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Section on Computer Techniques and Optimization		133/1			133/2			133/3			133/4	

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Review

ELEMENT-SELECTIVE DETECTION FOR CHROMATOGRAPHY BY PLASMA EMISSION SPECTROMETRY

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(Received 16th March 1981)

SUMMARY

High-temperature argon and helium plasmas provide stable sources for emission spectroscopy. These plasmas have proven useful as a means of selective detection for column chromatography. This review is based on a survey of the literature from 1965, when the first communication on this topic appeared. The species for which liquid and gas chromatographic effluents are monitored include metal ions and elements not traditionally determined by atomic spectrometry such as C, H, N, P, O, S, and the halogens. Applications to a wide variety of samples are discussed.

The use of some feature of the chemical nature of the substances eluted as a label for monitoring and identifying chromatographic peaks is as old as the origin of the name "chromatography" would imply. However, it is only since the early 1960s that this aspect of separation science has been the object of intense scrutiny. Selective detection usually enhances the detection limit of the method, minimizes interferences from possible overlapping bands, and provides important qualitative information about the species of interest. A recent review [1] offers an excellent survey of this principle of detection.

Atomic spectroscopy has the potential of being one of the most selective, consistent, and versatile techniques of this kind because it exploits differences in the elemental composition of the analytes. While atomic absorption and fluorescence represent particularly convenient approaches [2—4], they are confined to the determination of a limited number of compounds and are not readily adaptable to multi-element tracking. The development of the plasma as a stable spectroscopic source capable of exciting intense emission from all of the elements of the periodic table with linearity over four or five orders of magnitude suggests that plasma emission spectrometry may well become the method of choice for element-selective chromatographic detection.

In 1965, McCormack et al. [5] introduced the effluent from a gas chromatograph into a microwave-induced plasma (m.i.p.) and monitored a variety of atomic line and diatomic band emissions to achieve selective detection. Over 50 publications have subsequently appeared. Applications of this

technique have involved the use of the inductively coupled plasma (i.c.p.), the direct current plasma (d.c.p.), and the m.i.p. for selective monitoring of effluents from gas or liquid chromatographic separations. Krull and Jordan [6] have recently published an overview of the interface between chromatography and plasma emission spectrometry.

INSTRUMENTAL

While it is not the primary intent of this review to examine fully all the theoretical and operational aspects of the plasma, the discussion that follows may prove useful to the reader unfamiliar with the field. Excellent reviews of the inductively-coupled plasma [7—10], the microwave-induced plasma [11, 12, 12a], and the direct current plasma [13, 14] are available in the literature.

The plasma consists of a mass of predominantly ionized gas at a temperature of 4000—10000 K. This state can be maintained directly by an electrical discharge through the gas (d.c.p.) or indirectly via inductive heating of the gas by means of an electromagnetic field established using power generated at radio frequencies (i.c.p.) or microwave frequencies (m.i.p.). Analyte excitation results from electron impact and from collisions with metastable atoms of the plasma support gas (usually argon or helium) in accord with the Penning ionization scheme. The relative contributions of these two processes is uncertain at present.

A recent form of the d.c.p. is illustrated in Fig. 1. It consists of two graphite anodes and a thoriated tungsten cathode configured as an inverted "Y". The power supply is capable of providing about 15 A at 40—60 V d.c. Gas flow requirements (argon) are on the order of 1.5 l min^{-1} over each elec-

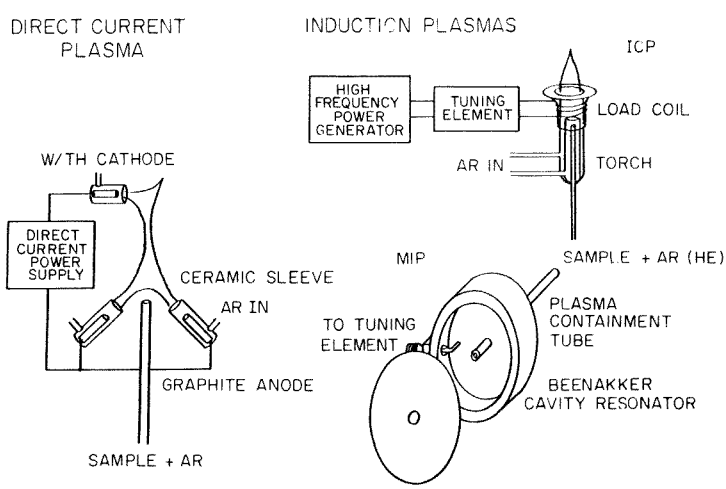

Fig. 1. Plasma sources for elemental emission spectroscopy.

trode with an additional 1 (with g.c.) to 6 (with l.c.) l min^{-1} being necessary for sample introduction. The electrodes and electrode sleeves are subject to degradation, and may need to be replaced frequently.

