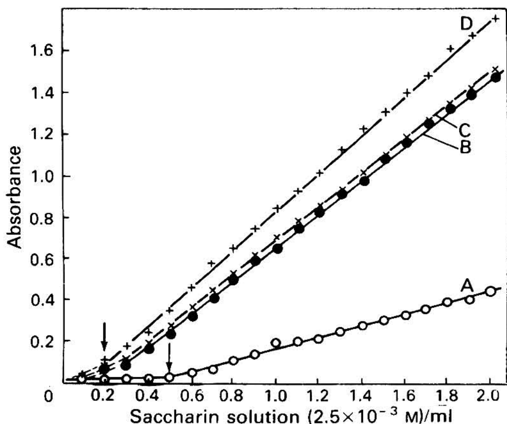

Fig. 4. Calibration graphs. 1.00 g of ferroin-impregnated silica gel matrix. A, Aqueous solutions containing 1.25×10^{-4}–5.0×10^{-4} M saccharin; B, 60% aqueous ethanol solutions containing 7.5×10^{-5}–5.0×10^{-4} M saccharin; C, 80% aqueous methanol solutions containing 7.5×10^{-5}–5.0×10^{-4} M saccharin; and D, 70% aqueous acetone solutions containing 5.0×10^{-5}–5.0×10^{-4} M saccharin. Shaking time, 5 min. Absorbances measured at 510 nm in a 1.0-cm cell.

Interferences

The influence of food additives was examined and it was shown that there are no interferences from other sweetening agents, such as glucose, sucrose and dulcin. The recovery of saccharin, in the presence of large amounts of these compounds (1 600 times the amount of saccharin), was 100%. However, sodium cyclamate, sorbic acid, benzoic acid, citric acid and sodium chloride gave positive errors. The presence of sodium hydrogen carbonate resulted in a very low recovery of saccharin but this compound may be eliminated easily by acid treatment.

Applications

Two commercial samples of saccharin tablets, A and B, from different manufacturers were examined in order to check the validity of the proposed procedure for saccharin determination. The saccharin content of each of ten tablets was determined in order to find out the

degree of variation in saccharin content from tablet to tablet. It was found that the addition of 0.8 ml of sulphuric acid (2% *V*/*V*) to each tablet was sufficient to eliminate the hydrogen carbonates and to adjust the pH to between 4.9 and 5.2, the pH range over which the ferroin is desorbed quantitatively.

Table II shows the correlation between the amount of saccharin found and the amount declared on the label and Table III shows some of the statistical data. The results showed a variation in content of saccharin from tablet to tablet but this variation is within the permitted amounts.[17] The advantage of the direct method is that it is quicker for a large number of samples for routine analysis; hence only one calibration graph is needed.

TABLE II

ANALYSIS OF SACCHARIN TABLETS

Experimental conditions: 2.0 ml of saccharin sample solution (one tablet per 100 ml); 1.0 ml of distilled water; 7.0 ml of acetone; 1.00 g of ferroin-impregnated silica gel matrix; shaking time, 5 min; filtration; and absorbance measurement at 510 nm. Absorbance values for these solutions range from 0.355 to 0.520 absorbance unit.

Tablet sample A				Tablet sample B			
Sample No.	Mass of tablet/mg	Saccharin found/mg	Recovery,* %	Sample No.	Mass of tablet/mg	Saccharin found/mg	Recovery,* %
1	59.4	12.720	101.60	1	60.06	11.793	94.34
2	61.8	11.220	89.76	2	59.10	11.908	95.26
3	59.5	14.200	113.60	3	59.40	13.969	111.75
4	59.4	12.590	100.72	4	60.10	11.335	90.68
5	59.9	12.365	98.92	5	60.00	12.595	100.76
6	59.7	13.510	108.08	6	59.00	12.137	97.10
7	59.1	13.970	111.76	7	57.60	12.137	97.10
8	60.7	13.510	108.08	8	60.70	11.221	89.77
9	57.5	15.115	120.92	9	58.70	13.740	109.92
10	61.6	13.970	111.76	10	59.80	11.221	89.77

* Based on a claim of 12.5 mg of saccharin per tablet.

The proposed method can be adapted to the determination of saccharin in soft drinks after removal of the interfering materials. These substances can be eliminated by a preliminary treatment, for example, in the low calorie soft drinks and beverages containing benzoates and sorbates as preservatives, by pre-extraction with carbon tetrachloride. Chlorides can be eliminated by passing the sample through a column of anion-exchange resin before the extraction of saccharin with diethyl ether.

The standard deviations given in Table I reflect the combined precision in the weighing, adsorption, desorption and spectrophotometric processes, whereas the larger standard deviations given in Table III are indicative of the variations of the saccharin content from tablet to tablet.

TABLE III

STATISTICAL ANALYSIS OF RESULTS OBTAINED FOR SACCHARIN TABLETS

Statistical parameter	Tablet sample A	Tablet sample B
Mean mass of tablet/mg	59.860	59.500
Mean saccharin found/mg	13.300	12.206
Mean recovery, %	106.520	97.640
Standard deviation/mg	1.120	0.976
Relative standard deviation, %	8.350	7.996
Standard deviation from the declared value/mg	1.400	1.024
Relative standard deviation from the declared value, %	11.200	8.195

Conclusions

A new technique, which ends with a spectrophotometric measurement, has been developed for the determination of saccharin. The method is based on the quantitative formation of the ion-association complex $[(Fe\ phen_3)^{2+}(saccharin)_2^-]$ when aqueous ethanol, aqueous methanol or aqueous acetone solutions of saccharin are shaken with ferroin-impregnated silica gel. The absorbance of the characteristic orange colour of the desorbed ferroin ion-association complex is measured at 510 nm.

The method is simple and less time consuming than other methods; accuracy and reproducibility are good and the use of reagents is kept to a minimum. Large amount of glucose, sucrose and dulcin do not interfere with the determination. Interfering compounds such as sodium cyclamate, sorbic acid, benzoic acid, citric acid and sodium chloride need to be removed prior to the determination.

El-Sayed A. K. Yacoub thanks his employer, The University of Gezira, Wad Medani, Sudan, for study leave.

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Assay of Hydrazine in Isoniazid and its Formulations by Difference Spectrophotometry

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A rapid procedure is described for the determination of hydrazine at the very low levels that may occur as a result of hydrolysis in certain isoniazid formulations. The method is based upon the measurement of the absorbance difference between a solution of the 4-dimethylaminobenzalazine derivative of hydrazine and a similarly prepared solution containing 3% *V/V* acetone. The absorbance difference, which is proportional to the concentration of hydrazine, is unaffected by the presence of a large excess of isoniazid. The choice of experimental conditions which provide the maximum accuracy, sensitivity and specificity is discussed.

Keywords: *Hydrazine determination; ultraviolet spectrophotometry; difference spectrophotometry; isoniazid; isoniazid formulations*

Isoniazid (isonicotinic acid hydrazide), an important tuberculostatic drug, is found on degradation to yield a number of products including hydrazine,[1] which has been recognised as a carcinogenic and hepatotoxic substance.[2] Consequently, drug regulatory authorities are becoming increasingly aware of the need to control the levels of hydrazine in isoniazid and other hydrazide drugs and in their formulations.

Although many methods exist for the assay of hydrazine in a variety of samples, few published procedures[3–5] deal specifically with the determination of hydrazine in drugs. This paper describes the development of a difference spectrophotometric assay of hydrazine in isoniazid and its formulations. The method is based on the measurement of the absorbance at 456 nm of a solution of the yellow 4-dimethylaminobenzalazine derivative produced by the reaction of hydrazine with 4-dimethylaminobenzaldehyde in hydrochloric acid solution,[6] relative to that of an equimolar solution of the reactants containing 3% *V/V* acetone. The absorbance difference due to the reduced concentration of the 4-dimethylaminobenzalazine in the presence of acetone, which also reacts with hydrazine to form the colourless dimethylketazine, is rectilinearly related to the concentration of hydrazine. Although isoniazid also reacts with the colour reagent to form the 4-dimethylaminobenzylidene derivative that interferes in a simple colorimetric assay of hydrazine, the conditions of the assay have been chosen to give zero absorbance difference for isoniazid and consequently the assay is specific for hydrazine.

Experimental

Reagents

The reagents were obtained from BDH Chemicals Ltd.

Isoniazid. Recrystallised twice from ethanol.

4-Dimethylaminobenzaldehyde for hydrazine determinations.

4-Dimethylaminobenzaldehyde reagent. Dissolve 4-dimethylaminobenzaldehyde (20 g) in hydrochloric acid (sp. gr. 1.18, 172.5 ml) and dilute to 1 l with water.

Acetone (AnalaR).

Apparatus

Absorbance values were measured in 1, 2 or 4 cm path length cells using a Cecil 505 or a Perkin-Elmer 552 double-beam spectrophotometer.

Standard Solutions

Dissolve hydrazine sulphate (0.2031 g, equivalent to 0.050 g of hydrazine base) in water and dilute to 250 ml. Dilute 5 ml of this solution to 1 l with water, thus obtaining a standard

solution of hydrazine containing 1 μg ml^{-1}. Transfer 5 ml of the standard solution to two calibrated flasks (50 ml). To one flask add 4-dimethylaminobenzaldehyde reagent (20 ml), starting a stop-clock when half of the reagent has been added. Dilute to 50 ml with water and shake the flask vigorously for 10 s. Transfer the solution to a 4-cm cell and exactly 2 min after the addition of the reagent, measure the absorbance of the solution at 456 nm relative to air. To the other flask add 1.5 ml of acetone and continue the assay as described above from the words "add 4-dimethylaminobenzaldehyde reagent . . . ." Calculate the absorbance difference at 456 nm (ΔA_{456}) as the absorbance of the 4-dimethylaminobenzalazine solution relative to that of the solution containing acetone.

Measure the absorbance at 456 nm of a solution of 4-dimethylaminobenzaldehyde reagent diluted 2 + 3 with water and of a similar solution containing 3% *V*/*V* of acetone.

Liquid Formulations of Isoniazid

Dilute an amount of the sample, equivalent to 50 mg of isoniazid, to 25 ml with water. Transfer equal aliquots up to 10 ml in volume, depending on the concentration of hydrazine in the sample, to two calibrated flasks (50 ml) and continue the assay as described for the standard solutions from the words "To one flask . . . ." Samples containing very high levels of hydrazine will require an intermediate dilution and/or measurement in a shorter path length cell.

Isoniazid Tablets

Weigh and powder 20 tablets. Shake some of the powder, equivalent to 200 mg of isoniazid, with 0.01 M hydrochloric acid (80 ml) in a calibrated flask (100 ml) for 5 min and dilute to the mark with water. Filter the solution through No. 1 filter-paper, discarding the first 10 ml of filtrate, and continue the assay as described above from the words "Transfer equal aliquots . . . ."

Calculate the concentration of hydrazine in the sample solutions from the ΔA_{456} of the sample solutions corrected for the small ΔA_{456} of the blank solutions with reference to the net ΔA_{456} of the standard solutions of hydrazine. If required, express the concentration of hydrazine in the sample relative to the content of isoniazid as a percentage by mass after assaying the isoniazid by the official procedure of the British Pharmacopoeia (1980)[7] or British Pharmaceutical Codex (1973).[8]

Results and Discussion

Composition of the Colour Reagent

The visible absorption spectra of 4-dimethylaminobenzalazine and the 4-dimethylaminobenzylidene derivative of isoniazid, formed by treating hydrazine and isoniazid, respectively, with a reagent containing 4-dimethylaminobenzaldehyde and hydrochloric acid, are shown in Fig. 1. The end absorption of the isoniazid derivative ($\lambda_{max.}$ 404 nm) overlaps the absorption maximum at 456 nm of the hydrazine derivative and is a source of interference in a simple colorimetric assay of hydrazine.

In order to determine the optimum composition of the colour reagent for the difference spectrophotometric assay of hydrazine, the absorbances at 456 nm of solutions of hydrazine and isoniazid were measured separately and in a mixture of hydrazine (0.02% *m*/*m*) in isoniazid after reaction with different colour reagents in which the concentrations of 4-dimethylaminobenzaldehyde and hydrochloric acid were varied independently. The maximum absorptivity of the hydrazine derivative was obtained with a reagent containing a high concentration of 4-dimethylaminobenzaldehyde and a low concentration of hydrochloric acid [Fig. 2(*a*)]. However, these conditions also increase the absorptivity of the isoniazid derivative relative to that of the hydrazine derivative, so that the maximum absorptivity of the hydrazine derivative relative to that of the isoniazid derivative is given by a reagent containing a low concentration of 4-dimethylaminobenzaldehyde and a high concentration of acid [Fig. 2(*b*)].

A high concentration of 4-dimethylaminobenzaldehyde is required for the maximum formation of the derivatives in the presence of each other [Fig. 2(*c*)]. The total A_{456} of the mixture, expressed as a percentage of the sum of the individual values, exceeded 99% in solutions containing 0.8% *m*/*V* of 4-dimethylaminobenzaldehyde and 0.8 M hydrochloric acid.

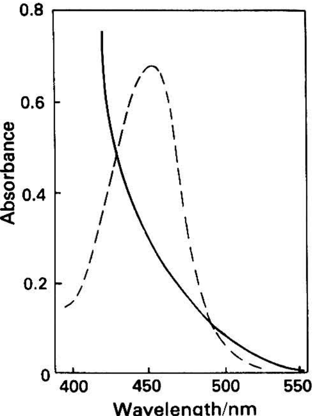

Fig. 1. Visible absorption spectra of 4-dimethylaminobenzalazine (3.125 μM, broken line) and the 4-dimethylaminobenzylidene derivative of isoniazid (3.65 mM, solid line).

The composition of the colour reagent was therefore chosen to give a final concentration of 0.8% *m/V* of 4-dimethylaminobenzaldehyde in 0.8 M hydrochloric acid. These concentrations give quantitative formation of the derivatives in the presence of each other and result in a compromise between maximum absorptivity of the hydrazine derivative and its maximum absorptivity relative to that of the isoniazid derivative.

After the rapid formation of the 4-dimethylaminobenzylidene derivative of isoniazid, within several seconds, the A_{456} of the solution was observed to increase slowly at a rate depending on the acidity of the solution [Fig. 2(*d*)]. After standing for 16 h the solution, on dilution, exhibited a distinct absorption band with a $\lambda_{max.}$ at 456 nm. The increase in A_{456} is therefore caused by hydrolysis of the 4-dimethylaminobenzylidene derivative followed by formation of the aldazine derivative in the presence of excess of 4-dimethylaminobenzaldehyde. A reaction time of 2 min precisely has been chosen as it has been found in this and earlier work[4] to allow complete formation of the hydrazine derivative and to minimise problems owing to the instability of the isoniazid derivative.

Effect of Acetone on the A_{456} of the Derivatives

The effect of increasing concentrations of acetone added to solutions of hydrazine and isoniazid before the colour reagent is shown in Fig. 3. The acetone almost abolishes the colour of the hydrazine derivative, probably as a result of the formation of the colourless dimethylketazine in equilibrium with the aldazine derivative. The presence of acetone in the isoniazid solutions also has a small effect on the A_{456} of its coloured derivative, giving a slightly reduced A_{456} at concentrations less than 3% *V/V* and a slightly greater A_{456} at concentrations above 3% *V/V*. The A_{456} of a solution of the isoniazid derivative containing 3% *V/V* of acetone, however, is identical with that of an equimolar solution containing no acetone. The concentration of acetone in the reference solutions of the derivatives was therefore chosen as 3% *V/V* as this provides a satisfactory absorbance difference for the hydrazine and zero absorbance difference for isoniazid, conditions that are necessary for the successful application of difference spectrophotometry.

The apparent reduction in the A_{456} of the isoniazid derivative at concentrations of acetone less than 3% *V/V* may be a result of the decrease in the A_{456} of the hydrazine derivative generated after 2 min by hydrolysis of the isoniazid derivative. 4-Dimethylaminobenzalazine produced by hydrolysis in the colour reagent does not interfere with the absorbance difference of hydrazine in the sample as the conditions of the assay, *viz.*, time of measurement and the concentrations of acetone and colour reagent, have been selected to give zero absorbance difference for isoniazid.

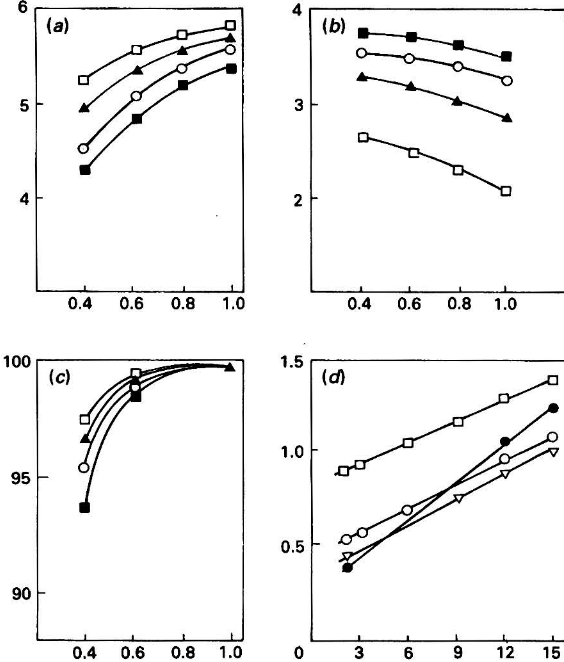

Fig. 2. Optimisation of concentrations of 4-dimethylaminobenzaldehyde (4-DMAB) and hydrochloric acid in the colour reagent. (*a*) Molar absorptivity ($\times 10^{-4}$) of hydrazine (ordinate) in various concentrations (% *m*/*V*) of 4-DMAB (abscissa) and hydrochloric acid. (*b*) Relative molar absorptivity ($\times 10^{-3}$) of hydrazine to isoniazid (ordinate) in various concentrations (% *m*/*V*) of 4-DMAB (abscissa) and hydrochloric acid. (*c*) Absorbance of a mixture of hydrazine (0.02% *m*/*m*) in isoniazid, as a percentage of the sum of the individual absorbance values (ordinate), in various concentrations (% *m*/*V*) of 4-DMAB (abscissa) and hydrochloric acid. (*d*) Absorbance at 456 nm (ordinate) of isoniazid (0.5 mg ml^{-1}) in colour reagents containing 0.8% *m*/*V* of 4-DMAB and various concentrations of hydrochloric acid as a function of time (abscissa, min). -●-●-, 1.6 M HCl; -▽-▽-, 1.2 M HCl; -■-■-, 1.0 M HCl; -○-○-, 0.8 M HCl; -▲-▲-, 0.6 M HCl; -□-□-, 0.4 M HCl. Some data points have been omitted for clarity.