Figure 1 also illustrates the design of induction plasmas. A high-frequency power generator supplies power to an induction device (an induction coil or a cavity resonator) which serves to maintain the plasma. To avoid interference with communication bands, high-frequency power generators in the United States that are commercially available for plasma work are constrained to specific frequencies such as 27.2, 912 and 2450 MHz. At the same time, these supplies are designed to provide power efficiently when the load impedance (the induction device plus the plasma) has a specific value, usually 50 ohms. This impedance is a function of frequency, the physical parameters of the induction device, and the composition of the plasma. As a result, tuning components such as stub tuners or variable capacitors are introduced in the transmission line to match the impedance of the load to that of the source. In Europe, "free running" generators are available which automatically compensate for a mismatch between the source and the load by altering the frequency of the output while maintaining the power level constant. In such cases, tuning components are unnecessary.

A block diagram of a plasma emission detector suitable for use with a gas chromatograph is presented in Fig. 2. Liquid chromatographic effluents may be routed directly into a nebulizer prior to the i.c.p. [15, 16] or the d.c.p. [17, 18]. Interfacing a gas chromatograph involves the removal of the nebulizer and the addition of supplementary plasma gas [19, 20]. These interfaces require simple fittings that are readily available, although care must be taken to minimize the dead volume. The m.i.p. is currently unsuited to normal liquid chromatography because it cannot tolerate sample introduction rates in excess of a few mg s^{-1}; this is due to the low power levels of operation (usually less than 120 W). Only limited success has been realized by

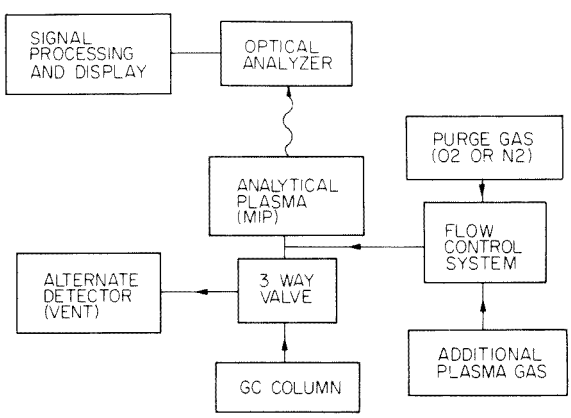

Fig. 2. Block diagram of the g.c./m.i.p. couple.

direct nebulization of aqueous aerosols [21—23]. However, the m.i.p. has enjoyed considerable use as a detector for gas chromatography (g.c.) and will be discussed below.

Emission from the plasma is focussed on the entrance slit of a suitable spectrometer. Since the plasma is a very rich and intense source, the quality of the results is significantly dependent on the quality of the spectrometer chosen.

Most publications have dealt with the use of the m.i.p. as a g.c. detector. Prior to 1976, the cavity resonators available would sustain an argon plasma at atmospheric pressure but helium plasmas could only be sustained at reduced pressure (1—10 Torr). Helium is a more desirable plasma support gas because it provides a simpler background spectrum yet produces a higher energy excitation medium. Not only is the helium plasma more robust than the argon plasma, it has improved the intensity and linearity of the emission produced by halogens and certain other elements. Two recent cavity designs [12, 23] permit a more efficient transfer of microwave power from the generator to the plasma, and will sustain the atmospheric helium plasma. A comparative study [24] has shown the TM_{010} cavity resonator of Beenakker [23, 25] to be superior to other designs. The application of this cavity to gas chromatography has only recently been illustrated [26—30]. Some improvements in design have been introduced by van Dalen et al. [31]. Because this cavity is relatively new, most g.c./m.i.p. to date has involved the use of other cavities.

METALS AND METALLOIDS

The i.c.p., m.i.p., and d.c.p. are well suited to the determination of metals. Detection limits, reproducibility, and linearity of response are generally comparable or superior to flame atomic absorption and emission. It is not surprising then, that plasma emission sources have shown great promise as chromatographic detectors for metals. Elemental hydrides, chelated metals, organometallics, and metal-containing proteins have all been separated by chromatography and quantified by plasma emission spectroscopy.

The feasibility of the separation and detection of metal chelates by g.c./m.i.p. has been investigated. Dagnall et al. [32] and Kawaguchi et al. [33] have shown the promise of this technique utilizing the acetylacetone and trifluoroacetylacetone (TFA) chelates of aluminum, beryllium, chromium, copper, iron, gallium, scandium, and vanadium. These authors used argon as the plasma support gas. Serravallo and Risby [34] compared the argon plasma to that of helium for chelates of chromium. Helium was found superior despite the low pressure requirement at the time of the work. Chromium yielded more intense and more numerous emission lines in the helium plasma. Application to real samples was made by Black and Sievers [35] who have determined 3 ppb chromium in blood as the 1,1,1-trifluoro-2,4-pentanedione complex. Also, copper and aluminum in zinc

were determined after extraction with TFA in chloroform from a neutralized aqua regia solution [36].

High-performance liquid chromatography (h.p.l.c.) has been used to separate copper complexed with nitriloacetate and ethylenediamine with detection by i.c.p. spectrometry [15]. Time-resolved minimum detectable concentrations were determined for twenty-four elements. Although the limits of detection were somewhat higher in the chromatographic mode than with continuous aspiration, only Pb, Na, and Mg were not within the same order of magnitude.

Copper, nickel, and cobalt diethyldithiocarbamates were separated by h.p.l.c. and monitored with a spectrometer in series with a d.c. argon plasma emission source [17, 18]. When a 5:15:80 mixture of acetonitrile:diethyl-ether:Skelly B (petroleum hydrocarbon mixture) was used, separation and quantification of the metal carbamates posed no major problems. However, when only the Skelly B hydrocarbon mixture was used as the solvent, the electrodes became coated with carbon black and the plasma was extinguished. A novel aerosol-nebulizer interface was applied to avoid this problem with a slight loss of sensitivity and reproducibility. The d.c. argon discharge plasma was also used to monitor the g.c. effluent of metal complexes of Ni, Pd, Cr, and Zn [20].

The m.i.p. spectroscopic detection of volatile elemental hydrides separated by gas chromatography [37, 38] has been investigated. Carbon dioxide and hydrochloric acid, products of the hydride generation procedure which are spectral interferents, were separated from the hydrides before elemental detection. In this way, Ge, As, Se, Sn, and Sb could be determined in nanogram amounts with a single element detection system. These eluents were also quantified simultaneously via a direct reading spectrograph after the chromatographic separation [38]. Although detection limits were somewhat higher, sub-microgram detection limits were retained. Hydrides of Ge, As, and Sb have been separated and determined utilizing a computer-controlled slew scanning monochromator [39]. A general review of hydride generation for atomic spectroscopy has appeared recently [40]. Detection limits of hydrides exceed those with solution nebulization and are comparable with m.i.p. and i.c.p. as seen in Table 1.

Several papers have appeared dealing with determination of environmentally important compounds by plasma emission spectroscopy. Work has been focussed on the methylmercury(II) species, tetraalkyllead (TAL) compounds, and methylcyclopentadienylmanganesetricarbonyl (MMT).

Mercury compounds have served as useful analytes for testing the microwave plasma. Because methylmercury compounds are simple and are relatively free of hydrocarbon emission in the ultraviolet region, the use of mercury emission (253.7 nm) makes detection by m.i.p. straightforward. Bache and Lisk [41] applied this system to methylmercury(II) chloride in fish. A linear response from 0.1 to at least 100 ng with a selectivity of 10000 to 1 compared to eicosane was obtained. Grossman et al. [42] have discussed the

TABLE 1

Comparison of detection limits (given in $\mu g\ ml^{-1}$) for hydride-forming elements by atomic emission spectroscopy

	Inductively-coupled plasma/solution nebulization [9]	Inductively-coupled plasma/as hydrides [40]	Microwave-induced plasma/as hydrides [37]
Ge	0.15	—	0.00015
As	0.04	0.0008	0.00035
Se	0.03	0.0008	0.00125
Sn	0.3	—	0.002
Sb	0.2	0.001	0.0005
Te	0.08	0.001	—
Pb	0.008	—	—
Bi	0.05	0.0008	—

selectivity for dimethylmercury relative to many other compounds. Selectivities of 10^3 were typical of the m.i.p. Comparison of the m.i.p. to the electron capture detector (e.c.d.) for mercury gave results comparable to within 5% [43]. Another advantage of the m.i.p. is that its molar response is similar for all methylmercury species while the e.c.d. response varies considerably with the number of methyl groups. Talmi [44] and Talmi and Norvell [45] have described the rapid determination of organomercury compounds in aqueous samples, biological tissues, and insects, with detection limits in the picogram to sub-picogram range. Quimby et al. [27] have recently applied the TM_{010} resonance cavity to the detection of diphenylmercury with similar results.

Gasoline additives, MMT and TAL, have received considerable recent attention. Quimby et al. [27] determined microwave plasma emission detection limits of 0.25 and 0.49 pg s^{-1} respectively, monitoring the metal emission lines. Five isomers of methyllead and ethyllead were determined in the atmosphere and in gasoline by this method [46]. Approximately 1% hydrogen was added to the plasma support gas to prevent the formation of lead deposits on the quartz plasma containment tube. Cyclopentadienylmanganesetricarbonyl (CMT) was used as an internal standard for the determination of MMT in gasoline by d.c.p. spectrometry [47]. Interferences were essentially absent as shown in Fig. 3A, B. The effluent was split for a detector response comparison with the flame ionization detector (f.i.d.). As is seen in Fig. 3C, plasma emission detection is far superior to the f.i.d. for the direct determination of MMT in gasoline.