Adherence to Beer's Law

Beer's law graphs for hydrazine showed that a rectilinear relationship exists between the measured ΔA_{456} and the concentration of hydrazine in the range 0–0.20 μg ml^{-1}. The regression equation was $y = 4.92x + 0.0013$, where y is the ΔA_{456} in a 4-cm cell and x μg ml^{-1} is the concentration of hydrazine (correlation coefficient 0.99998). An almost identical line ($y = 4.90\,x + 0.0056$; correlation coefficient 0.99998) was obtained for a similar series of solutions of hydrazine containing isoniazid (500 μg ml^{-1}).

Specificity

To assess further the specificity of the method to samples containing isoniazid, a series of solutions containing hydrazine at 1 μg ml^{-1} and increasing concentrations of isoniazid, 0–10 mg ml^{-1}, were assayed by the procedure, the final concentration of hydrazine in each solution being 0.1 μg ml^{-1}. The ΔA_{456} of all solutions containing isoniazid fell within 99.7–101.0% of that of the standard solution of hydrazine containing no isoniazid, confirming that

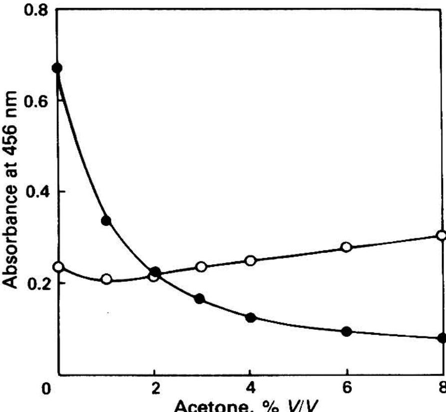

Fig. 3. Effect of acetone on the absorbance at 456 nm of the coloured derivatives of (closed circles) hydrazine (0.1 μg ml^{-1}) and (open circles) isoniazid (500 μg ml^{-1}).

the ΔA_{456} measured is independent of the level of isoniazid up to 250% of the specified analytical concentration of isoniazid.

Isonicotinic acid, isonicotinamide and bisisonicotinoylhydrazine,[9] which, in addition to hydrazine, have been identified in degraded solutions of isoniazid,[1] were found to give zero absorbance difference and therefore do not interfere in the assay.

The procedure has been found to be unsuitable for isoniazid formulations containing sodium aminosalicylate as this substance also reacts with the colour reagent to give a heavy orange precipitate.

Accuracy, Precision and Limit of Detection

A series of standard solutions containing hydrazine and isoniazid, prepared to simulate solutions of isoniazid that had undergone varying degrees of hydrolytic decomposition, were assayed by use of the procedure. The results in Table I show that an excellent recovery of hydrazine was achieved and that the method is satisfactory even in 0.008% hydrolysed samples.

The standard deviations of the absorbance difference given by a solution of isoniazid (5 mg ml^{-1}) and hydrazine (1 μg ml^{-1}) (S_A) and a similar solution of isoniazid containing no hydrazine (S_B), each assayed ten times by the procedure, were found to be 2.70×10^{-3} and 9.68×10^{-4}, respectively. The relative standard deviation, calculated from the corrected standard deviation $[\sqrt{(S_A{}^2 + S_B{}^2)}]$,[10] was 0.574% and the limit of detection ($2S_B$)[10] of hydrazine in isoniazid corresponded to a concentration of 7.7×10^{-5}% *m/m*. These results show that the present method is the most accurate, precise and sensitive of the methods available for the assay of hydrazine in isoniazid.

Assay Results

In order to test the application of the method, the levels of hydrazine were measured in samples of isoniazid drug substance and in several formulations, both freshly prepared and

TABLE I

ASSAY OF HYDRAZINE IN STANDARD MIXTURES OF HYDRAZINE AND IZONIAZID

Concentration of hydrazine in isoniazid, added/μg g^{-1} ..	20	50	100	200	500	1000	2000	5000	10000
Concentration of hydrazine in isoniazid, found/μg g^{-1} ..	19.3	49.3	99.8	198.2	505.0	1001	1986	4980	10010
Accuracy, %	96.4	98.5	99.8	99.1	101.0	100.1	99.3	99.6	100.1

stored for periods up to 7 years. The results in Table II show that the commercial samples of the drug substance and tablets examined and the freshly prepared elixir and injection contain negligible amounts of hydrazine. Considerably higher levels of hydrazine were, however, found in the liquid formulations stored at room temperature for long periods or maintained at elevated temperatures, including a sample of injection sterilised by heating in an autoclave at 121 °C for 15 min. These results confirm that hydrazine is formed in isoniazid solutions owing to hydrolytic decomposition and that the extent of the hydrolysis is readily measured by using the difference spectrophotometric assay.

TABLE II

LEVELS OF HYDRAZINE IN IZONIAZID DRUG SUBSTANCE AND FORMULATIONS

Sample	Content of isoniazid (stated)	Storage/treatment	Concentration of hydrazine ($\mu g\ g^{-1}$ of isoniazid)
Drug substance*	—	—	0
Drug substance*	—	—	0
Tablets*	100 mg	7 years at room temperature	0
Tablets*	100 mg	5 years 10 months at room temperature	10.2
Tablets*	50 mg	5 years 10 months at room temperature	13.3
Elixir†	10 mg ml^{-1}	—	0
Elixir†	10 mg ml^{-1}	100 min at 100 °C	390.0
Elixir†	10 mg ml^{-1}	6.5 h at 55 °C	49.7
Injection†	25 mg ml^{-1}	—	0
Injection†	25 mg ml^{-1}	Autoclaved at 121 °C for 15 min	248.6
Injection*	25 mg ml^{-1}	—	242.4
Injection*	25 mg ml^{-1}	—	401.1
Injection*	25 mg ml^{-1}	5 years at room temperature	1846
Injection*	25 mg ml^{-1}	5 years at 35 °C	7558

* Commercial samples.
† Extemporaneously prepared samples.

The assistance of Mr. B. Sims, Roche Products Ltd., in supplying isoniazid samples is gratefully acknowledged.

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Highly Sensitive Spectrophometric Determination of Trace Amounts of Aluminium with Chromazol KS and Cetylpyridinium Bromide

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A highly sensitive spectrophotometric method has been developed for the determination of aluminium, based on the formation of a ternary complex with chromazol KS (CALKS) and cetylpyridinium (CP) bromide in aqueous solution in the presence of 25–40% of ethanol. The pH range for the formation of the ternary complex is 5.8–6.7, and the wavelength of maximum absorbance is 625 nm. The ternary system obeys Beer's law for between 0.02 and 0.32 μg ml^{-1} of aluminium. A high molar absorptivity of $\epsilon = 1.02 \times 10^5$ l mol^{-1} cm^{-1} and a Sandell's sensitivity of 0.00026 μg cm^{-2} have been obtained. The complex has the composition $Al(OH)(CALKS)_2(CP)_6$, as established by Job's method of continuous variation and the equilibration shift method. The method has good selectivity and so can be applied to the direct spectrophotometric determination of trace amounts of acid-soluble aluminium in steel.

Keywords: *Aluminium determination*; *spectrophotometry*; *chromazol KS*; *cetylpyridinium bromide*

The addition of cationic surfactants to a solution containing a binary complex usually causes a marked change in colour, resulting in a bathochromic shift in the absorption spectra with an increase in the absorbance. In recent years, most studies of these changes have been confined to acidic triphenylmethane dyes, there being fewer studies on the azo dyes.

Chromazol KS (CALKS) [trisodium salt of 2-(2-hydroxy-3-carboxy-5-sulphobenzolazo)-1,8-dihydroxynaphthalene-3,6-disulphonic acid] is a monoazo reagent based on chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid). Basargin *et al.*[1,2] have proposed this as a spectrophotometric reagent for the determination aluminium. We[3] have used the reagent for the determination of aluminium in steel, but the sensitivity was not very good.

CALKS

The addition of cetylpyridinium bromide (CPB) to a solution of the binary complex of aluminium with chromazol KS in the presence of ethanol causes a bathochromic shift in the absorbance with a two-fold increase in the molar absorptivity over the binary complex; in the absence of ethanol the effect is not as marked.

This paper reports the results of a study of the colour reactions of this ternary complex and proposes a procedure for the determination of trace amounts of acid-soluble aluminium in steel.

Experimental

Reagents

Chromazol KS solution, 0.1%. The reagent was synthesised as previously reported.[1] Dissolve 0.5000 g of chromazol KS (prepared in the chemical plant of Shanghai Normal University) in 500 ml of water. This solution is stable for at least 1 year.

EDTA - manganese(II) solution. Weigh out 37.2 g of the disodium salt of EDTA and 8.5 g of manganese sulphate, dissolve in 300 ml of water, with heating, and neutralise with dilute (1 + 1) ammonia solution using bromocresol purple as the indicator, the colour change being from yellow to purple. Cool and dilute to 1 l with water.

Buffer solution (pH about 6.2). A mixture of 500 ml of 2 M sodium acetate solution and 10 ml of 36% acetic acid.

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

Ethanolic cetylpyridinium bromide solution, 0.5% *m/V* in ethanol.

Aluminium standard solution. Dissolve, with heating, 0.1000 g of spectroscopic-grade aluminium in 10 ml of 6 M hydrochloric acid, cool and transfer into a 1000-ml calibrated flask and dilute to the mark. Pipette 20 ml of this solution into a 200-ml calibrated flask and dilute to the mark to give a solution containing 10 $\mu g\ ml^{-1}$ of aluminium.

Sulphuric - nitric acid mixture. Water plus 50 ml of sulphuric acid and 8 ml of nitric acid in a total volume of 1000 ml.

All other chemicals used were of analytical-reagent grade.

Apparatus

A 721 spectrophotometer (Third Analytical Instrument Factory, Shanghai), wavelength range 360–800 nm, was employed for absorbance measurements. The pH measurements were made with a pHs-2 pH meter (Second Analytical Instrument Factory, Shanghai), pH range 0–14 and accuracy to within a pH of 0.02, consisting of a glass - calomel electrode assembly.

Results and Discussion

Spectral Characteristics

In Fig. 1 lines A, B and C show the absorption spectra of chromazol KS, the binary complex of aluminium - chromazol KS and the ternary complex of aluminium - chromazol - CP^+, respectively, at pH 6.2.

The wavelength of maximum absorbance of the aluminium - chromazol KS complex is at 600 nm. When the ternary complex is formed with the addition of cetylpyridinium bromide a bathochromic shift moves the wavelength of maximum absorbance to 625 nm, and is accompanied by a marked increase in the absorbance.

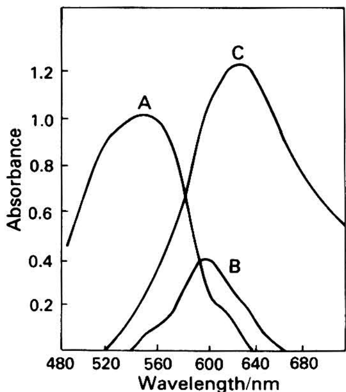

Fig. 1. Absorption spectra of Al - Chromazol KS - CP^+ system. A, 5 ml of 0.1% Chromazol KS added to 3 ml of buffer solution (pH 6.2) and 10 ml of ethanol, diluted to 50 ml and measured against water in a 1-cm cell; B, as A, but 16 μg of Al added and measured against reagent blank; C, as B, but 4 ml of 0.5% CPB added and measured against reagent blank.

Effect of Varying the Conditions on Colour Development

Effect of pH

The effect of pH on the absorbance of the complex was studied at a wavelength of 625 nm on solutions containing 1.6 ml of the aluminium standard solution (16 μg of aluminium), 5 ml of 0.1% chromazol KS solution, 10 ml of ethanol and 4 ml of 0.5% CPB solution, diluted to 50 ml.

Fig. 2 shows the dependence of the absorbance on pH. The optimum pH suitable for the formation of the ternary complex was 5.2–6.7.

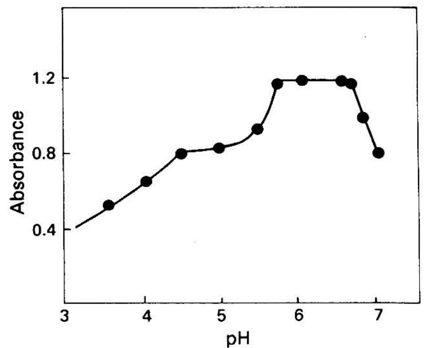

Fig. 2. Dependence of absorbance on pH. λ = 625 nm; l = 1 cm; measured against reagent blank; ethanol concentration = 28%.

Effect of ethanol concentration

If CPB alone was added to the solution of the binary complex of aluminium and chromazol KS, with no ethanol present, then the colour change was not marked. If the solution of the complex contains ethanol, on addition of CPB to the solution a change of colour is observed and there is an increase in the absorbance.

The maximum of absorbance was obtained for an ethanol concentration of between 25 and 40% (Fig. 3). Any further increases in the concentration of ethanol caused a marked decrease in the absorbance.

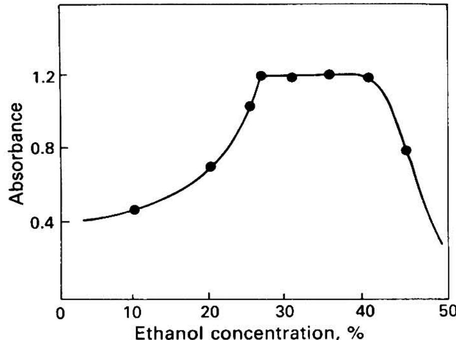

Fig. 3. Effect of ethanol concentration. λ = 625 nm; pH = 6.2; l = 1 cm; measured against reagent blank.

Effect of reagent concentrations

The maximum absorbance was obtained with concentrations of chromazol KS and CPB of 0.0010–0.0014% and 0.003–0.007%, respectively.

The final concentrations used in the method were 0.001% of chromazol KS and 0.004% of cetylpyridinium bromide.

Stability of Colour of Ternary Complex in Solution

When chromazol KS solution and CPB solution are added to a solution of aluminium, under the experimental conditions given in the proposed procedure and at room temperature (10–30 °C), 10 min are needed for full colour development. The colour is very stable, its absorbance varying by not more than ±2% after 40 h.

Composition of Ternary Complex

A molar ratio for aluminium to chromazol KS in the ternary complex of 1:2 was determined by Job's method of continuous variation (Fig. 4) and the equilibrium shift method [Fig. 5(*a*)]. A molar ratio for aluminium to CP^+ in the complex of 1:6 was established by the equilibrium shift method [Fig. 5(*b*)].

The composition of the ternary complex was therefore $Al(OH)(CALKS)_2(CP)_6$.

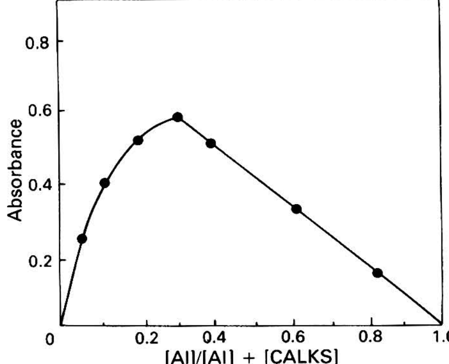

Fig. 4. Composition of the complex as established by Job's method. [Al] + [CALKS] = 2×10^{-4} M; [CPB] = 1×10^{-3} M; pH = 6.2; λ = 625 nm; l = 2 cm.

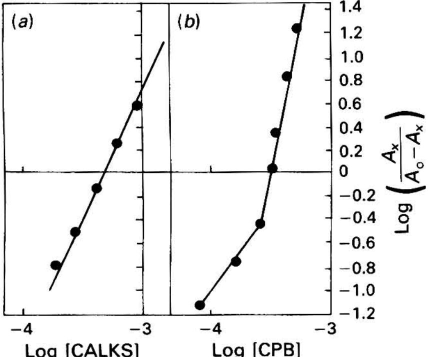

Fig. 5. Composition of the ternary complex as established by the equilibrium shift method. [Al] = 10 µg per 50 ml; pH = 6.2; λ = 640 nm; l = 1 cm.

Beer's Law and Sensitivity of Reaction

The calibration graph was found to obey Beer's law in the range 1–16 µg of aluminium in 50 ml of solution. The molar absorptivity of the reaction was calculated to be 1.02×10^5 l mol^{-1} cm^{-1} at 625 nm. Sandell's sensitivity was 0.000 26 µg cm^{-2}.

Interference Study

The selectivity of the proposed method was investigated by the determination of 16 µg of aluminium in the presence of a series of other ions.

The following ions did not interfere: 50 mg of manganese(II); 2 mg of lead(II), zinc(II), calcium(II) and magnesium(II); 1 mg of antimony(III) and chromium(VI); 500 µg of nickel(II), titanium(IV), chromium(III) and bismuth(III); 250 µg of tungsten(VI) and molybdenum(VI); 100 µg of niobium(V), cerium(III) and zirconium(IV).