Morita et al. [16] have demonstrated the use of an i.c.p. with a direct reading system as a multi-element detector for a protein molecular weight calibration kit and for vitamin B_{12}. The liquid chromatographic effluents were directed to the crossflow nebulizer of the i.c.p. Peak ratioing of the response for C, P, and Co in vitamin B_{12} ($C_{63}H_{90}CoN_{14}O_{14}P$) yielded an

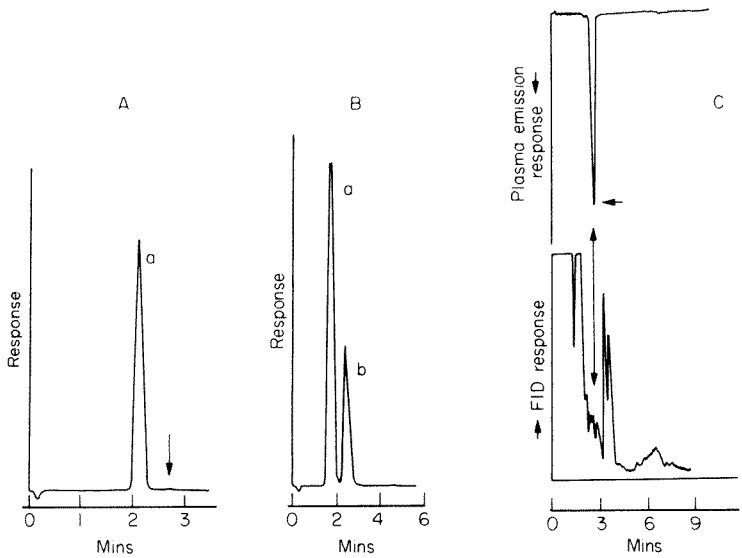

Fig. 3. Chromatograms using the d.c. argon plasma for detection with the 279.83-nm Mn line [47]. A, Leaded gasoline with 600 ng of methylcyclopentadienylmanganese tricarbonyl (a) added as internal reference. B, Cyclopentadienylmanganese tricarbonyl (a) (100 ng) and 50 ng of methylcyclopentadienylmanganese tricarbonyl (b) in 5 μl of isooctane solution. C, Dual detector chromatogram of 5-μl unleaded gasoline containing cyclopentadienylmanganese tricarbonyl [47]; effluent split 1:1 between f.i.d. and argon d.c.p. detectors. Reprinted with permission from ref. 47. Copyright, American Chemical Society.

atomic ratio of $C_{64.2}P_{0.93}Co_1$. Elemental ratios in excellent agreement with expected values were also obtained for cytochrome c. Unseparated peaks caused by ferritin and catalase can be resolved by monitoring phosphorus which is contained in the former compound.

GASES

Radio-frequency and microwave excitation sources have found use in the determination of gases. Boos and Winefordner [48] used a radio-frequency (8 MHz) plasma to monitor gases from an exponential dilutor. Molecular emission bandheads were used to monitor CO, CO_2, SO_2, NH_3, NO, NO_2, N_2, and CH_4. Gas chromatographic separation was not utilized although its use was suggested.

Dagnall et al. [49, 50] separated atmospheric gases by g.c. using the m.i.p. as an emission source for detection. The atomic lines of carbon and sulfur could be used for CO, CO_2, and SO_2 while N_2 and NO_2 were monitored via molecular emission bandheads. Oxygen could not be determined because it caused a negative background interference. McCormick et al. [5] noted that water could be monitored by utilizing the OH emission band.

ORGANIC COMPOUNDS

Plasma emission spectroscopy shows great promise as a detector for organic compounds. The advantage lies in its ability to observe emission from most of the elements in these compounds. That several emission lines are generally available for each element aids in reducing interferences. By proper selection of lines, the emission of one element can usually be monitored while avoiding that of another.

This versatility of response mode is not available with other chromatographic detectors, except the mass spectrometer. While mass spectrometry is very sensitive, considerable experience is required for interpretation of the complex data produced. In its simplest form, plasma emission data analysis is straightforward. With computer control and the proper spectrometer, the data output can yield considerable information.

In many applications, an element characteristic of the compound of interest which is absent from the bulk sample is monitored. This is particularly advantageous with complex samples in which the analyte contains sulfur, silicon, antimony, arsenic, selenium, or a halogen. An excellent example of the single element concept is shown by Bostick and Talmi [51]. The g.c. effluent of a mixture of several *n*-alkanes and trimethylsilane derivatives was split between an m.i.p. and an f.i.d. The f.i.d. responded to all compounds in the sample. The m.i.p. detector responded only to the silanes. Moreover, the m.i.p. chromatogram showed a considerably smaller background from the solvent than did the f.i.d.

Feldman and Batistoni [52] have shown the advantages of monitoring more than one element using a helium glow-discharge detector. The chromatographed species included a mixture of hydrocarbons, silicon-containing compounds, and a compound containing arsenic and silicon. Results are shown in Fig. 4. Selectivity ratios of silicon (288.16 nm) and arsenic (228.81 nm) to carbon were 325:1 and 296:1, respectively.

Talmi and Bostick [53] have determined a series of arsenic acids by first converting the compounds to their corresponding arsines and using a g.c./m.i.p. system. The sensitivity ratio of equimolar amounts of monoethylarsine and dimethylarsine was 1.01:1.00, virtually independent of substituent groups. With instrumental precision at 2.1%, the major portion of the relative standard deviation of the technique was due to sample preparation. In a similar paper, arsenic and antimony were determined as the triphenyl compounds [54].

Talmi and Andern [55] used an argon g.c./m.i.p. to quantify selenium in environmental samples. Comparison was done with National Bureau of Standards certified standards and results from neutron activation. Excellent agreement was obtained as shown in Table 2. Relative standard deviations ranged from 5% for orchard leaves to 0.3% for fly ash.

With the increased emphasis in analytical chemistry on the determination of insecticides, fungicides, herbicides, etc., plasma spectroscopy promises to

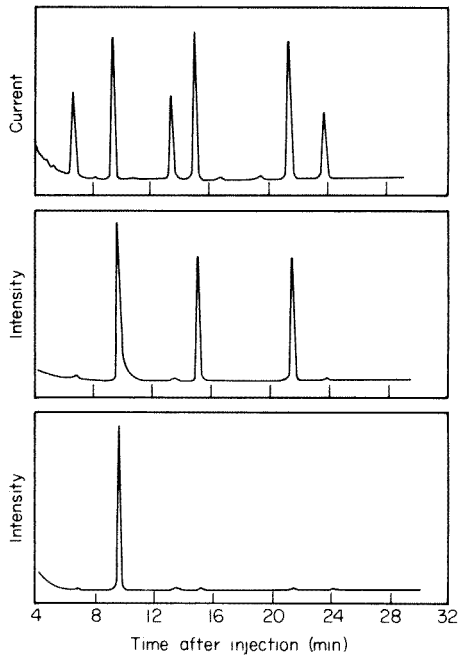

Fig. 4. Gas chromatograms of a silylated mixture of aliphatic acids, phenylarsonic acid, and hydrocarbons [52]. Top curve: flame ionization detector. Center and bottom curves: glow discharge detector with monochromator set for Si (288.1 nm) and As (228.8 nm) respectively. Reprinted with permission from ref. 52. Copyright, American Chemical Society.

TABLE 2

Determination of selenium in environmentally based samples [55]

Sample	Conc. of Se found (ppm)	Reported Se conc. (ppm)
Bovine Liver (NBS 1577)	0.98 ± 0.03	1.1 ± 0.1[a]
Orchard Leaves (NBS 1571)	0.083 ± 0.004	0.08 ± 0.01[a]
Coal (NBS 1632)	2.86 ± 0.13	2.9 ± 0.3[a]
Fly Ash (NBS 1633)	9.35 ± 0.03	9.4 ± 0.5[a]
		9.1 ± 0.2[b]
Human hair	0.97	0.95[b]
Air	0.088 ± 0.002	0.090[b]

[a]National Bureau of Standards certified value. [b]Value obtained by neutron activation.

play an important role. The first application of g.c./m.i.p. to complex samples [56] appeared simultaneously with the initial g.c./m.i.p. paper by McCormick et al. [5]. The work dealt with organophosphorus insecticide residues. Work has continued and pesticide constituents including H, C,

Se, N, P, O, S, Cl, Br, I, and F have been monitored by g.c. plasma spectroscopy.

Bache and Lisk [56] used an argon m.i.p. in work with 18 phosphorus-containing insecticides at the 253.6-nm phosphorus atomic line. Detection limits comparable to the electron affinity detector were obtained. Samples varied from grapes to lettuce to bees to whole chicken with excellent results. The system was modified for low pressure and was optimized for baseline-to-analyte signal ratio to extend the detection limits even further [57]. Using helium as the plasma support gas, Bache and Lisk found that eight phosphorus lines could be easily detected [58]. The 253.6-nm line was the most intense in both the argon and helium plasmas. Application of the helium plasma to complex samples (onions, carrots, and chicken flesh) was demonstrated [58, 59]. Moye [60] utilized the advantages of both support gases and found that the maximum peak height for the 253.6-nm line of parathion was obtained when 85% helium and 15% argon were used as the plasma support gas.

No scavenger gases were used in the above-mentioned work to rid the quartz plasma containment tube of carbon deposits. It is worth noting that van Dalen et al. [61] found that nitrogen is superior to oxygen for this purpose. Phosphorus oxides react with the walls of the tube to cause erratic behavior. With the use of nitrogen, this is avoided and detector response is doubled.