The interfering effect of 1 mg of copper(II) was eliminated by the addition of thiourea. Iron(III) also interferes; however, the effect of a large amount of iron(III) can be eliminated by using the EDTA - manganese(II) solution.

Large amounts of citrate, fluoride and tartrate cause a reduction in the absorbance, and thiocyanate and perchlorate produce a cloudy solution.

The method has good selectivity and can be applied to the direct spectrophotometric determination of trace amounts of aluminium in steel.

Procedure for Spectrophotometric Determination of Acid-soluble Aluminium in Iron and Steel

Dissolve 0.2000 g of the sample in 20 ml of the sulphuric - nitric acid mixture and heat. Add 5 ml of 15% ammonium persulphate solution and boil for 1.5 min. Add 2–3 drops of 10% hydrogen peroxide, cool, transfer into a 100-ml calibrated flask and dilute to the mark with water.

Pipette 10 ml of this test solution into a 50-ml calibrated flask. Add 5 ml of the EDTA - manganese(II) solution and stand for 3–5 min. In the following order, add 4 ml of 30% sodium acetate solution, 3 ml of pH 6.2 buffer solution, 5 ml of 0.1% chromazol KS solution, 10 ml of ethanol, 5 ml of water and 4 ml of 0.5% CPB solution. Dilute to the mark with water and shake.

Measure the absorbance of the solution after 10 min, against a reagent blank, at 625 nm.

Precision of the Method

In order to determine the precision of the proposed method for the determination of aluminium in steel, the same standard steel sample containing 0.061% of aluminium was determined ten times. The standard deviation of the result was 0.0007%. The results for the analyses of several standard steel samples are given in Table I.

TABLE I

ANALYSIS OF STANDARD STEEL SAMPLES

Steel sample	Acid-soluble aluminium content, %		Elements other than aluminium and iron, %
	Certified	Found	
Industrial pure iron BH 1504–1	0.014	0.0135 ± 0.0005	C 0.087, Si 0.0146, Mn 0.40, P 0.0064, S 0.016, Ni 0.028, Cr 0.0053, Cu 0.085
Medium carbon steel 221	0.036	0.0363 ± 0.0006	C 0.37, Si 0.282, Mn 0.67, P 0.013, S 0.005, Cr 0.189, Cu 0.14, Co 0.013, Sn 0.012, Ni 0.202, N 0.0105
Alloy steel 70–12	0.037	0.0376 ± 0.0006	C 0.20, Si 0.023, Mn 0.052, P 0.006, S 0.015, Cr 0.13, Ni 0.52 Mo 0.053, V 0.035, Cu 0.067
Low-alloy steel 7126	0.061	0.0608 ± 0.0008	C 0.10, Si 0.26, Mn 0.94, P 0.013, B 0.0135, V 0.23, Cu 0.043, Mo 0.0025
Industrial pure iron BH 1506–1	0.074	0.0731 ± 0.0014	C 0.120, Si 0.034, Mn 0.41, S 0.0085, P 0.0059, Ni 0.049, Cr 0.0104, Cu 0.108
Low-alloy steel 7135	0.081	0.083 ± 0.0020	C 0.065, Si 0.38, Mn 0.96, P 0.174, Mo 0.10, R. E.* 0.014, Ti 0.061, Cr 0.016

* Rare earth elements.

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Rapid Determination of Residual Chlorine by Flow Injection Analysis

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Flow injection determination of residual chlorine in solution has been carried out spectrophotometrically by methyl orange decolorisation and by formation of a yellow complex with *o*-tolidine. The carrier streams are 0.180 mM methyl orange in pH 2 buffer solution or 0.01 M hydrochloric acid, and 3.0 mM *o*-tolidinium dichloride (in 2 M hydrochloric acid), respectively. A sampling rate of 288 samples per hour has been obtained with the latter reagent and the detection limit is 0.08 p.p.m. of chlorine. Influence of flow parameters such as flow-rate, tube length and diameter and the effect of interferents on the determination have been investigated. A study of the hyprochlorite - ammonium - *o*-tolidine reaction system has been performed and a method for the simultaneous determination of NH_4^+ and OCl^- is described.

Keywords: *Chlorine determination; flow injection analysis; methyl orange;* o-*tolidine*

Flow injection analysis, developed by Růžička and Hansen,[1,2] employs Skeggs' concept of continuous flow measurements,[3] but without air segmentation. It has received considerable attention recently, as it provides a simple approach to automation of chemical analyses. Further, the technique has the advantages of good sensitivity, reproducibility and a high sampling rate. The theory of flow injection analysis and the influence of experimental parameters on analyses have been discussed and its applications in spectrophotometric, potentiometric, turbidimetric, fluorimetric and other analytical measurements have been reviewed by Růžička and Hansen[4] and Betteridge.[5]

Iodimetric and amperometric methods[6] and several colorimetric procedures,[7] such as the DPD (*NN*-diethyl-*p*-phenylenediamine oxalate) method proposed by Palin,[6] and the leuco crystal violet method,[6] are available for the assay of chlorine (free and/or combined) in solution. The decrease in the colour of azo dyes,[8,9] by chlorination, has been utilised for determining residual chlorine in solution. The reaction of methyl orange with chlorine depends on the pH of the medium and the addition order of reagents. Limitations and advantages are discussed by Boltz.[9] Athavale *et al.*[10] have employed the procedure for determining residual chlorine in solution. Laitinen and Boyer[11] attribute the decrease in absorbance of dye solutions, at 505 nm, to two competitive reactions involving aromatic substitution and azo-link cleavage. A sensitive method for determining chlorine involves the oxidation of *o*-tolidine in acid solution[9] and the yellow product, believed to be a monomeric quinone imine (holoquinone),[12] then absorbs at 438 nm. The colour intensity reaches a maximum almost immediately and decreases thereafter at a moderate rate. Flow injection determination of halogens, in solution, has not been investigated. A recent paper by Ramasamy *et al.*[13] describes the determination of bromine and chlorine based on two consecutive reactions at a gas - solid interface.

This paper reports the adaptation of the methyl orange and *o*-tolidine reactions to the determination of chlorine in solution, using a simplified flow injection apparatus. The proposed methods are straightforward and provide a high sampling rate. Minor variations in reagent strengths or mode of addition of reagents have no influence. Based upon our investigations of the hypochlorite - ammonium - *o*-tolidine reactions, in the presence and absence of sodium arsenite, a method has been developed for the simultaneous determination of OCl^- and NH_4^+. The techniques would be useful for assaying total residual chlorine in town and industrial water supplies, swimming pools, in water used as a secondary coolant in nuclear reactors[10] and waste water.

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Experimental

Distilled, de-ionised water was used in preparing the solutions. Its chlorine demand was found to be negligible. As different procedures have been proposed for the methyl orange method, optimum reagent strengths were ascertained by trial experiments, in which a constant amount of halogen (14.0 p.p.m. of chlorine) was injected into streams containing different combinations of dye, hydrochloric acid or pH 2 buffer (hydrochloric acid - potassium chloride solution, 7.4×10^{-3} M - 5.0×10^{-2} M, respectively). Values of ΔA (*i.e.*, $A - A_0$, where A_0 is the initial absorbance of methyl orange and A that of the bleached dye, at 510 nm) were constant for a 0.120 mM solution of methyl orange, with hydrochloric acid and pH 2 buffer.

Reagents

Methyl orange. Eastman ACS reagent was dried overnight at 105 °C, stored over phosphorus(V) oxide and used without further purification. A freshly prepared solution of methyl orange (0.180 mM) was employed for the flow injection determination of chlorine.

o-Tolidine. A 3 mM solution of *o*-tolidinium dichloride in 2 M hydrochloric acid was prepared[12,14–17] and stored in amber glass bottles.

Caution—*o*-Tolidine is highly toxic and is classified by the International Agency for Research on Cancer as a carcinogen. The chemical should be handled with extreme care. Avoid inhalation or contact with the skin.

Chlorine standards. Stock solutions of calcium hypochlorite and chlorine water (Fisher Reagents) were prepared and standardised iodimetrically.[9,17] Hypochlorite standards were fairly stable and the solutions of chlorine were stable for about 4 h.

Other reagents. Sodium arsenite (29.9 mM) and ammonium chloride (10.5 mM) (Fisher reagent grade) solutions were prepared in distilled water.

Instrumentation

A Bausch and Lomb (Spectronic 21) spectrophotometer, coupled with a Servo recorder (Kipp and Zonen, The Netherlands), was used for absorbance measurements at 510 nm with methyl orange (438 nm for the tolidine reagent). The carrier stream was pumped by a Buchler Polystaltic Pump. Construction of a flow cell and the flow injection apparatus used have been described previously.[18] The manifold was made from polyethylene tubing (2.0 and 1.0 mm i.d.). The mixing coil (0.8 mm i.d.) was 240 cm long for methyl orange (Fig. 1A) and 60 cm long for *o*-tolidine (Fig. 1B). Methyl orange reagent was originally pumped as a single stream using a solution in pH 2 buffer or 0.01 M hydrochloric acid (flow-rate 1.2 ml min^{-1}), but base-line drifts were observed after long periods of operation. This was eliminated by pumping methyl orange solution and hydrochloric acid - potassium chloride solution (1.48×10^{-2} M - 0.1 M) in two separate streams (2.8 and 1.6 ml min^{-1}, respectively), as shown in Fig. 1A. The *o*-tolidine reagent was pumped in a single stream (2.8 ml min^{-1}). For the hypochlorite - ammonium - *o*-tolidine investigations, in the presence and absence of sodium arsenite, a confluence joint was positioned between the injection and detection points, as shown in Fig. 1C.

Results and Discussion

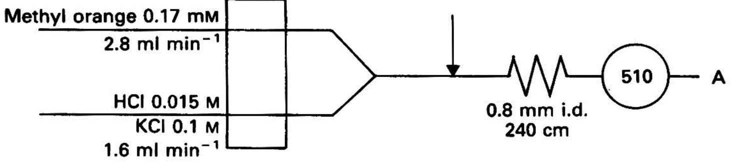
The flow-rate was kept constant and a continuous recording of transmittance (T) *versus* time was made during the flow injection determination of chlorine.

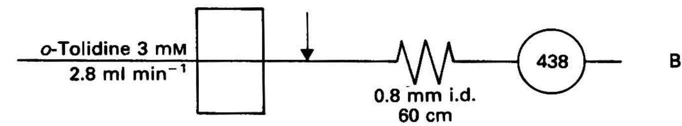
Methyl Orange

The calibration graph, obtained by plotting ΔA *versus* chlorine content of the solutions, was linear. The data were analysed by linear regression, assuming both variables were subject to error.[19] For hypochlorite solutions the regression equation was found to be

$$\Delta A = -0.00410\ (\pm 0.00020)C_{\text{Cl}} - 0.0166\ (\pm 0.00014)$$

where C_{Cl} is the chlorine content, and the correlation coefficient, R, is 0.9994. These results apply to a chlorine range of from 3.25 to 37.30 p.p.m.





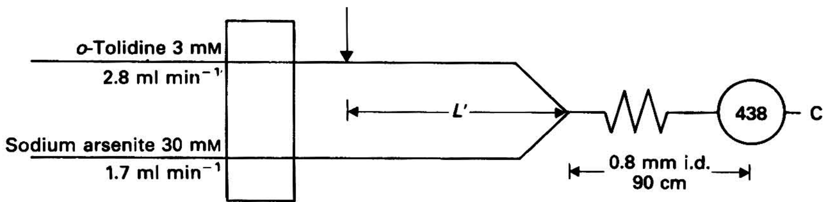


Fig. 1. Flow injection analysis system for: A, determination of chlorine using methyl orange; B, determination of chlorine using *o*-tolidine; and C, determination of chlorine, in the presence of NH_4^+, using *o*-tolidine.

Injection of a sample blank gives rise to a peak due to dilution of the reagent stream. Although it is possible to detect 1.4 p.p.m. of chlorine (corresponding to 63%*T*, relative to the blank peak at 62%*T*) the lower limit of the linear range for the method was found to be 3.25 p.p.m. of chlorine. Samples having a higher chlorine content than 40 p.p.m. caused complete decolorisation of the methyl orange and therefore 37 p.p.m. represents the highest practical chlorine content that can be determined with this particular concentration of methyl orange and the flow conditions described. Limited solubility of methyl orange at pH 2, less than 0.20 mM, prevents more concentrated solutions being used, which, in turn, places an absolute upper limit on the chlorine content of the sample.

For chlorine solutions the regression equation was

$$\Delta A = -0.00397\ (\pm 0.000314)C_{Cl} - 0.0113\ (\pm 0.0002)$$

with a correlation coefficient, *R*, of −0.9975. The regression equation applies to a chlorine range of from 2.9 to 38.40 p.p.m. The detection limit, as described above, was 1.4 p.p.m. of chlorine, while the lower limit of the linear range was 2.9 p.p.m.

A sampling rate of 160 h^{-1} has been achieved (Fig. 2). Base-line stability was excellent with no carry-over at any stage. However, drifts in base line were noticed at a sampling rate of 210 h^{-1}. The peak height decreased on decreasing the flow-rate to 1.9 ml min^{-1} and on increasing the tube length to 300 cm, owing to increased residence time and dispersion of the sample zone. Attempts to decrease both residence time and dispersion resulted in significant departures from linearity (R = −0.984 for chlorine water and −0.964 for hypochlorite). Peak broadening was apparent when the internal diameter of the tube was increased to 1.0 and 1.5 mm. Flow injection peaks became more symmetrical with tubes of 0.5 mm i.d., but this resulted in lowering the absorbance of methyl orange, thereby limiting the range of halogen concentration that could be studied.

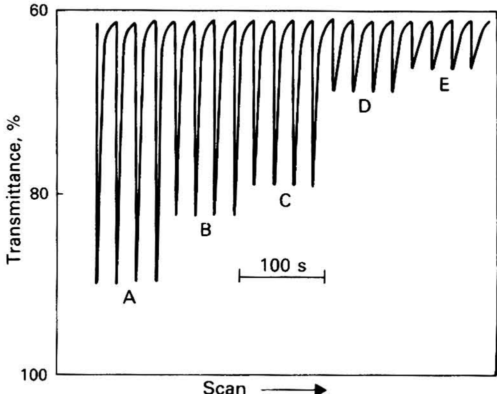


Fig. 2. Determination of chlorine using methyl orange. Chlorine, as hypochlorite, standards are A 35.9, B 27.3, C 23.3, D 8.9 and E 4.3 p.p.m. of chlorine. Sampling rate is 160 h^{-1}, sample volume 60 μl.

o-Tolidine

Reproducibility of the flow injection peaks was excellent at a rate of 216 samples per hour. Fig. 3 shows that peak heights were reproducible at a higher sampling rate of 288 h^{-1}, although the trace did not return to the base line under these conditions. Regression analysis of the data obtained at the higher sampling rate gave the following for hypochlorite solutions:

$$A = 0.03521\ (\pm 0.0008)C_{Cl} + 0.00436\ (\pm 0.0002)\ (R = 0.9992)$$

and for chlorine solutions:

$$A = 0.02004\ (\pm 0.0014)C_{Cl} + 0.00531\ (\pm 0.0006)\ (R = 0.9981)$$

where A is the absorbance, calculated from the peak height. The regression equation applies

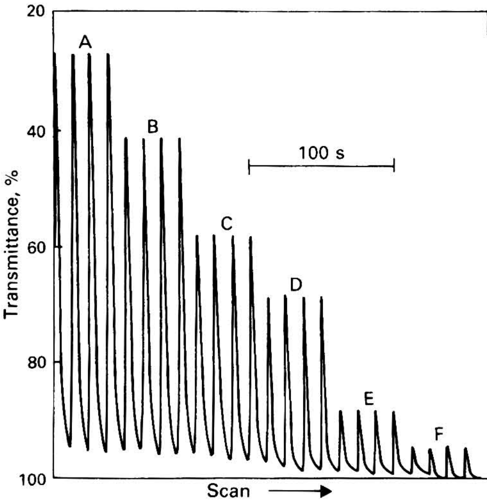


Fig. 3. Determination of chlorine using *o*-tolidine. Chlorine, as hypochlorite, standards are A 15.6, B 10.9, C 6.6, D 4.5, E 1.5 and F 0.6 p.p.m. of chlorine. Sampling rate is 288 h^{-1}, sample volume 60 μl.

to a chlorine range of from 0.18 to 18.1 p.p.m. The detection limit, taken as twice the signal to noise ratio, was 0.08 p.p.m. while the lower limit of the linear range was 0.18 p.p.m. For both determinations with *o*-tolidine decreasing the flow-rate to 0.4 ml min^{-1} and increasing the tube length to 210 cm resulted in a decrease of the peak height. It has been noted[9,12] that the *o*-tolidine - chlorine reaction product is unstable. However, under the conditions used here for the formation of the holoquinone, the observed decreases in peak height may be attributed to dispersion rather than holoquinone decomposition. This conclusion has been confirmed in our studies of the hypochlorite - ammonium - *o*-tolidine reaction system. Peak shape was not adversely affected by changing the internal diameter of the mixing tube but sample carry-over became troublesome with tubing of greater than 1.25 mm i.d.

Interferences

Common cations and anions do not interfere in either method below the levels quoted in Table I; concentrations of the additives are given in parts per million. The standard deviations of the chlorine recoveries have been calculated at the 95% confidence level for five replications. Bromine interferes and is recovered along with the chlorine; iodine masks the reagents leading to a chlorine recovery of about 55%. Amino acids form *N*-chloro compounds with chlorine.[20] Marks and Joiner[14] observed that hippuric acid and glycine do not interfere whereas chloro-derivatives of cystine are slow to react with *o*-tolidine. The effects of some amino acids on the chlorine determinations are shown in Table II. All recoveries, except for hippuric acid, are low.