Bache and Lisk [58] characterized four sulfur emission lines in the m.i.p. using dimethylsulfoxide. Although the technique was very sensitive (5×10^{-11} g s^{-1}), the selectivity to phenanthrene was relatively poor (22:1) with the low-pressure helium plasma. This system was applied to synthetic samples of seven sulfur-containing pesticides and five sulfa drugs [59]. This subsequently was applied to disyston, ronnel, and thinet extracts of natural samples. Derivatives of carbamate pesticides have also been determined by using a sulfur emission line [62]. Dagnall et al. [63] listed two other sulfur emission lines and a C=S bandhead which they used to monitor sulfur via the m.i.p.; the bandhead gave the better response for some compounds. Detection limits were extended to subnanogram levels utilizing the 247.9-nm carbon line and the 257.6-nm sulfur bandhead [64]. It is interesting to note that insertion of a platinum coil into a 1/4-wave foreshortened cavity increased atomic emission five-fold at both of these lines while a 25% decrease in emission was noted at 257.6 nm with the Evenson 1/4-wave cavity. This was attributed to incomplete molecular fragmentation in the foreshortened cavity. Braun et al. [65] have shown linearity of response over four orders of magnitude for sulfur. An argon d.c.p. has recently shown excellent selectivity and linearity of response over three decades when interfaced for g.c. [66].

Recently, Brown and Fry [67] have monitored oxygen-containing compounds by g.c./i.c.p. in the near-infrared region. An extended torch was used to avoid entrainment of atmospheric gases. A detection limit of 650 ng was obtained.

The halogens provide an interesting comparison for the argon and helium plasmas as emission detectors. Since the helium provides higher excitation energies than those of most atoms, atomic or ionic lines are more easily excited than in the argon plasma. Also, more complete molecular fragmentation is provided by the helium plasma because of the higher energy metastable states of helium compared to those of argon. An excellent discussion of these states is given by Beenakker [26].

Iodine in methyl iodide was the first halogen to be monitored via the atomic emission line [5]. This was done with an argon g.c./m.i.p. Iodinated herbicides were subsequently determined in soils and grains [68].

The argon m.i.p. detector has been utilized to provide the atomic emission lines of bromine from appropriate halogenated compounds whereas chlorine lines could not be obtained [49]. The 85% He:15% Ar m.i.p. used by Moye [60] to obtain excellent results for phosphorus did not produce detectable atomic emission lines for chlorine. Windsor and Denton [69] used the atomic lines of F, Cl, Br, and I to detect g.c. effluents by i.c.p. spectroscopy with argon as the support gas, but detection limits in all cases were poorer than those obtained with the helium m.i.p. Fry et al. [70, 71] later extended the detection limits of F, Cl and Br by more than 100 fold, by utilizing low energy near-infrared lines. A comparison of detection limits for a variety of helium and argon plasmas is presented in Table 3.

The initial detailed study of the halogens was done by Bache and Lisk [58] in 1967 using a helium m.i.p. The work lists five atomic emission lines for both chlorine and bromine and nine for iodine. Data for over twenty compounds were given with sensitivities for the most intense elemental lines.

Halogen-containing pesticides have been successfully determined with the helium m.i.p. detector [59, 62]. Quimby et al. [28] later determined trihalomethane in drinking water. Detection limits of less than 1 ppb and selectivities greater than 10^2 were obtained. Mulligan et al. [30] have also utilized g.c./m.i.p. to monitor polybrominated biphenyl and related compounds with comparable results.

The most advanced g.c./m.i.p. system to date was applied by Quimby et al. [29] for the determination of aqueous chlorination products of humic sub-

TABLE 3

Detection limits (as ng) for halogens by chromatography with plasma emission spectroscopy

Halogen	Argon i.c.p.	Argon m.i.p.	Helium m.i.p.	Helium d.c.p.
I	24[a]	20[d]	1[e]	4.5[f]
Br	50[b]	160[d]	0.4[e]	45[f]
Cl	50[b]	40[d]	1[e]	45[f]
F	1000[c]	—	—	4.1[f]

[a]Taken from ref. 19. [b]Taken from ref. 71. [c]Taken from ref. 70. [d]Taken from ref. 49. [e]Taken from ref. 28. [f]Taken from ref. 52.

stances. A capillary g.c. column was used to separate this complex sample which was eluted into a TM_{010} microwave cavity. The detection limit of pentachlorophenol was estimated at 40 ppt (ng l^{-1}).

Halogens have also been monitored via a helium d.c. discharge plasma emission detector [72]. Atomic lines were utilized for F, Cl, Br, and I with detection limits in the 10^{-13} g s^{-1} range. Selectivities are from 40:1 to 70:1 for F, Br, and Cl in the compounds studied. Poor selectivities are obtained for iodine. Braman and Dynako [72] stated that this problem is due to extraneous line and band interferences at the 608.2 and 546.4-nm analytical wavelengths used. Had another iodine line been used, this problem might have been reduced. Feldman and Batistoni [52] modified this detector cell to prevent deposits of decomposition products.