Determination of Chlorine in the Presence of Ammonium Ions

The determination of chlorine in the presence of ammonium ions has received much attention and several methods have been proposed.[6] The *o*-tolidine - arsenite procedure (OTA) is based on reaction rate differences between *o*-tolidine and free chlorine (which are

TABLE I

RECOVERY OF CHLORINE THROUGH FLOW INJECTION ANALYSIS IN THE PRESENCE OF SOME CATIONS AND ANIONS

Interferent added	Methyl orange method: Concentration of interferent added, p.p.m.	Methyl orange method: Chlorine recovered, p.p.m.*	*o*-Tolidine method: Concentration of interferent added, p.p.m.	*o*-Tolidine method: Chlorine recovered, p.p.m.*
SO_4^{2-}	2560	5.36 ± 0.09	1280	5.09 ± 0.05
PO_4^{3-}	276	5.66 ± 0.08	552	5.33 ± 0.04
HPO_4^{2-}	1000	5.43 ± 0.05	300	4.86 ± 0.05
CH_3COO^-	156	5.08 ± 0.12	1440	4.99 ± 0.06
NO_3^-	1210	5.06 ± 0.08	276	5.20 ± 0.03
NO_2^-	100	4.84 ± 0.16	0.6	4.96 ± 0.10
$Cr_2O_7^{2-}$	90	4.80 ± 0.10	10	4.74 ± 0.08
Fe^{3+}	66	5.07 ± 0.12	6.6	4.92 ± 0.09
Cu^{2+}	41	4.90 ± 0.10	4.1	4.90 ± 0.09

* The original chlorine content for each determination was 5.1 p.p.m.

TABLE II

RECOVERY OF CHLORINE IN THE PRESENCE OF AMINO ACIDS

Interferent added	Concentration of interferent added, p.p.m.	Chlorine recovered, p.p.m.*: Methyl orange method	Chlorine recovered, p.p.m.*: *o*-Tolidine method
Hippuric acid	6.2	4.96 ± 0.09	5.20 ± 0.14
Glycine	3.8	3.65 ± 0.06	1.20 ± 0.07
Histidine	6.2	1.0 ± 0.09	1.0 ± 0.07
Glutamic acid	8.8	0.6 ± 0.08	0.6 ± 0.07
Leucine	6.3	0.6 ± 0.05	0.6 ± 0.03

* The original chlorine content for each determination was 5.1 p.p.m.

fast) compared with chloramines (which are slow). The OTA method involves the determination of total chlorine (free chlorine plus chlorine as chloramines) followed by a separate determination of free chlorine. This second step is achieved by the addition of sodium arsenite solution 5 s after *o*-tolidine has been added to the sample. Chloramines are reduced by sodium arsenite thereby preventing their reaction with the reagent. The standard procedure is prone to error. Difficulty in maintaining the same short time delay between the addition of reagent and sodium arsenite solution results in poor reproducibility. Moreover, there is some reaction between chloramines and *o*-tolidine prior to the addition of sodium arsenite.

Flow injection analysis provides a method by which sodium arsenite may be mixed with the sample and reagent in a highly reproducible manner. The optimum confluence point for the addition of sodium arsenite was established as follows: L' (see Fig. 1C) was increased from 7 to 107 cm in 10-cm increments; for each L' the $[OCl^-]$ to $[NH_4^+]$ ratio was varied and either water or sodium arsenite solution introduced at the confluence point. Fig. 4 presents a representative selection of the observed trends. For each L' the absorbance of the reaction mixture falls to a minimum, when $[OCl^-]:[NH_4^+]$ equals 2, both in the presence and absence of sodium arsenate. This corresponds to the familiar "break-point chlorination" stage.

Consider each side of the minima shown in Fig. 4. When the $[OCl^-]$ to $[NH_4^+]$ ratio is greater than 2, including $[NH_4^+] = 0$, the absorbance decreases linearly for all L' values as $[OCl^-]:[NH_4^+]$ decreases to 2. It is also observed that for each L' value when $[NH_4^+] = 0$, the absorbance decreases in a manner consistent with increasing dispersion of the sample bolus. A different pattern is observed when sodium arsenite solution is added to the stream. When L' is less than 27 cm insufficient reaction time is available for all the free hypochlorite

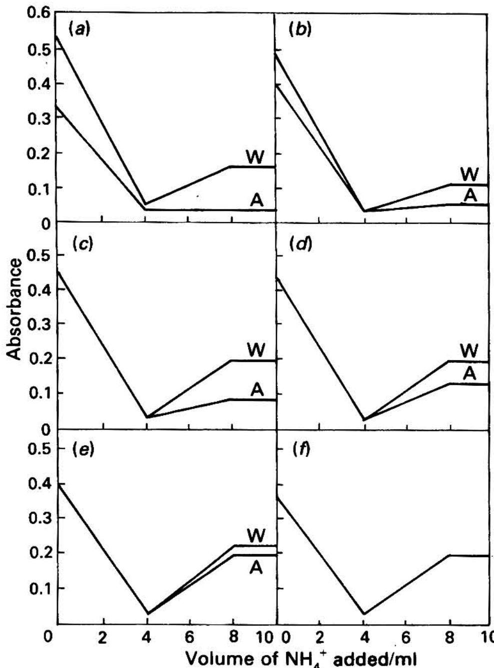


Fig. 4. Investigation of OCl^-, NH_4^+, *o*-tolidine reaction. All solutions contained 5.0 ml of 16.8 mM OCl^- and various volumes of 10.5 mM NH_4Cl solution. Each solution was analysed, using the flow injection analysis system shown in Fig. 1C, with water (W) or $NaAsO_2$ (A) added at the confluence point. L' is (*a*) 7, (*b*) 17, (*c*) 27, (*d*) 47, (*e*) 67 and (*f*) 87 cm.

to react with the *o*-tolidine. A lower absorbance is observed under these conditions. However, when L' becomes greater than 27 cm the addition of sodium arsenite has no measurable impact on the reaction, as evidenced by the coincidence of the two lines. The general trend of decreasing absorbance up to a value of the $[OCl^-]$ to $[NH_4^+]$ ratio of 2 is in accord with previous observations. These indicate the existence of a complex series of reactions between ammonium ions and excess of hypochlorite giving products, such as nitrogen and dinitrogen oxide, which do not react with *o*-tolidine. Hence, the apparent decrease in hypochlorite concentration. The specific reaction conditions used for these studies (flow-rate 1.75 ml min^{-1}, 0.8 mm i.d. tube) give reaction times for hypochlorite and *o*-tolidine, prior to the addition of sodium arsenite, of 1.21, 2.92 and 4.65 s for L' values of 7, 17 and 27 cm, respectively. This part of the study shows that a confluence point placed at least 27 cm downstream from the injection point allows sufficient time for the reaction between *o*-tolidine and free chlorine to proceed to completion.

To the right of the minima, where $[OCl^-]:[NH_4^+]$ is less than 2, mono- and di-chloramine predominate. It is seen from Fig. 4 that for short reaction times both chloramines have been reduced by sodium arsenite, irrespective of the $[OCl^-]$ to $[NH_4^+]$ ratio. In the absence of sodium arsenite, when the ratio of $[OCl^-]$ to $[NH_4^+]$ has fallen to 1 further increases in the ammonium ion concentration do not alter the observed absorbance. This may be attributed to the complete conversion of hypochlorite into NH_2Cl. When sodium arsenite solution is added at the confluence point as L' is increased from 27 to 67 cm only a fraction of the chloramines are free to react with *o*-tolidine before they are reduced. For L' values of greater than 87 cm the chloramine - *o*-tolidine reactions are complete and consequently sodium arsenite has no measurable impact on the observed absorbance. The graph obtained when L' is 107 cm is identical in form with that obtained when L' is 87 cm but all absorbances are slightly less, owing to increased dispersion of the sample bolus.

An additional set of experiments were performed at a constant L' value of 40 cm. Within each subset hypochlorite concentration was held constant and the $[OCl^-]$ to $[NH_4^+]$ ratio varied from 4:1 to 1:3, and included samples where $[NH_4^+] = 0$. The graphs obtained were identical in form with those of Fig. 4, where L' was 37 cm. For each hypochlorite concentration chosen, sharp break points were observed at $[OCl^-]$ to $[NH_4^+]$ ratios of exactly 1.0 and 2.0, in the presence and absence of sodium arsenite. Moreover, when the ratio of $[OCl^-]$ to $[NH_4^+]$ was greater than 2.0 the slope of the line was identical, irrespective of the hypochlorite concentration taken.

These two sets of experiments show that all the graphs display two distinct breakpoints, which are most evident when L' is about 40 cm. The inherently high level of reproducibility of reaction conditions afforded by flow injection analysis have enabled an analytical method to be devised for the analysis of chlorine samples that may, or may not, contain ammonium ions.

An aliquot of the sample is taken and diluted appropriately. A sample plug is injected into the reagent stream with a confluence point 40 cm downstream. Injections are made in the presence and absence of sodium arsenite. One of two results will be observed. Either the absorbance is not affected in the presence of sodium arsenite, or it is decreased. Fig. 5 illustrates the first possibility. Samples are prepared, containing ammonium ions, using a standard-additions procedure. Injections of these samples are made, by either water or sodium arsenite solution being added to the reaction coil at the confluence point. A graph is constructed and the extrapolations made (the broken lines in Fig. 5). As it has been established that the distances AB and AB′, measured on the abscissa, always correspond to a change in the $[NH_4^+]$ to $[OCl^-]$ ratio from 0.5 to 1.0, the number of moles of added ammonium ions from A to B(B′), labelled x moles NH_4^+ in Fig. 5, is exactly half of the original number of moles of hypochlorite present in the sample. The absolute position of the ordinate may now be established, thereby fixing the abscissa scale, and the original ammonium content of the sample may also be established. In other words, the number of moles of ammonium ion in the sample is given by subtracting the number of moles added to A from the number of moles between A and B(B′).

The second possibility is that the $[OCl^-]$ to $[NH_4^+]$ ratio in the sample is less than 2.0, giving rise to a decrease in the absorbance in the presence of sodium arsenite. In these situations sufficient standard chlorine solution is added to the sample to ensure that the $[OCl^-]$ to $[NH_4^+]$ ratio is greater than 2.0. The procedure described above is then followed

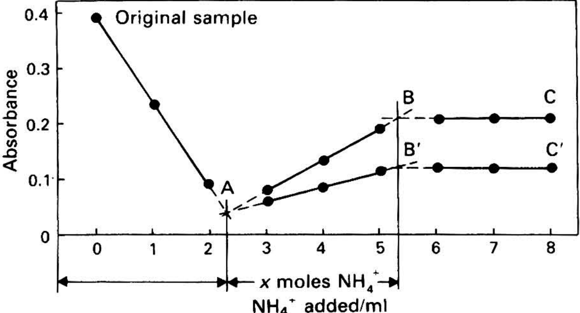


Fig. 5. A typical graph of data obtained from the determination of chlorine in the presence of NH_4^+. Line ABC is for water, line AB′C′ is for $NaAsO_2$ added at the confluence point. $L = 40$ cm (Fig. 1C). Analysis results of these data are shown in Table III, sample 1.

with due account being taken of the added hypochlorite when calculating the original chlorine content. Table III presents results of the determination of hypochlorite in the presence of ammonium ions for four samples. Results for sample 1, Table III, are taken from the data depicted in Fig. 5.

Similar exploratory experiments were performed with *o*-tolidine being replaced by methyl orange. However, the effect of sodium arsenite was found to be erratic and further studies were abandoned. However, in the absence of sodium arsenite the appearance of "break-point chlorination" was readily discernible.

TABLE III

TYPICAL DETERMINATIONS OF HYPOCHLORITE IN THE PRESENCE OF AMMONIUM IONS

Details	Sample 1	Sample 2	Sample 3	Sample 4
Volume of OCl^-/ml*	—	—	6.00	10.00
Volume of NH_4^+ to A/ml†	2.30 ± 0.13‡	4.25 ± 0.14‡	1.96 ± 0.08‡	3.17 ± 0.12‡
Volume of NH_4^+ to B(B′)/ml	5.35 ± 0.17‡	9.83 ± 0.21‡	5.27 ± 0.17‡	9.68 ± 0.19‡
Moles of NH_4^+ in sample—				
Found	0.796×10^{-5}	1.40×10^{-5}	1.41×10^{-5}	3.52×10^{-5}
Originally	0.83×10^{-5}	1.44×10^{-5}	1.44×10^{-5}	3.31×10^{-5}
Moles of OCl^- in sample—				
Found	6.44×10^{-5}	11.7×10^{-5}	2.45×10^{-5}	6.17×10^{-5}
Originally	6.20×10^{-5}	12.4×10^{-5}	2.30×10^{-5}	6.20×10^{-5}

* 0.00757 M standard OCl^- solution.
† 0.01056 M standard NH_4^+ solution.
‡ Mean and standard deviation of three replicate determinations.

Determination of Chlorine in Sea Water

Two synthetic samples, prepared to simulate sea water[21] [SSW(I) and SSW(II)], and natural sea water (NSW) taken from the Gulf of Mexico, all containing known amounts of added chlorine, were analysed (Table IV). It can be seen that halogen recoveries by the methyl orange method are low with NSW, reflecting its chlorine demand. However, all samples show decreased chlorine recoveries by *o*-tolidine, probably due to reactions of the constituent salts with this reagent in addition to the chlorine demand of NSW.

Conclusion

This work describes two methods for assaying residual chlorine in solution, using a simple flow injection apparatus. Advantages are the insensitivity to small variations in reagent concentrations or their mode of mixing, the absence of a time of equilibration and a high sampling rate. The *o*-tolidine method appears to be superior and demonstrates clearly the

TABLE IV

RECOVERY OF CHLORINE IN THE SYNTHETIC SEA WATER (SSW) AND NATURAL SEA WATER (NSW)

Sample	Volume/ml	Chlorine added, p.p.m.	Chlorine recovered, p.p.m. Methyl orange method	Chlorine recovered, p.p.m. *o*-Tolidine method
SSW I	75	14.6	14.2	10.5
	50	17.2	16.4	14.0
	50	14.6	14.5	10.8
SSW II	50	14.6	14.2	13.4
	50	17.2	17.1	16.6
NSW	10	14.6	13.2	11.9
	20	14.6	11.6	11.6
	10	10.9	9.3	8.2
	30	17.2	13.2	11.9

usefulness of the flow injection method for spectrophotometric determinations involving unstable intermediates. The method is suitable for routine analyses, as it is sensitive (0.18 p.p.m. of chlorine) and gives a high sampling rate of 288 samples per hour. Both methods are useful for assaying the more active fraction of residual chlorine. For the concentration ranges examined, optimum results are obtained for the following experimental conditions: methyl orange method, L = 240 cm, flow-rate = 2.8 and 1.6 ml min^{-1} for methyl orange and pH 2 buffer, respectively, concentration range determined 1.4–38.4 p.p.m. of chlorine; *o*-tolidine method, L = 60 cm, flow-rate = 2.8 ml min^{-1}, concentration range determined 0.18–18.1 p.p.m. of chlorine. A procedure has been developed for the determination of chlorine, in the presence of ammonium ions, using *o*-tolidine and a merged stream of sodium arsenite solution.

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SHORT PAPERS

Improving the Yield, Purity and Molecular Integrity of Skeletal Muscle RNA, Isolated by Phenol Extraction

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Keywords: *RNA extraction*; *RNA degradation*; *ribonuclease inhibitors*

In the course of our study[1,2] on the turnover of previously labelled ribosomal ribonucleic acid (RNA) in gastrocnemius muscle of prednisolone-treated rats, considerable difficulty was encountered in extracting undegraded ribosomal RNA from the tissue. The tough and fibrous nature of this muscle necessitated drastic homogenisation procedures during which degradative enzymes, principally ribonucleases, were released from sub-cellular organelles, and then hydrolysed the RNA. While such RNA degradation did not limit measurements of the total RNA present in the tissue, it imposed constraints on RNA turnover studies that required prior fractionation of the various RNA species.[2] The major problem was that, with extensive RNA degradation, procedures that depended on molecular size to separate the RNA species could not be performed without error. Furthermore, such RNA degradation severely reduced the yield of RNA.

The difficulty in obtaining undegraded RNA from skeletal muscle may explain the relative dearth of reports on ribosomal RNA turnover in this tissue at a time when data are continuing to accumulate on RNA metabolism in more easily processed organs, such as the liver. In this paper a method is described by which RNA can be extracted from gastrocnemius muscle in a form suitable for turnover measurements of ribosomal RNA. The yield, purity and molecular integrity of the RNA obtained by this method are compared with those of RNA extracted using three other procedures.

Experimental

Reagents and Materials

AnalaR-grade chemicals and reagents were used wherever possible.

Water-saturated phenol reagent. Prepared by mixing phenol crystals with twice their volume of distilled water, stirring the mixture for 45 min at 20 °C, and standing the cloudy solution in the dark for 18 h at 4 °C. The lower phenol layer was collected and protected from atmospheric oxidation by the addition of 0.1 % *m*/*V* quinolin-8-ol. The reagent was then stored in a dark bottle at 4 °C.

Gastrocnemius muscle. Excised rapidly from the animal, weighed, frozen in liquid nitrogen and pulverised into fine fragments in a liquid nitrogen cooled stainless-steel mortar. The total RNA content of the tissue was obtained by subjecting fragments from the left gastrocnemius muscle to alkali digestion[3] and collecting the RNA fraction.

Extraction of RNA

The technique used to extract RNA was a modification of the procedure described by Perry *et al.*[4] who extracted messenger RNA from the polyribosomes of L-cells. The fragments from the right gastrocnemius muscle were added to 10 volumes of medium A (0.1 M sodium chloride and 0.001 M EDTA in 0.01 M sodium acetate buffer solution, pH 6.0) containing 0.2 of a volume of a 0.5% *m*/*V* solution of poly(vinyl sulphate) in medium A. The slurry was homogenised using a Silverson Homogeniser (Silverson Machines Ltd., Buckinghamshire) operated at top speed. This procedure was performed in two bursts, each lasting 30 s, and a 2-min interval was interposed between the bursts to maintain the temperature of the homogenate, which was immersed in an ice-bath at a temperature of between 0 and 4 °C throughout. An aliquot of

0.1 of a volume of 5% *m*/*V* sodium dodecyl sulphate (SDS) in medium A was added to the homogenate and shaken for 10 min at 20 °C.

A mixture of the phenol reagent and re-distilled chloroform (1 volume), in a 1:1 volume ratio, was added to the slurry and, after shaking for 25 min and cooling to 2 °C, was centrifuged at 1500 *g* for 10 min at 4 °C. Thereafter, one re-extraction each of both the aqueous and organic phases was performed. The aqueous pool was mixed with 2 volumes of absolute ethanol and made 0.2 M with respect to sodium chloride. RNA was allowed to precipitate from this mixture at −15 °C, overnight. The material was collected by centrifuging and washed successively with 67% *V*/*V* ethanol and diethyl ether.

RNA was also isolated from muscle fragments by hot[5] and cold[6] phenol procedures as well as by a cetyltrimethylammonium bromide (CTA) method.[7]

Each RNA extract was dissolved in 5.0 ml of 0.01 M sodium acetate buffer solution, pH 5.1.

Assays of RNA

RNA was measured by the orcinol method,[8] using yeast RNA as the standard. The ratio of absorbances at 260 and 280 nm of a solution of the extract in the acetate buffer provided a measure of its purity. Sucrose gradient analysis of the sample was performed using a pre-cooled 3 × 23 swing-out aluminium rotor (MSE Ltd., England) under the experimental conditions described in Fig. 1.

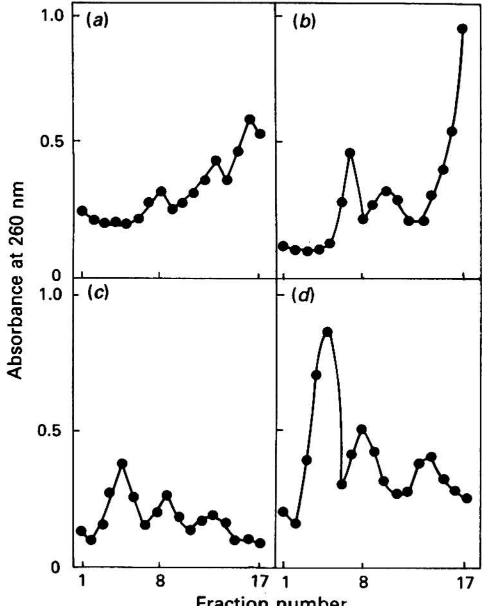

Fig. 1. Sucrose gradient profiles of RNA extracted with (*a*) hot phenol, (*b*) cold phenol, (*c*) CTA and (*d*) phenol - chloroform: 1.0 mg of RNA extract in 2.0 ml of 0.05 M sodium acetate buffer solution, pH 5.1, was layered over 15 ml of a 5–20% *m*/*V* sucrose gradient and centrifuged at 60000 *g* for 7 h at 5 °C.

Results

Data presented in Table I show that, of the total RNA present in the tissue, 79% could be recovered using phenol - chloroform mixtures according to the procedure described under Experimental. When we used the hot phenol extraction method,[5] which worked well for the softer and more easily homogenised rat diaphragm muscle, it gave a low (61%) recovery of

TABLE I

COMPARISON OF YIELD AND PURITY OF RNA EXTRACTED FROM RAT GASTROCNEMIUS MUSCLE BY VARIOUS TECHNIQUES

RNA extracts were obtained from right gastrocnemius muscle; RNA was assayed by the orcinol method[8]; results are mean values from 2–4 rats ± the standard error of the mean.

Extract isolation technique*	Muscle RNA/mg	Recovery, %†	A_{260}/A_{280}
Hot phenol method[5]	1.2 ± 0.13	61.3 ± 2.3	1.55
Total RNA content	1.9 ± 0.12	—	—
Cold phenol method[6]	1.1 ± 0.06	57.9 ± 3.8	1.80
Total RNA content	1.9 ± 0.11	—	—
CTA method[7]	0.80 ± 0.06	43.6 ± 6.1	1.95
Total RNA content	1.81 ± 0.07	—	—
Phenol - choroform mixtures	1.5 ± 0.03	78.9 ± 3.6	1.98
Total RNA content	1.9 ± 0.05	—	—

* Total RNA content refers to RNA from left gastrocnemius hydrolysed by alkali digestion.[3]
† Recovery, % = RNA content of extract/total RNA content × 100.

RNA from gastrocnemius muscle. A technique[6] in which the phenol extraction step was performed at 4 °C did not improve the yield of RNA although it produced a purer extract as indicated by the ratio of absorbances at 260 to 280 nm (Table I). An even purer extract could be obtained with the CTA method,[7] which, however, yielded only 44% of the total RNA present in the tissue. Minor modifications of this CTA method failed to improve the yield of RNA. Mixtures of phenol and chloroform, used in the manner described, consistently gave the highest and purest yield of RNA from gastrocnemius muscle.

When samples of the RNA extracts were subjected to sucrose gradient analysis, extracts obtained using the phenol - chloroform mixtures showed no evidence of significant RNA degradation in their sedimentation profile, as measured by the absorbance at 260 nm [Fig. 1(*d*)]. The proportions of the three RNA peaks were in the ratios 3.8:1.6:1, in reasonable agreement with the reported relative ratios of 28S, 18S and 4S RNAs, respectively, in mammalian tissues.[9,10] The result was reproducible with all the RNA extracts prepared by this method. When extracts isolated by the CTA method were analysed on the sucrose gradient, the same three peaks (corresponding to the two ribosomal RNAs and transfer RNA) were observed [Fig. 1(*c*)].

However, the results from this method could not be predicted with confidence and, inexplicably, extreme variability was observed between experiments. The sedimentation profile of the RNA extracts obtained using the hot or cold phenol procedures differed markedly, with some loss of material from the presumptive 28S ribosomal RNA peak, suggesting some degradation of this RNA species [Fig. 1(*a*) and (*b*)]. There was a steep rise in the ultraviolet absorption, at 260 nm, near the top of these gradients [Fig. 1(*a*) and (*b*)] due, presumably, to RNA degradation products and contamination of the extract with SDS.

The results of studies[2] on ribosomal RNA turnover, carried out using extracts obtained with phenol - chloroform mixtures, were consistently reproducible. The same conclusion could not be made using the extracts prepared by the other RNA isolation procedures investigated.

Discussion

Procedures that required extraction of ribosomes to study ribosomal RNA turnover were found unsuitable for gastrocnemius muscle because the muscle preparation, in combination with sucrose at the high salt concentration (0.3 M potassium chloride) necessary to solubilise muscle proteins, formed a viscous homogenate from which ribosomes could not be satisfactorily extracted.[1] However, ribosomal RNA turnover studies that involved alkali digestion of the entire tissue were subject to error as the RNA estimated in this manner is composed of various RNA species whose turnover rates may differ.[11]

Furthermore, tritium, a commonly used radioisotopic label for RNA in these experiments, can be incorporated into other cellular components (*e.g.*, glycogen), which remain in the alkali digest and complicate interpretation of results. Therefore, our investigations of gastrocnemius muscle ribosomal RNA turnover required prior isolation of RNA from the tissue, undegraded and free from cellular contaminants, before other procedures[2] to remove unwanted RNA species were performed.

Most of the current methods used for isolating RNA in mammalian tissues still rely on phenol extraction procedures. However, as the originator of this technique later noted,[12] ribonucleases can survive phenol extraction and, on removal of the reagent, can degrade RNA. It would appear that the effectiveness of a particular ribonuclease inhibitor depends on the prevailing experimental conditions and on the source of the ribonuclease in question. The phenomenon of RNA lability is also linked with the preferential solubility of certain RNA species in phenol and results given elsewhere[4] suggest that the inclusion of chloroform in the organic phase mediates effective deproteinisation of RNA - protein complexes thus eliminating the co-precipitation of these two entities. The choice of pH appears to be a critical experimental consideration and it may be significant that when gastrocnemius muscle was homogenised in a medium buffered at pH 6.0, no RNA degradation was observed [Fig. 1(*d*)]. This is a pH at which alkaline ribonucleases, which predominate in the muscle,[13] have minimum activity.

The inclusion of sodium chloride in the homogenisation medium was designed to solubilise muscle proteins, particularly myosin, with which ribosomes tend to co-precipitate in a medium of low ionic strength[14] with a resultant diminution in the RNA yield. However, the excessive use of this salt may reduce the efficiency of ribonuclease inhibitors[15] with consequent loss of RNA through hydrolytic processes. A compromise between two sodium chloride optimum concentrations, one at which muscle proteins are most soluble and another at which ribonucleases are least active, is therefore desirable. In this study, a homogenisation medium 0.1 M in sodium chloride proved ideal.

SDS is a detergent which, once introduced, is not easily removed from solutions. It is, however, also a potent ribonuclease inhibitor and does not appear to contaminate RNA extracts significantly if used at a concentration that does not exceed 0.5% *m*/*V* in the medium. The findings from this study suggest that the use of SDS at this concentration, along with phenol - chloroform mixtures in the presence of dodecyl sulphate under the conditions described, prevented ribosomal RNA degradation during its isolation from rat gastrocnemius muscle.

It is recognised that tissue to tissue variations may be encountered in the activity of intracellular ribonucleases and, therefore, in the lability of the RNA entity during nucleic acid extraction procedures. A tailoring of extraction techniques to suit the RNA source is, therefore, recommended and may involve the use of a different combination of protein precipitants, as has been demonstrated by Chirgwin *et al.*[16]

I thank Dr G. A. J. Goodlad for his advice and interest.

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Spectrophotometric Determination of Beryllium(II) Using a Trisazosalicylic Derivative of Triphenylamine

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Keywords: *Beryllium(II) determination; spectrophotometry; triphenylamine trisazosalicylic acid*

The most common reagents used for the determination of beryllium are triphenylmethane derivatives[1,2] and azo reagents (such as Beryllon II[3] and Beryllon III[4]).

In this study, we have used a trisazosalicylic derivative of triphenylamine as a reagent for the spectrophotometric determination of beryllium. This type of reagent has not been reported in the literature as being used for analytical purposes. We have also used this reagent for the determination of uranium(VI).[5]

Some trisazoic derivatives of triphenylamine are photoconductive materials.[6] The azo derivatives of salicylic acid give colour reactions with beryllium(II), which can be detected in the visible region.[7,8]

Baiulescu and Nistreanu[9,10] have used some bisazo derivatives of salicylic acid for the spectrophotometric determination of beryllium. They have shown that although the reagents are very sensitive, the selectivity of the reaction was improved by using masking agents.

Experimental

Reagents

Beryllium metal. Spectroscopic grade.

Beryllium chloride solution. Prepared by dissolving 0.01 g of beryllium in 1 ml of concentrated hydrochloric acid (analytical-reagent grade). The solution was diluted to 100 ml with water in a calibrated flask. A working solution of 10 $\mu g\ ml^{-1}$ was then prepared; 1 ml of concentrated hydrochloric acid was added during dilution, to prevent hydrolysis.

Buffer solution, pH 10. Boric acid - potassium chloride - sodium hydroxide standard buffer solution (Merck) was used in all determinations.

EDTA stock solution, 0.2 M. Analytical-reagent grade. Dilute solutions were prepared as required.

Metal ion solutions. Solutions of potential interfering metal ions were taken from standard 1000 p.p.m. solutions.

Triphenylamine trisazosalicylic acid

The reagent was synthesised as follows: trinitrotriphenylamine was synthesised, as described by Walter[11]; the trinitro-derivative was reduced using tin(II) chloride in a hydrochloric acid medium, as described by Neunhoeffer and Heitmann[12]; the derivative was then diazotised and coupled.

Diazotisation. A 0.5094-g sample of triaminotriphenylamine was added to a 100-ml beaker containing 1.32 ml of concentrated hydrochloric acid and 1.32 ml of distilled water. This mixture was shaken and a solution of 0.37 g of sodium nitrite in 1.5 ml of water was added to it drop by drop. During the addition, about 2 g of ice were introduced to the reaction mixture, in order to keep the temperature between 0–5 °C. A diazonium salt was obtained and the excess of nitrous acid acid was neutralised with a small amount of urea.

$$(H_2N-C_6H_4-)_3N.3HCl \xrightarrow{NaNO_2 + HCl} (N{\equiv}\overset{+}{N}-C_6H_4-)_3N.3Cl^- \quad (1)$$

Coupling. The solution of the diazonium salt thus obtained was added drop by drop to the following mixture: 0.732 g of salicylic acid plus 0.42 g of sodium hydroxide dissolved in 5.5 ml of water and about 5 g of ice, this keeping the temperature at between 0 and 6 °C during the

addition. The precipitate that formed was removed by filtration, leaving a colourless filtrate that was slightly alkaline, having a pH of 8.5. The precipitated reagent was recrystallised from ethanol. The compound is crystalline, with an intense red colour, insoluble in water, but soluble in ethanol. The purity of this compound was established by elemental analysis and nuclear magnetic resonance spectrometry.

Trisazosalicylic compound

Apparatus

The visible spectra were recorded using a Varian Superscan-3 ultraviolet - visible spectrophotometer.

Results and Discussion

Ligand Spectra

The solutions used for the measurement of the spectra were prepared by diluting 2 ml of the reagent (a 0.05% solution in ethanol) and 10 ml of pH 10 buffer solution with distilled water in a 25-ml calibrated flask. The spectra were recorded using a buffer - water mixture as the blank, the maximum absorbance of the ligand being at 450 nm.

Complex Spectra

Samples for absorbance measurements, were prepared as follows. A mixture of the beryllium solution to be measured, 2 ml of the reagent (a 0.05% solution in ethanol) and 10 ml of the pH 10 buffer solution was diluted to 25 ml with distilled water in a calibrated flask.

By derivative spectrometry it has been established that the absorption maxima of the compound formed between the reagent and beryllium(II) are at 521 and 392 nm. In our study we used the maximum at 521 nm. The colour develops instantaneously and is stable for at least 24 h. Fig. 1 shows the absorption spectra, (*A*) for the ligand and (*B*) for the complex in the range 400–600 nm.

To establish the dependence of the absorbance on the concentration of beryllium, samples are prepared, as above, with different concentrations of beryllium.

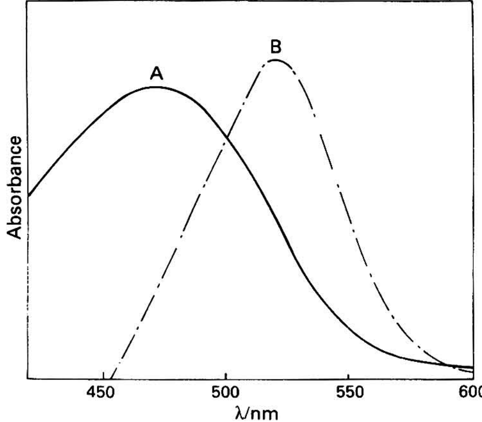

Fig. 1. Absorption spectra (A) for the ligand and (B) for the complex (400–600 nm).

Using the proposed reagent, it is possible to determine beryllium(II) at concentrations between 0.01 and 0.3 $\mu g\ ml^{-1}$. The molar absorptivity at 521 nm is found to be 12800 $l\ mol^{-1}\ cm^{-1}$, which indicates that the reagent is very sensitive for the determination of beryllium.

Determination of the Molar Ratio of Beryllium(II) to Reagent in the Compound

By using the molar-ratio method, the ratio of beryllium(II) to reagent in the compound was found to be 1:1.

Study of Interferences

In considering the selectivity of the reagent, we used EDTA as a masking agent for interferences.

In the first stage, we studied the determination of beryllium(II) using the proposed reagent in the presence of EDTA, under the same conditions as described previously but with the addition of 2 ml of 0.01 M EDTA solution. The absorbance curves were recorded. From these results, we concluded that the absorbance maximum is at the same wavelength (521 nm) as without EDTA, the absorbance values being slightly increased and the system still obeys Beer's law.

In the second stage, we mixed the beryllium(II) solution with different amounts of other metal ion solutions, and carried out the same absorbance measurements in the presence of EDTA. The results indicate the feasibility of determining beryllium(II) at 521 nm in the presence of copper(II), nickel(II), manganese(II), iron(III) and aluminium(III); the maximum permitted excesses of these interfering ions, expressed as ratios (beryllium to ion), were 1:100, 1:10, 1:100, 1:10, and 1:50, respectively.

Taking these results into consideration, the third stage was to determine beryllium(II) in two of its alloys, *i.e.*, copper and beryllium and aluminium and beryllium alloys.

For the copper - beryllium alloy, a sample of 0.517 g of the alloy is carefully weighed, dissolved in 2 ml of concentrated hydrochloric acid, then the solution is diluted with distilled water to 100 ml in a calibrated flask. A 0.5-ml aliquot of this solution is mixed with 2 ml of the reagent, 2 ml of the EDTA solution and 10 ml of the pH 10 buffer solution, and this mixture is then diluted to 25 ml with distilled water in a calibrated flask. The colour does not develop quickly, and the time required for its complete development depends mainly on the order of addition of the reagent and the EDTA; if the reagent is added before the EDTA, shaking for 1 h is necessary for the colour to develop, but if the EDTA is added before the reagent, shaking for 30 min is sufficient.