ELEMENTAL RATIOS

The use of elemental ratios along with chromatographic retention times has the potential of making analyte identity a more systematic task. If the analyst had the empirical formula associated with each peak, most of the time spent analyzing the chromatogram could be utilized elsewhere. This approach is well suited for the laboratory which deals with a variety of sample types.

Several workers have used two different detectors. McLean et al. [73] and Lowings [74] have split g.c. effluent for simultaneous use of the f.i.d. and m.i.p. detectors. Hydrogen was monitored spectroscopically while carbon was monitored with the f.i.d. detector. It was found that the H/C response factors were relatively independent of molecular structure for the compounds studied. Experimental H:C ratios were generally within 1% of the theoretical value. Two monochromators were used to determine the ratios of C to S, P, Cl, I, and Br by g.c./m.i.p. [75].

While the determination of two elements in a compound provides qualitative insight, the determination of additional elements increases identification capabilities even more. This concept has been utilized by Bonnekessel and Klier [76] and Windsor and Denton [69] using m.i.p. and i.c.p., respectively. Each method required a direct reading multichannel spectrometer. A simultaneous chromatogram of 22 compounds is shown in Fig. 5. A sampling of results obtained by this method is shown in Table 4. Results are normalized to one of the atoms in each compound. It is clear that molecular structure is not an important factor in the results and that the results match well with the theoretical values.

CONCLUSION

In the past, plasma spectroscopy has been shown to be a versatile and useful means of chromatographic detection. It is expected that the technique will attain greater use as a chromatographic detector in the future.

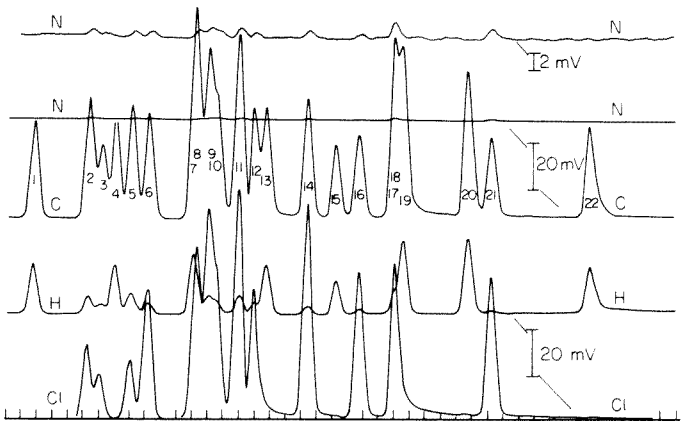

Fig. 5. Multielement chromatogram of n-paraffins and chlorine and nitrogen derivatives of benzene, toluene and phenol [76].

TABLE 4

Normalized experimental elemental ratios

Molecular formula	C	H	Cl	I	N
C_4H_9I[a]	4.06	9.00	—	1.00	—
C_6H_5I[a]	5.92	4.69	—	1.00	—
C_4H_9Cl[a]	3.98	8.97	1.00	—	—
$C_{15}H_{32}$[b]	15.00	34.80	0.03	—	—
$C_6H_4NO_2Cl$[b]	6.00	4.10	1.03	—	0.94
$C_6H_3NO_2Cl_2$[b]	6.00	3.02	2.02	—	0.93
$C_6H_2NO_2Cl_3$[b]	6.00	2.02	2.99	—	1.09
$C_6HN_2O_4Cl_3$[b]	6.00	1.23	3.00	—	1.92

[a]Taken from ref. 69. [b]Taken from ref. 76.

Useful applications now exist and many possibilities can be considered for further research. Future work may include development of the helium i.c.p. Nitrogen has already been used as a plasma support gas [77]. Relatively inexpensive hardware is required for the d.c.p. and further investigation is in order. Reduction of the sample volume in the plasma will undoubtedly improve the detection limit of the technique. Solution nebulization into the m.i.p. should be an important objective for researchers in the coming decade. This would greatly enhance the attractiveness of this detector for multipurpose use. A particularly important and exciting area of study will be to pursue further the determination of halogens as a monitor for pesticides and other high-molecular-weight organic compounds.

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FLOW INJECTION SYSTEMS WITH INDUCTIVELY-COUPLED ARGON PLASMA ATOMIC EMISSION SPECTROMETRY
Part 1. Fundamental Considerations

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SUMMARY

Flow injection systems with inductively-coupled argon plasma atomic emission spectrometry are proposed. Effects of flow rates, injected volumes and mixing coil lengths are investigated and conditions for the measurement of the flow injection transient signal are discussed. The peak profile measured with the spectrometer corresponds well with the estimate of the true sample zone distribution near the inlet of the spectrometer made by a zone-sampling process; thus the plasma is not a limiting factor in the proposed systems. For plant analysis, the system provides nearly zero sample dispersion and so the inherent sensitivity of the spectrometric method is preserved. The results obtained for 10 elements in the NBS Orchard Leaves reference material (SRM 1571) are in good agreement with the certified values. For determinations of calcium and magnesium in dolomitic limestones, cadmium is used as internal standard and so the merging zones configuration is employed. The proposed system provides medium sample dispersion and permits about 100 samples to be analysed per hour. Relative standard deviations of 1.34% and 1.23% were calculated for the calcium and magnesium data, respectively. The analytical results compare favorably with those obtained by normal i.c.p. spectrometry with pneumatic sample aspiration, after manual sample dilution.