The optimum conditions for the assay were with equal volumes of EDTA (0.01 M) and reagent (0.05% in ethanol) solutions; 4 ml of this mixture were taken and added to the sample solution. Under these conditions, the colour was completely developed after 15 min of shaking. The results of the analysis were compared with the median values obtained using emission spectroscopy and atomic-absorption spectroscopy to determine beryllium(II) in the alloy.

These results are as follows: beryllium found using the proposed reagent, 1.82 and 1.79%; the median value for beryllium content using other techniques, 1.87%.

For the second alloy (aluminium - beryllium), 0.08 g of sample is weighed, dissolved in 1 ml of concentrated hydrochloric acid and diluted to 25 ml with water. The same reagent mixture is made as for the copper - beryllium alloy, but the order of addition of the reagents is as follows: sample solution, buffer solution [a white precipitate of aluminium(III) hydroxide appears], EDTA solution (which dissolves the precipitate) then the reagent. The absorbance is measured after 1 h of shaking.

The amount of beryllium found was 2.44–2.48%, and the median result for the analysis of this alloy, using atomic-absorption and -emission spectroscopy carried out in our laboratory, shows that the alloy contains 2.81% of beryllium.

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Titrimetric Determination of the Yield of Sulphide Formed by Alkaline Degradation of Cephalosporins

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Keywords: *Cephalosporins; alkaline degradation; sulphide; titrimetry*

Recently we reported a spectrophotometric method for the determination of cephalosporins based on alkaline degradation and conversion of the resulting sulphide into methylene blue.[1] As sulphide is not a primary degradation product of penicillins, the method is selective for cephalosporins.

Different cephalosporins were shown to give different but reproducible yields of sulphide. The yield varied from 14.0% for cefuroxime to 64.4% for cephalexin. These yields were calculated assuming that the yield of methylene blue in the reaction of sulphide with *NN*-dimethyl-*p*-phenylenediamine and iron(III) as oxidising agent, which had been determined as 52.2% using standard sulphide solutions, was unchanged in the presence of the cephalosporin degradation products and that the degradation products did not react with the cyclisation reagents.

In this paper a procedure is described in which the sulphide formed by degradation of various cephalosporins has been determined by potentiometric titration with standard lead nitrate solution using an Orion sulphide ion-selective indicator electrode. This has confirmed the yields reported previously, which were determined spectrophotometrically. The opportunity was also taken to check that the loss of sulphide by atmospheric oxidation during the degradation and the colorimetric or titrimetric procedures is negligible. The addition, before the degradation, of ascorbic acid as antioxidant causes the degraded solutions of most cephalosporins to become more strongly coloured, but the yield of sulphide is unchanged.

The range of cephalosporins studied has been extended to include cephaloridine, cephoxazole, cefaclor and cefazolin, and the yields of sulphide that they produce are reported here for the first time. The structures of these cephalosporins are given in Table I.

Experimental

The spectrophotometric procedure has been described previously.[1] The degradation was carried out in 0.5 M sodium hydroxide solution with the cephalosporins at about the 4×10^{-4} M level.

In this study, when using the titrimetric method, degradations of cephalosporins were carried out in 1 M sodium hydroxide solution at the 5×10^{-3} M level. The yields of sulphide as determined spectrophotometrically are the same when 0.5 or 1 M sodium hydroxide solution is used. The use of the lower sodium hydroxide concentration is more convenient in the spectrophotometric method as the degraded solution has to be acidified, whereas sulphide is usually measured potentiometrically in 1 M sodium hydroxide solution. Better potentiometric end-points were obtained at the higher concentration of the cephalosporins, but similar yields of sulphide were obtained at lower levels.

TABLE I

STRUCTURES OF CEPHALOSPORINS EXAMINED

R—NH— (cephem nucleus: S, N, O=, COOH, R')

	R	R'
Cephaloridine	(thiophen-2-yl)—CH_2CO—	—CH_2—N^+(pyridinium)
Cephoxazole ..	3-(2-chlorophenyl)-5-methylisoxazol-4-yl—CO— (Cl, N, O, CH_3)	—CH_2OCOCH_3
Cefaclor	C_6H_5—CH(NH_2)CO—	—Cl
Cefazolin..	(tetrazol-1-yl, N=N, N)—CH_2CO—	—CH_2—S—(5-methyl-1,3,4-thiadiazol-2-yl, N—N, S, CH_3)

Thus, for use with the titrimetric procedure approximately 5×10^{-3} M solutions of cephalosporins in 1 M sodium hydroxide solution containing 20 g l^{-1} of ascorbic acid were degraded at 100 °C for the length of time recommended previously. Aliquots (25 ml) of these degraded solutions were diluted in a water-jacketed titration vessel held at 25 °C, with 100 ml of 1 M sodium hydroxide solution containing 20 g l^{-1} of ascorbic acid and 10% *V*/*V* of glycerol. The solutions were titrated with 10^{-2} M lead nitrate solution. An Orion sulphide ion-selective electrode (94–16) was used as the indicator electrode. A saturated calomel reference electrode was connected to the solution in the titration vessel by means of a 0.1 M potassium nitrate salt bridge. Potentiometric measurements were made with a Radiometer PHM64 pH meter.

The rate of formation of sulphide from the four cephalosporins for which data had not been obtained previously was determined spectrophotometrically. Ascorbic acid could not be added to these solutions as the deeper colours produced interfered with the spectrophotometric determination. Absorbance measurements were made with a Unicam SP8-100 spectrophotometer.

Results

The rate of formation of sulphide from cephaloridine, cephoxazole, cefaclor and cefazolin as determined spectrophotometrically is illustrated in Fig. 1, and this may be compared with similar data given previously for other cephalosporins.[1] The apparent molar absorptivities of these degraded cephalosporins at 667 nm ($\lambda_{max.}$ for methylene blue) are 0.68, 0.55, 1.6 and 1.0×10^4 l mol^{-1} cm^{-1} at recommended hydrolysis times for complete formation of sulphide of 60, 60, 40 and 40 min, respectively. The coefficients of variation for six determinations at the levels indicated in Fig. 1 were 0.2, 1.5, 1.7 and 1.0%, respectively. The yields of sulphide determined spectrophotometrically for all of the cephalosporins are compared in Table II with the yields obtained by potentiometric titration. Clearly close agreement was obtained.

Lead nitrate is being used increasingly in place of silver nitrate for the titration of sulphide[2] mainly because there is less adsorption of sulphide ion on lead sulphide than on silver sulphide and this gives more accurate results. The addition of glycerol has been advocated recently as increasing the stability of the potential readings,[3] and this was confirmed in our study. The

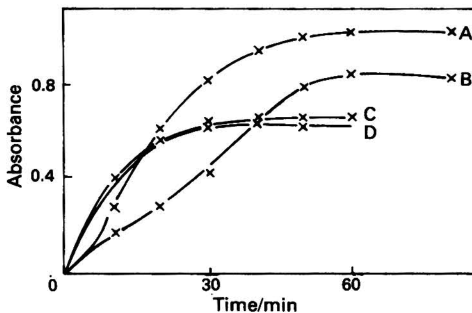

Fig. 1. Effect of time of heating in 0.5 M sodium hydroxide solution on the formation of sulphide as indicated by the methylene blue reaction. A, Cephaloridine (74 p.p.m.); B, cephoxazole (69 p.p.m.); C, cefazolin (29 p.p.m.); and D, cefaclor (18 p.p.m.).

degraded cephalosporin solutions gave similar titration curves to those of standard sulphide solutions of the same sulphide concentration. A slight instability of the potential readings was observed near the end-point but a sharp end-point jump, usually in excess of 200 mV, was observed.

During degradation the 5×10^{-3} M cephalosporin solutions frequently became coloured. Solutions of the α-aminobenzylcephalosporins, cephalexin, cefaclor and cephaloglycin, showed a strong yellow colour that was unchanged in the presence of ascorbic acid; the same occurred for solutions of 7-ACA and 7-ADCA, although these solutions were paler in colour. The solutions of the other cephalosporins were colourless in the absence of ascorbic acid but were coloured when it was present. Cefuroxime and cephalonium solutions containing ascorbic acid were yellow, whereas those of cephoxazole and cefazolin were brown. Those of cephaloridine took on a slight green colour initially but this became red - brown. Those of cephalothin went through green and red to give a strong red - brown colour when cool.

TABLE II

MOLAR YIELD OF HYDROGEN SULPHIDE FORMED IN 0.5 AND 1.0 M SODIUM HYDROXIDE SOLUTION AS DETERMINED SPECTROPHOTOMETRICALLY AND BY TITRATION WITH LEAD NITRATE SOLUTION

	Molar yield, %	
	Spectrophotometrically	Titrimetrically*
Sodium sulphide	100.0	100.0
Cephalexin	64.4	65.8
Cephradine	63.7	61.9
Cephaloglycin	18.9	19.5
Cephalothin	19.8	19.7
Cefuroxime	14.0	14.2
Cephalonium	16.0	17.4
7-ACA†	47.4	48.1
7-ADCA‡	57.9	57.3
Cephaloridine	18.9	18.1
Cephoxazole	15.7	14.8
Cefaclor	43.1	43.5
Cefazolin	26.9	25.1

* Mean of two or three titrations all within 1% error.
† 7-Aminocephalosporanic acid.
‡ 7-Aminodeacetoxycephalosporanic acid.

Discussion

The values determined spectrophotometrically and titrimetrically for the yields of sulphide obtained when cephalosporins are degraded in sodium hydroxide solution are similar.

The degradation products of the cephalosporins would not be expected to interfere in the titrimetric method; thiols if present would give a separate potential jump at a less negative potential. Thus, it is probable that the degradation products neither react colorimetrically with the cyclisation reagents nor interfere with the formation of methylene blue from sulphide.

Clearly the titrimetric method can be used to determine cephalosporins but the spectrophotometric method is probably simpler. The spectrophotometric method has been automated using an air-segmented flow system, and this procedure will be published after evaluation. The possibility of using a potentiometric flow-injection system incorporating a sulphide ion-selective electrode for the determination of cephalosporins is being studied.

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Investigations on the Determination of Germanium in Organogermanium Compounds Using Carbon Furnace Atomisation

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Keywords: *Germanium determination*; *organogermanium*; *carbon furnace atomisation*; *atomic-absorption spectrophotometry*

It is difficult to attain high sensitivity in the determination of germanium by flame atomic-absorption spectrophotometry.[1,2] The low sensitivity has been attributed to the formation of highly stable, volatile, germanium monoxide in the flame and to the inefficiency of germanium hollow-cathode lamps.[1]

Electrothermal atomic absorption was first examined for the determination of germanium by Johnson *et al.*[2] using both carbon-rod and carbon-tube atomisers. A graphite tube was preferred because the residence time of material in a high temperature environment was greater than when using a rod. Germanium dioxide is first reduced by carbon to the volatile monoxide, which can sublime without further reduction; a rapid increase in temperature has been recommended to prevent a loss of sample in molecular form. Ohta and Suzuki[3] reported improved sensitivity at lower temperature, using a tungsten micro-tube atomiser with argon - hydrogen as the purge gas. Pronounced interferences from diverse elements and acids were noted and prior solvent extraction of germanium was recommended. Solvent extraction was also used with carbon-tube atomisation for the determination of germanium in *Ginseng radix*.[4]

Pelosi and Attolini[1] determined trace amounts of germanium in cadmium sulphide indirectly as molybdenum using a germanomolybdate reagent in a time-consuming, alternate element amplification procedure.

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The aim of the present study was to seek a rapid or convenient determination of germanium in organogermanium compounds by use of the sensitivity achievable with carbon tube furnace atomisation, coupled with the ease of thermal and/or chemical pre-treatment of samples, which it was considered might produce a procedure that was, overall, simpler than existing spectrophotometric procedures.[5,6] As it was found not possible to mineralise samples reproducibly in the furnace, the final method involves atomisation in a pyrolytically coated graphite tube following the mineralisation of the organogermanium sample by means of oxygen flask combustion.

Experimental

Apparatus

The data were obtained by using a Perkin-Elmer 403 double-beam atomic-absorption spectrometer fitted with background correction facility and a Perkin-Elmer HGA76 graphite furnace; a Model 56 chart recorder was used to monitor signals. Sample solutions were introduced to the graphite tube with an Eppendorf microlitre pipette. The graphite tubes were coated pyrolytically using methane - argon (1 + 9) at a flow-rate of 0.5 l min^{-1} for 10 min at an ashing temperature of 2100 °C.[7] Tantalum carbide coated tubes were prepared according to the procedure of Hocquellet and Labeyrie.[8]

Final Procedure

Mix 2–7 mg of the organogermanium compound with approximately the same amount of sucrose (as a combustion aid) and combust the mixture in a 500-ml flask containing 10 ml of 0.3 M sodium hydroxide solution plus 10 drops of 30% hydrogen peroxide solution. After this combustion shake the flask for 15 min. Transfer the solution into a 100-ml calibrated flask with the aid of 0.3 M sodium hydroxide solution and dilute to volume with the same solution.[5] Mix the solution and dilute it 1 + 9 with 0.3 M sodium hydroxide solution.

Atomise 10-µl aliquots in a pyrolytically coated graphite tube under the following conditions (for an HGA76 furnace).

	Dry	Ash	Atomise	Clean
Temperature/°C ..	100	700	2700	2700
Time/s	25	5	5	5
Gas mode	Flow	Flow	Stop	Flow

Determine the amount of germanium from a calibration graph constructed using 10-µl aliquots of standard solutions containing 0–1.5 p.p.m. of germanium in 0.3 M sodium hydroxide solution. These standard solutions should be prepared using pure germanium dioxide.[5]

Results and Discussion

Preliminary Studies

Direct determination of germanium in organogermanium compounds

A series of 20-µl aliquots of solutions containing 10 p.p.m. of germanium (germanium dioxide and compounds 1, 2 and 3 in Table III) were atomised at a series of temperatures from 2000 to 2700 °C. Different absorbance maxima and temperature profiles were obtained for each compound, results were not reproducible and it was noted that the response for the organogermanium compounds and for germanium dioxide increased with usage of the carbon tube. The response from normal graphite tubes increased 100% over 100 firings.

Effect of condition of tube on germanium response

Further experiments with aliquots of germanium dioxide solution showed that both pyrolytically and tantalum carbide coated tubes showed a reduced change of sensitivity with use (10% per 100 firings). The pyrolytically coated tubes were less sensitive than tantalum carbide coated tubes but gave the most reproducible results and hence were used in the rest of the study.

Matrix modification

In an attempt to overcome the variation in response from compound to compound a series of matrix modification experiments were carried out. Prior wet oxidation (2 ml of concentrated sulphuric acid plus 2 ml of nitric acid, heated for 1 h) did not lead to consistent results owing to variable amounts of free acid and sodium salts in sample solutions. These were present because of the dissolution with sodium hydroxide solution of germanium dioxide deposited on the walls of the flasks during the acidic digestion of the organometallic compounds. The enhancement effect of various acids on the germanium response was noted earlier by Ediger[9] and that of sodium ion by Mino *et al.*[10]

In order to avoid the problems encountered in the external wet oxidation, mineralisation within the carbon tube furnace was examined. Volumes (10 μl) of 10 p.p.m. solutions of the organometallic compounds were injected, followed by 10-μl amounts of 20% perchloric acid; the furnace was then dried (100 °C for 30 s). The furnace was allowed to cool, a further 10 μl of 20% perchloric acid were added and the samples again dried (100 °C for 60 s), ashed (650 °C for 25 s) and atomised (2700 °C for 5 s). Eight out of the nine compounds listed in Table III gave the same response but very low results (20% of that expected) were obtained for compound 5, hence it was concluded that it was not possible to analyse organogermanium compounds by this method. Because neither direct atomisation of the compounds nor in-tube or external prior wet mineralisation were satisfactory oxygen flask combustion was examined. The concentration of sodium hydroxide for the absorbent solution was chosen as 0.3 M on the basis of a high enchancement with minimum background effect (Table I) that was observed when 10-μl aliquots of 1 p.p.m. germanium solution were atomised in the presence of varying concentrations of sodium hydroxide.

TABLE I

EFFECT OF SODIUM HYDROXIDE ON GERMANIUM AND BACKGROUND SIGNALS

	Molarity of sodium hydroxide					
	0.0	0.1	0.3	0.5	0.7	0.9
Peak height Ge + NaOH	6	39	69	71	65	62
Peak height NaOH	0	3	5	14	25	39

A further advantage of the addition of sodium hydroxide was the dramatic increase in the precision of the data, although a similar effect was noted with 10^{-3} M calcium nitrate solution; the data are shown in Table II.

TABLE II

EFFECT OF MATRIX MODIFICATION ON THE PRECISION OF GERMANIUM SIGNALS

Sample	Coefficient of variation, %
10 μl of 10 p.p.m. Ge	23.8
20 μl of 1 p.p.m. Ge in 10^{-3} M $Ca(NO_3)_2$	4.6
10 μl of 1 p.p.m. Ge in 0.3 M NaOH	3.7

The other alkali and alkaline earth metals behaved similarly. The enhancing effect of sodium hydroxide has been shown to be due to the formation of sodium germanate during the ashing stage.[10]

Determination of Germanium in Organogermanium Compounds

Results obtained by use of the finalised procedure are given in Table III. The results are less precise in terms of standard deviation than those obtained earlier by spectrophotometry; it is possible to obtain comparable confidence of mean values by generating more data by means of repetitive injection, which is readily achieved by using an autosampling accessory. The advantage of the method as described is in its over-all simplicity and the over-all speed of an atomic-absorption method when compared with that of ultraviolet - visible spectrophotometry.

TABLE III

ANALYSIS OF ORGANOGERMANIUM COMPOUNDS

Reference number	Compound*	Germanium expected, %	Germanium found, %†
1	Ph_4Ge	19.07	19.2
2	$Ph_4Ge_2I_2$	20.53	20.8
3	$(Ph_3Ge)_2O$	22.90	23.2
4	$(Me\text{-}C_6H_4)_6Ge_2$	21.00	21.0
5	$(PhCH_2)_2GeMe_2$	29.20	27.6
6	$Ph_3Ge_2Me_3$	34.47	34.0
7	$(Me\text{-}C_6H_4)_3GeBr$	17.06	17.2
8	$Ph_4Ge_2F_2$	29.50	28.9
9	$(PhGeO)_2O$	41.82	41.8

* Compounds prepared at Queen's University of Belfast. C, H analysis showed that the compounds were all above 99% pure.

† Each value is the average of three parallel determinations.

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Differential-pulse Polarographic Determination of Red 10B Formed From the Permitted Food Colour Red 2G

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Keywords: *Red* 10*B*; *Red* 2*G*; *food colours*; *differential-pulse polarography*

Apparently, it is well known that under certain conditions the permitted food colour Red 2G degrades by deacetylation to the non-permitted food colour Red 10B, although little mention

appears to have been made of this reaction in the literature.[1] During a food colour degradation study in this laboratory a 0.5% solution of Red 2G was heated in a tightly-sealed autoclavable bottle at 85 °C. When it was finally removed from the oven after 5 months the hue of the solution was noticed to have changed markedly to a more pink shade and the differential-pulse polarographic peak in pH 9 buffer was found to have shifted completely from −0.77 V (the peak potential for Red 2G) to −0.85 V with a small increase in peak height. Red 10B was shown to give a polarographic peak at this latter potential. Subsequent studies of the effect of pH on the position of the polarographic peaks of the two colours showed that maximum separation of the peaks occurred at pH 9 but that the peaks were insufficiently separated for quantitative determination of the two colours.

Previously tetraphenylphosphonium chloride (TPPC) had been shown to shift the polarographic peak potentials of some food colours, and to be useful in effecting separation of some overlapping differential-pulse peaks.[2–4] This proved to be the case also with Red 2G and Red 10B. Optimum separation of the main peaks of the two colours in the presence of TPPC occurred at pH 2, but at this pH a minor peak formed by Red 10B interferes with the main Red 2G peak. For this reason pH 7 was chosen as the best compromise, giving maximum separation wthout interference from this minor peak. Without addition of TPPC the peak potentials at pH 7 of Red 2G and Red 10B were −0.66 and −0.77 V, respectively, whereas for a solution containing 600 p.p.m. of TPPC the peak potentials were −0.59 and −0.80 V, respectively. A procedure for the differential-pulse polarographic determination of Red 2G and Red 10B, based on this electrolyte, has been developed.

Experimental

Differential-pulse polarographic measurements were made with a PAR-174A polarographic analyser (Princeton Applied Research), using a dropping-mercury electrode, a saturated calomel reference electrode and a platinum counter electrode. A pulse amplitude of 50 mV and a drop time of 2 s were used.

Reagents

Standard Red 2G solution, 50 p.p.m. Prepare a 500 p.p.m. solution by dissolving 0.125 g of Red 2G in 250 ml of water in a calibrated flask. Dilute 10 ml of this solution to 100 ml in a calibrated flask.

Standard Red 10B solution, 50 p.p.m. Prepare this solution in the same way as the standard Red 2G solution but using Red 10B.

Tetraphenylphosphonium chloride (TPPC) solution, 6000 p.p.m. Dissolve 1.5 g of TPPC in 250 ml of water.

Britton - Robinson's buffer, pH 7.0 (0.04 M *in each of the three buffer components).* Dissolve 2.47 g of boric acid in 500 ml of distilled water containing 2.3 ml of glacial acetic acid, then add 2.7 ml of orthophosphoric acid and dilute to 1 l with water. Adjust the pH of the solution to 7.0 using 4 M sodium hydroxide solution.

Preparation of Calibration Graphs and Determination of Red 2G and Red 10B

Pipette aliquots (0–5 ml) of standard Red 2G or Red 10B solution or an aliquot of sample solution containing less than 250 μg of Red 2G and Red 10B into a 50-ml calibrated flask. Add 20 ml of buffer solution and 5 ml of TPPC solution and dilute to 50 ml with water. Mix the solutions thoroughly and obtain a differential-pulse polarogram between −0.20 and −1.10 V after deoxygenating the solution.

Results

Differential-pulse polarograms of a Red 2G standard at pH 9 and the diluted 0.5% solution of Red 2G that had been heated at 85 °C for 5 months are shown in Fig. 1. The shift in peak potential owing to the near-quantitative conversion into Red 10B is clearly seen. Differential-pulse polarograms of a mixture of Red 2G and Red 10B at pH 7 with and without the addition of TPPC are given in Fig. 2, where the separation owing to the addition of TPPC is apparent.

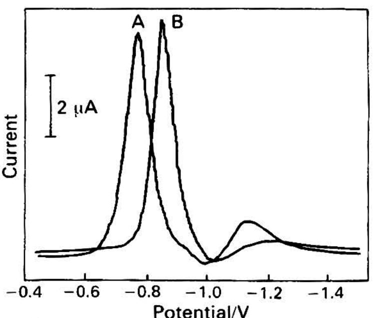

Fig. 1. Formation of Red 10B by heating a 0.5% solution of Red 2G for several months at 85 °C. Polarograms run at the equivalent of 4 p.p.m. initial Red 2G concentration in pH 9 Britton - Robinson buffer. A, Red 2G standard; and B, sample solution after 5 months.

Calibration graphs for the determination of Red 2G and Red 10B at pH 7 from 0.5 to 5 p.p.m. are rectilinear and independent of the presence of the other food colour if this is present at levels less than 5 p.p.m. At higher levels of the food colours (*e.g.*, 25 p.p.m.), a lowering of the base line between the two food colours occurs owing to an adsorption effect and the height of the peak for each food colour is no longer independent of the concentration of the other food colour (see Table I). Calibration graphs for each food colour at a constant level of the other food colour remain rectilinear. Coefficients of variation for the determination of Red 2G and Red 10B using the recommended procedure are typically less than 3%.

As an illustration of the application of this procedure to the determination of the amount of Red 10B formed from Red 2G an accelerated heat degradation study was set up. Solutions of Red 2G (100, 250, 500 and 1000 p.p.m) in water were heated in sealed glass phials at 130 °C. Typical polarograms obtained after 24 h are shown in Fig. 3. Clearly after one day, at all concentrations, less than 20% of the Red 2G remains, a slightly greater proportion of the Red 2G having degraded the higher the initial Red 2G concentration. Further, similar amounts of Red 10B and Red 2G are present after one day, a greater proportion of Red 10B being present the higher the initial Red 2G concentration. Consideration of the

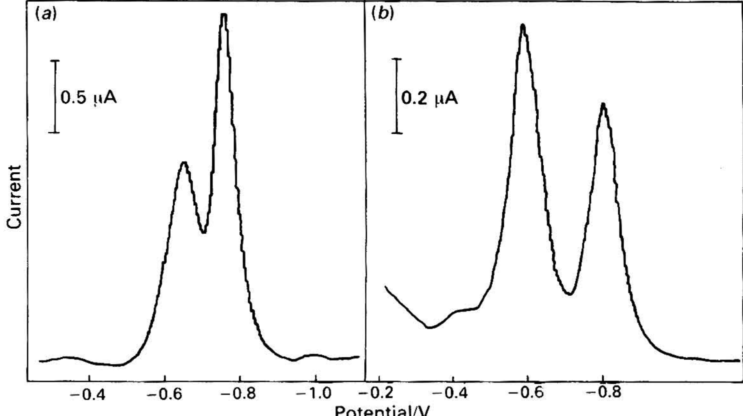

Fig. 2. Effect of tetraphenylphosphonium chloride (TPPC) on the differential pulse polarograms of a mixture of Red 2G and Red 10B in pH 7 Britton - Robinson buffer. TPPC concentration: (*a*) 0 and (*b*) 500 p.p.m.

TABLE I

INTERDEPENDENCE OF DIFFERENTIAL-PULSE PEAK CURRENTS OF RED 2G AND RED 10B AT HIGHER CONCENTRATIONS

(*i*) *Variation of apparent peak current of Red 2G (25 p.p.m.) with Red 10B concentration—*

Red 10B concentration, p.p.m.	0	5	10	15	20	25
Apparent peak current of Red 2G/μA	9.5	10.3	9.5	9.5	8.4	6.4

(*ii*) *Variation of apparent peak current of Red 10B (25 p.p.m.) with Red 2G concentration—*

Red 2G concentration, p.p.m.	0	5	10	15	20	25
Apparent peak current of Red 10B/μA	9.1	10.4	11.5	11.9	12.8	12.4

polarograms over the following three days indicated that the Red 10B degrades much more slowly than Red 2G. This was confirmed by a study of the degradation of a Red 10B sample under the same solution conditions: 89% of the Red 10B in a 500 p.p.m. solution remained unchanged after 24 h at 130 °C. The pH of a 1000 p.p.m. Red 2G solution was shown to have decreased from 7.4 to 3.4 on degradation, but this did not appear to be the cause of the increased proportion of Red 10B formed at higher Red 2G initial concentrations as the same amount of Red 10B was formed when the Red 2G was degraded in a solution buffered at pH 7.2.

Discussion

The polarographically reducible product observed in these studies has identical polarographic properties with Red 10B. Half-peak widths and peak potentials are identical over a range of pH values in the presence or absence of tetraphenylphosphonium chloride. Further confirmation that the product is Red 10B is indicated by paired-ion HPLC results, which will be reported in a later paper.

The recommended procedure is given as a convenient method of determining the extent of deacetylation of Red 2G during food processing. The procedure represents another example of the use of tetraphenylphosphonium chloride to cause separation of mutually interfering food colour peaks. A minor disadvantage of the procedure is that it cannot be used at concentrations of food colours much above 5 p.p.m., owing to adsorption characteristics causing interference between the two differential-pulse peaks.

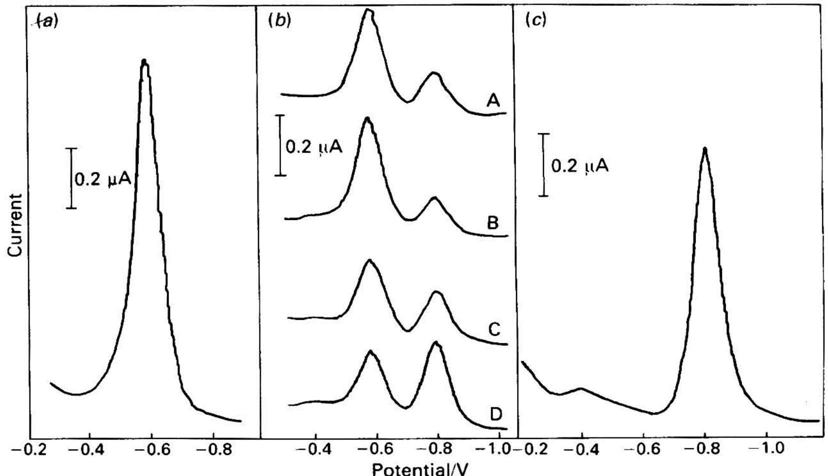

Fig. 3. Polarograms showing degradation of solutions of Red 2G and Red 10B at 130 °C in sealed vials after 24 h. All polarograms run at an actual or equivalent initial concentration of 4 p.p.m. (*a*) Red 2G standard; (*b*) degraded Red 2G solutions, initial Red 2G concentration (A) 100, (B) 250, (C) 500 and (D) 1000 p.p.m.; and (c) Red 10B standard.

The brief degradation study made on Red 2G indicates a major conversion into Red 10B by deacetylation on heating for prolonged periods at 85 °C, whereas heating at 130 °C gives extensive degradation of the azo group of Red 2G. The azo group in Red 10B is less susceptible to degradation than that in Red 2G.

We thank Dr. N. T. Crosby of the Laboratory of the Government Chemist for advice and one of us (M.R.W.) thanks the Science Research Council and the Laboratory of the Government Chemist for financial assistance.

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Determination of Morphine and Morphine-6-nicotinate by an *In Situ* Reaction on Chromatographic Plates with Dansyl Chloride

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Keywords: Morphine and morphine-6-nicotinate determination; morphine-3,6-dinicotinate; dansylation; derivatisation by overspotting

Nicomorphine (morphine-3,6-dinicotinate) has proved to be a very powerful analgesic, which shows a much more advantageous spectrum of activity than morphine.[1] When stored correctly, morphine-3,6-dinicotinate, the active substance in various Vilan preparations, remains virtually unhydrolysed. Only as a consequence of improper or too long a storage, or under the influence of heat, do morphine-6-nicotinate and morphine form as decomposition products. In order to achieve a sensitive method of detection of these products, dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride) has been used for derivatisation. This reagent has been used for several years for the fluorimetric determination of primary and secondary amines or compounds with a phenolic group.[2] The reaction with dansyl chloride is usually carried out in solution. Frei *et al.*[3] have determined carbamates and urea herbicides by means of thin-layer chromatography by overspotting the sample spots with dansyl chloride. The reaction time was 60 min at room temperature. This paper describes a modified application of this technique, using morphine and morphine-6-nicotinate as examples. It is thus possible to carry out quickly a quantitative determination of phenolic compounds in the presence of other easily decomposable phenolic esters.

Experimental

Apparatus

The fluorimetric measurements were made with a Perkin-Elmer spectrofluorimeter, MPF 44, equipped with a thin-layer chromatographic attachment.

Chromatographic plates. Silica gel G plates, 20 × 20 cm, 0.25-mm layers, without fluorescence indicator, from Macherey-Nagel (Düren, West Germany) were used. The plates were washed once with the eluent and activated before use.

Reagents

All of the reagents used were of analytical-reagent grade.

Reagent for derivatisation. Dansyl chloride (2 mg) obtained from E. Merck, Darmstadt, West Germany was dissolved in 1 ml of acetone.

Dipping solution. Triethylamine - 3% *V/V* solution of hydrogen peroxide - ethanol (30 + 30 + 20).

Solvent system. Chloroform - methanol - water (30 + 55 + 10). The hR_F ($R_F \times 100$) value for morphine was 36 and for morphine-6-nicotinate it was 47.

Stock solutions. Morphine (10 mg) and morphine-6-nicotinate (10 mg) were dissolved in 50 ml of methanol - water (1 + 1). From this solution, amounts of between 10 and 1 ml, corresponding to 200–20 ng per 2 µl, were pipetted into 20-ml flasks and diluted to 20 ml with methanol - water. The 20-ml flasks each contain 5 mg of morphine-3,6-dinicotinate to balance the effect that this substance, which is present in the samples, has on the fluorescence intensity.

Procedure

Aliquots of 2 µl of the standard and the sample solutions are applied to the thin-layer chromatographic plate with 2-µl microcaps. These substance spots are also overspotted with 2 µl of the dansyl chloride solution. Immediately afterwards, the chromatogram is placed in a drying cupboard at 100 °C. After 8 min the plate is developed in the solvent system in the dark, and is air dried in the dark for about 5 min. The thin-layer chromatographic plate is then dipped quickly into the dipping solution and is dried for 30 min in a vacuum oven at room temperature. The dansylates are marked under the ultraviolet lamp at 366 nm and measured at an excitation wavelength of 330 nm and at an emission wavelength of 510 nm.

Results and Discussion

When dansyl chloride reacts with the appropriate substances in solution, the resulting hydrochloric acid can be eliminated by using buffers. Comparative analyses with and without buffers showed that better results could be obtained without any additional application of the buffer to the thin-layer chromatographic plate. The application of 2 µl of the reagent in acetone is sufficient for derivatisation, although 2 µl of the sample solution in methanol - water were applied first. In spite of the different polarities of the solvents no difference in the resulting intensities of fluorescence could be found even if 3 or 5 µl of the reagent solution were used. The drying of the thin-layer chromatographic plate resulted in a strong reduction in the fluorescence of the dansyl derivatives. Through dipping the chromatogram into a mixture of triethylamine, hydrogen peroxide and ethanol, an optimum stabilisation could be achieved as well as a considerable enhancement of fluorescence intensity of the substance spots.

The optimum reaction time for the derivatisation of morphine and morphine-6-nicotinate is 8 min at 100 °C (see Table I); 55–60% of the maximum fluorescence intensity is achieved after only 1 min. Nachtmann *et al.*[4] were also able to observe different kinds of fluorescence behaviour of various dansyl derivatives.

TABLE I

KINETICS OF THE DANSYLATION OF MORPHINE AND MORPHINE-6-NICOTINATE

	Relative peak area after different reaction times/min								
Compound	1	3	5	8	10	15	20	30	40
Morphine	415	675	750	830	830	831	820	800	760
Morphine-6-nicotinate	215	275	320	348	350	345	325	300	250

It is possible that a steric effect is also the cause of the behaviour in this instance, because morphine-6-nicotinate is substituted with nicotinic acid in the 6-position. In order to eliminate any influence of morphine-3,6-dinicotinate on the dansylation, as a result of

differently distributed proportions in the substance spot, an adequate amount of morphine-3,6-dinicotinate was added to the standard solutions.

A better sensitivity, 800 pg for morphine and 5 ng for morphine-6-nicotinate, was obtained with the Macherey-Nagel material than with the Merck thin-layer chromatographic plates. The determination is linear for between 2 and 200 ng of morphine and for between 5 and 200 ng of morphine-6-nicotinate. The reproducibility of the method was tested with morphine as well as with morphine-6-nicotinate by a series of ten assays, each treated under identical conditions. The relative standard deviations obtained for each 50 ng per spot was 1.25% for morphine and 1.84% for morphine-6-nicotinate.

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Book Reviews

ANALYSIS OF NON-METALS IN METALS. PROCEEDINGS OF THE INTERNATIONAL CONFERENCE, BERLIN (WEST), JUNE 10–13, 1980. Edited by GUNTHER KRAFT. Pp. xiv + 546. Walter de Gruyter. 1981. Price DM148. ISBN 3 11 008443 0.

The properties of metals can be adversely affected by the presence of non-metals although in certain instances the effects may be beneficial. This applies not only to mechanical and metallurgical properties but also to nuclear ones, such as neutron capture cross-section. The effects can be produced by concentrations as low as 0.1 $\mu g\ g^{-1}$, whereas the content may rise to as much as 1 mg g^{-1} in certain metals. The determination of these levels of oxygen, nitrogen, hydrogen, carbon, sulphur, boron and fluorine imposes considerable strain on conventional chemical methods. In the last decade there have been notable improvements in accuracy and precision resulting from the introduction of more modern techniques such as hot extraction and activation analysis.

A four-day symposium to discuss the progress to date was called in June 1980 by the Fachgruppe Analytische Chemie der Gesellschaft Deutscher Chemiker. Other groups taking part were the Non-Metals in Non-Ferrous Metals team of the Bureau Communautaire des References of the Commission of the European Communities and The Chemists' Committee of the Gesellschaft Deutscher Metallhütten und Bergleute of the Verein Deutscher Eisenhüttenleute. This volume is a collection of some 36 papers given at the symposium, many with exhaustive references.

The first eight papers on non-metals in iron and steel are in German. In the next section of 15 papers on the determination of non-metals in metal by activation analysis two are in French, three in German and ten in English. In the final section on non-metals in non-ferrous metals two papers are in French, three in English and eight in German. Every paper carries an abstract in English, which in some instances is merely a brief résumé of the contents but in others it is a fairly full summary of the relevant details.

All the papers contain much information in graphic or diagrammatic form and in the non-English versions enable one to decide how much translation would be beneficial. A useful list of referents' names and addresses is given at the end of the book together with a subject index.

An important feature is the rapid publication achieved in a relatively short period of time from the ending of the conference. By this means an authoritative and up-to-date account is available from a group of specialist contributors on a subject that is exercising analysts more and more each year.

W. R. NALL

CHEMICAL TECHNICIANS' READY REFERENCE HANDBOOK. Second Edition. By GERSHON J. SHUGAR, RONALD A. SHUGAR, LAWRENCE BAUMAN and ROSE SHUGAR BAUMAN. Pp. xxiv + 867. McGraw-Hill. 1981. Price £27.95. ISBN 0 07 057176 7.

The authors' expressed aim was to produce an "omnibook" to provide anyone from undergraduate to graduate student to trained chemist with a guide to "every single step" when performing normal chemical laboratory procedures. This was a very broad remit to undertake and indeed impossible to achieve fully in that one laboratory's normal practice may by another be regarded as extraordinary or sophisticated. The breadth of material and wealth of detail make it difficult to summarise the contents briefly.

The book starts with and emphasises throughout responsibility and safety practices. All the basic operations such as heating, cooling, measuring temperature, evaporation of liquids, mechanical agitation, filtration, recrystallisation, sublimation, extraction, centrifugation, distillation, weighing, glassblowing and pH measurement are included, as are techniques of measurement of physical properties of materials in general, volumetric analysis and chromatography. Use of gases, ground-joint glassware and plastic laboratory ware is also detailed. Particularly useful are chapters on pressure and vacuum, electricity and laboratory tools and hardware. Chapters that deal with more theoretical material are in parts too compressed and thus considered to be less useful; these include basic concepts in organic chemistry, determination of elements in organic chemistry, the electromotive series (*i.e.*, electroanalysis) and that on the electromagnetic spectrum, which amazingly explains how to use a Duboscq colorimeter.

If what you want is included you will, or should, be pleased and save time in instructing or re-instructing yourself or whoever is to do the task. The instructions are written in clear, simple,

numbered statements, the equipment required is detailed and what it looks like is indicated (drawing or photograph). How the pieces are used in a procedure, the sequential steps in performing the task or acquiring the data, the precautions to be observed and finally how to do the calculations needed to be able to utilise the data are clearly explained. Should you need to remove a stuck glass stopper, safely empty a 50-gallon drum of diethyl ether, explain how a pressure regulator valve works or detect the colloidal state, there are no problems as all is explained.

Potential readers should not be put off by the title; for the British market, where the US use of the term technician may be misunderstood, a better title would have been simply "Chemical Laboratory Handbook." This excellent book, together with The Royal Society of Chemistry book "Hazards in the Chemical Laboratory," should be regarded as mandatory reading for all new graduates before they reach industry, where experiments are not always rigged to work even in expert hands, the boss is often busy and not inclined to be amused by failure, nor these days can afford to ignore hazardous practices. More experienced chemists will find the volume an excellent *aide memoire*.

D. THORBURN BURNS

APPLICATIONS OF GLASS CAPILLARY GAS CHROMATOGRAPHY. Edited by WALTER G. JENNINGS. *Chromatographic Science Series, Volume* 15. Pp. viii + 629. Marcel Dekker. 1981. Price SwFr186. ISBN 0 8247 1223 4.

This is a book of 15 chapters by 20 contributors. It has an introduction on the evolution of open tubular columns followed by system requirements. The remaining chapters deal with applications in xenobiotic research, air and air pollutants, water and water pollutants, pesticides, clinical medicine, beer, hops, grapes, wines, brandies, food and flavours, essential oils, fatty acids, bile acids, amino acids and steroid hormones. There is a large number of references (around 1 800 in total) and a fairly good index (this latter is a good point of the volume as books of this nature often dispense with an index).

The book is printed in a clear typescript that is relatively easy to read, and all the diagrams are clear, although in some instances rather small for the detail they possess. One hesitates to single out any individual contributor for praise or criticism. Suffice to say that in some instances rather more practical detail could be given and some of the chapters, together with their numerous references, may be regarded as short reviews rather than more practical applications.

In general this is a useful book for the experienced chromatographer dealing with a wide field of applications and would be recommended to libraries.

D. SIMPSON

TRACE ELEMENT ANALYTICAL CHEMISTRY IN MEDICINE AND BIOLOGY. PROCEEDINGS OF THE FIRST INTERNATIONAL WORKSHOP, NEUHERBERG, FEDERAL REPUBLIC OF GERMANY, APRIL 1980. Edited by PETER BRÄTTER and PETER SCHRAMEL. Pp. xviii + 851. Walter de Gruyter. 1980. Price DM180. ISBN 3 11 008357 4.

Most of us regard expensive books that are the record of a meeting held some time ago as a poor buy for our libraries, as they are often overtaken by developments in a rapidly changing subject. This book is a collection of papers based upon material presented at a Workshop on trace-element analysis in medicine and biology held in West Germany in April 1980. So the organisers have produced the book fairly rapidly and the contents are an up-to-date account of developments in this fashionable topic. Haste is evident in parts of the book, however; for example, the Panel Discussion that closed the meeting is badly in need of editing (the verbatim record of what the translators thought was said makes curious reading). This is a minor blemish in an otherwise well produced book. The quality of the Plenary lectures is high. The opening contribution from Professor Feinendegen is a review of the medical aspects of trace-element research and sets the scene for later, more specialised lectures. This scholarly account should also alert the more complacent amongst British and American workers that by no means all first-class research is published in English language journals. Inevitably, the quality of the contributed papers is more uneven and there is some repetition. A few contributors are more intent on justifying their own favourite analytical technique than selecting an appropriate method for the problem they are considering. For some of us there is an undue emphasis on neutron activation techniques, which, however successful in the past, would not be a realistic first choice nowadays.

There are several papers giving an account of atomic-spectrophotometric methods, including atomic fluorescence and carbon-furnace emission, also descriptions of electroanalytical and even catalytic rate reaction procedures. A whole section is devoted to X-ray emission (PIXE) and X-ray fluorescence techniques. A review chapter by Hislop surveys the wide choice of methods available to the analyst.

A most important section deals with the validity of results obtained by the sensitive techniques. Versieck and Cornelis remorselessly catalogue the extraordinary differences in values for metals found at parts per billion levels in healthy human serum. Referees should surely note this work and question any submission where the quoted controls are not in reasonable agreement with the lowest consensus mean values for human serum. Practical advice on reducing sample contamination is given in a chapter by Behne and other workers describe problems of sample dissolution prior to analysis. A section of the book is devoted to the growing range of biological reference materials, which, when properly used, should improve quality assurance programmes.

Although expensive for the individual reader, the book should be purchased for the libraries of all institutions with any interest in trace-element biology. G. S. FELL

METHODS FOR THE EXAMINATION OF WATERS AND ASSOCIATED MATERIALS. By THE STANDING COMMITTEE of ANALYSTS (to REVIEW STANDARD METHODS FOR QUALITY CONTROL OF THE WATER CYCLE); DEPARTMENT OF THE ENVIRONMENT, NATIONAL WATER COUNCIL. HM Stationery Office.

A Survey of Multielement and Related Methods of Analysis for Waters, Sediments and Other Materials of Interest to the Water Industry, 1980. Pp. 46. 1981. Price £3.20. ISBN 0 11 751529 9.

Dissolved Potassium in Raw and Potable Waters, Tentative Methods (1980 *Version*). Pp. 17. 1981. Price £2.40. ISBN 0 11 751545 0.

Zinc in Potable Waters by Atomic Absorption Spectrophotometry, 1980. Pp. 10. 1981. Price £1.70. ISBN 0 11 751541 8.

Dissolved Sodium in Raw and Potable Waters, Tentative Methods (1980 *Version*). Pp. 17. 1981. Price £2.40. ISBN 0 11 751546 9.

Chloro- and Bromo- Trihalogenated Methanes in Water, 1980. Pp. 16. 1981. Price £2.40. ISBN 0 11 751544 2.

Bromide in Waters, High Level Titrimetric Method, 1981 (*Tentative*). Pp. 8. 1981. Price £1.40. ISBN 0 11 751543 4.

These six booklets are part of the series that provides recommended methods for the determination of water quality, and which supersede the Department of the Environment volume on "Analysis of Raw, Potable and Waste Waters." The Standing Committee of Analysts is now providing a very useful set of up-to-date methods that can be readily applied by water laboratories in the UK and elsewhere. It is very encouraging to see the introduction of standard methods based on modern instrumental techniques and the above publications, which have just been released, contribute effectively to this process. Thus the method for halogenated methanes involves gas chromatography after solvent extraction, and the booklets on potassium and sodium both include two alternative methods using flame photometry and flame atomic-absorption spectrometry. The latter technique is also used for zinc determinations but in this instance following pre-concentration of the sample by evaporation in the presence of nitric acid. The method for bromide involves oxidation to bromate with sodium hypochlorite and determination of the bromate iodimetrically and has a limit of detection of 0.09 mg kg^{-1}. The information given about each method is extremely valuable and includes precision, range and speed, as well as accuracy data. The impression given is that the methods have been subjected to detailed evaluation and can therefore be used with confidence. The booklet on multielement methods is a brief summary/review and would be a useful document to give to new employees in a water laboratory. More detailed reading, using the references cited, would however be required to appreciate the theory and methodology of the methods described. J. M. OTTAWAY

Determination of Saccharin by Desorption of Ferroin from Silica Gel

Ferroin is strongly adsorbed on silica gel but its ion-association complexes are easily desorbed. A simple, rapid and accurate procedure for the determination of saccharin has been devised utilising these properties. The procedure is based on the quantitative formation of the ion-association complex [(Fe $phen_3)^{2+}(saccharin)_2^{-}$] when saccharin dissolved in water, 60% aqueous ethanol, 70% aqueous acetone or 80% aqueous methanol is shaken with ferroin-impregnated silica gel. The absorbance of the characteristic orange colour of the ferroin ion-association complex is measured at 510 nm.

There are no interferences from glucose, sucrose and dulcin even when these constituents are present at 1 600 times the concentration of saccharin. Sodium cyclamate, sorbic acid, benzoic acid, citric acid and sodium chloride give positive errors. Although the presence of sodium hydrogen carbonate results in a low recovery of saccharin, this compound may be eliminated easily by the addition of dilute sulphuric acid.

A procedure for the routine determination of saccharin in saccharin tablets is proposed.

Keywords: Saccharin determination; silica gel adsorption; tris(1,10-*phenanthroline*)*iron*(*II*)

E. ROY CLARK and EL-SAYED A. K. YACOUB

Department of Chemistry, University of Aston in Birmingham, Gosta Green, Birmingham, B4 7ET.

Analyst, 1982, **107**, 414–421.

Assay of Hydrazine in Isoniazid and its Formulations by Difference Spectrophotometry

A rapid procedure is described for the determination of hydrazine at the very low levels that may occur as a result of hydrolysis in certain isoniazid formulations. The method is based upon the measurement of the absorbance difference between a solution of the 4-dimethylaminobenzalazine derivative of hydrazine and a similarly prepared solution containing 3% *V*/*V* acetone. The absorbance difference, which is proportional to the concentration of hydrazine, is unaffected by the presence of a large excess of isoniazid. The choice of experimental conditions which provide the maximum accuracy, sensitivity and specificity is discussed.

Keywords: Hydrazine determination; ultraviolet spectrophotometry; difference spectrophotometry; isoniazid; isoniazid formulations

A. G. DAVIDSON

Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW

Analyst, 1982, **107**, 422–427.

Highly Sensitive Spectrophotometric Determination of Trace Amounts of Aluminium with Chromazol KS and Cetylpyridinium Bromide

A highly sensitive spectrophotometric method has been developed for the determination of aluminium, based on the formation of a ternary complex with chromazol KS (CALKS) and cetylpyridinium (CP) bromide in aqueous solution in the presence of 25–40% of ethanol. The pH range for the formation of the ternary complex is 5.8–6.7, and the wavelength of maximum absorbance is 625 nm. The ternary system obeys Beer's law for between 0.02 and 0.32 $\mu g\,ml^{-1}$ of aluminium. A high molar absorptivity of $\epsilon = 1.02 \times 10^5\,l\,mol^{-1}\,cm^{-1}$ and a Sandell's sensitivity of 0.00026 $\mu g\,cm^{-2}$ have been obtained. The complex has the composition $Al(OH)(CALKS)_2(CP)_6$, as established by Job's method of continuous variation and the equilibration shift method. The method has good selectivity and so can be applied to the direct spectrophotometric determination of trace amounts of acid-soluble aluminium in steel.

Keywords: *Aluminium determination*; *spectrophotometry*; *chromazol KS*; *cetylpyridinium bromide*

LIU SHAO-PU

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Analyst, 1982, **107**, 428–432.

Rapid Determination of Residual Chlorine by Flow Injection Analysis

Flow injection determination of residual chlorine in solution has been carried out spectrophotometrically by methyl orange decolorisation and by formation of a yellow complex with *o*-tolidine. The carrier streams are 0.180 mM methyl orange in pH 2 buffer solution or 0.01 M hydrochloric acid, and 3.0 mM *o*-tolidinium dichloride (in 2 M hydrochloric acid), respectively. A sampling rate of 288 samples per hour has been obtained with the latter reagent and the detection limit is 0.08 p.p.m. of chlorine. Influence of flow parameters such as flow-rate, tube length and diameter and the effect of interferents on the determination have been investigated. A study of the hyprochlorite - ammonium - *o*-tolidine reaction system has been performed and a method for the simultaneous determination of NH_4^+ and OCl^- is described.

Keywords: *Chlorine determination*; *flow injection analysis*; *methyl orange*; o-*tolidine*

D. J. LEGGETT, N. H. CHEN and D. S. MAHADEVAPPA

University of Houston, Department of Chemistry, Houston, TX 77004, USA.

Analyst, 1982, **107**, 433–441.

Improving the Yield, Purity and Molecular Integrity of Skeletal Muscle RNA Isolated by Phenol Extraction

Short Paper

Keywords: *RNA extraction*; *RNA degradation*; *ribonuclease inhibitors*

FRANCIS N. ONYEZILI

Department of Clinical Pathology, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria.

Analyst, 1982, **107**, 442–445.

Spectrophotometric Determination of Beryllium(II) Using a Trisazosalicylic Derivative of Triphenylamine

Short Paper

Keywords: *Beryllium(II) determination; spectrophotometry; triphenylamine trisazosalicylic acid*

M. E. M. KHALIFA
Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt.

Analyst, 1982, **107**, 446–449.

Titrimetric Determination of the Yield of Sulphide Formed by Alkaline Degradation of Cephalosporins

Short Paper

Keywords: *Cephalosporins; alkaline degradation; sulphide; titrimetry*

A. G. FOGG, M. A. ABDALLA and H. P. HENRIQUES
Chemistry Department, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU.

Analyst, 1982, **107**, 449–452.

Investigations on the Determination of Germanium in Organogermanium Compounds Using Carbon Furnace Atomisation

Short Paper

Keywords: *Germanium determination; organogermanium; carbon furnace atomisation; atomic-absorption spectrophotometry*

D. THORBURN BURNS and D. DADGAR
Department of Analytical Chemistry, The Queen's University of Belfast, Belfast, BT9 5AG, Northern Ireland.

Analyst, 1982, **107**, 452–455.

Differential-pulse Polarographic Determination of Red 10B Formed From the Permitted Food Colour Red 2G

Short Paper

Keywords: *Red* 10*B*; *Red* 2*G*; *food colours; differential-pulse polarography*

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Analyst, 1982, **107**, 455–459.

Determination of Morphine and Morphine-6-nicotinate by an *In Situ* Reaction on Chromatographic Plates with Dansyl Chloride

Short Paper

Keywords: *Morphine and morphine-*6-*nicotinate determination; morphine-*3,6-*dinicotinate; dansylation; derivatisation by overspotting*

R. WINTERSTEIGER
Institut für Pharmazeutische Chemie, Universität Graz, A-8010 Graz, Austria.

Analyst, 1982, **107**, 459–461.

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