After the inception of flow injection analysis (f.i.a.) in 1975 [1], this new approach in automated chemical analysis underwent fast development as indicated in comprehensive reviews and in a monograph [2]. In the earlier stages of development, simple systems involving colorimetry, potentiometry or turbidimetry were proposed and emphasis was given to agronomical and biomedical applications, where the number of samples to be analysed is normally high. In 1979, flow injection analysis was applied to atomic absorption and flame emission spectrometry [3]: the manifold in the merging zones configuration [4] was very simple and allowed controlled sample dispersion and addition of masking reagents within the analytical path. Other

applications also demonstrated the feasibility of flow injection analysis in conjunction with atomic absorption spectrometry [5—7].

It is well known that the use of a peristaltic pump for sample addition to an inductively-coupled argon plasma (i.c.p.) spectrometer improves some analytical characteristics in i.c.p. atomic emission spectrometry (i.c.p.e.s.), especially when sample viscosity is a problem [8, 9]. A draw-back of this procedure is, however, the cumbersome passage of the sample through the pump and the replacement of samples, which impair the analytical frequency. In a flow injection system, a sample carrier stream is continuously flowing, the samples being smoothly injected into it after the peristaltic pump so that high sampling rates can be achieved.

The aim of the work described here is to demonstrate the feasibility of f.i.a. in i.c.p.e.s. Two distinct situations are emphasized: first, when sample dispersion tends to zero and the inherent sensitivity of the spectrometer is preserved; and second, when sample dispersion is medium [10] in a typical flow injection system. This latter situation allows the incorporation into the i.c.p.e.s. technology of several processes which are easily performed in flow injection systems, such as controlled sample dispersion [10], sample splitting [11], preconcentration by solvent extraction [12] or by ion-exchange [13], dialysis [14], distillation [15], standard additions [16], etc. To demonstrate the potential of the f.i.a.—i.c.p.e.s. system, analyses of rock and plant materials are reported.

EXPERIMENTAL

Apparatus

An Ismatec MP-13 peristaltic pump furnished with tygon pumping tubes was employed. The proportional injector, with three 2:3:2 commutation sections [17] was made from perspex and consisted of two fixed external plates and a central sliding bar assembled tightly together by means of two screws with springs. Silicone rubber sheets were used between the plates to avoid leakage. Since the insertion holes were slightly conical, tubes were easily forced into the plates of the injector. The central plate of the injector was displaced by means of a brass lever, which could be manually or electronically operated. Details of the electronics involved have been given elsewhere [7, 13]. The manifold was made with polyethylene tubing (0.8 mm i.d.) of the non-collapsible wall type. The coils were made by winding suitable tubing lengths on a glass tube with a 2-cm external diameter. All connectors were made from perspex.

The flow injection system and the i.c.p. spectrometer were coupled when the peristaltic pump was on to avoid extinguishing the plasma. This was easily done by connecting the mixing coil to the inlet of the i.c.p. nebulizer (point x, Fig. 1). A Jarrell—Ash model 975 ICAP AtomComp with 40 analytical channels was employed with the parameters indicated in Table 1. The standard software in the PDP-8 computer was maintained. No back-

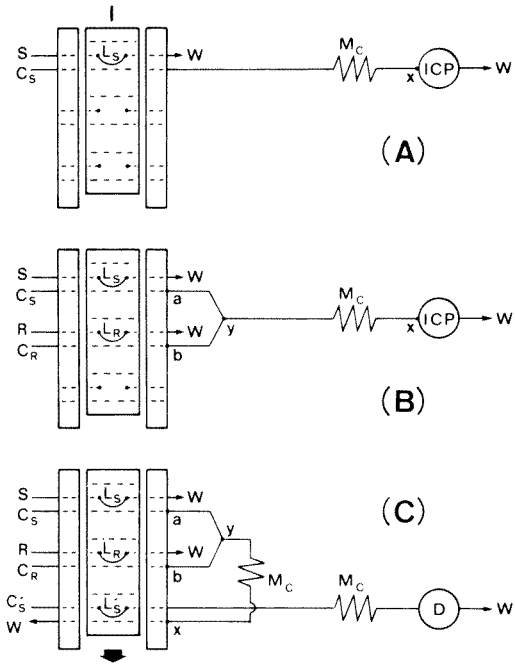

Fig. 1. Flow diagram of the systems employed for plant analysis (A), for rock analysis (B) and for estimating the true sample distribution near x (C). I represents the injector in the sampling position, with the sample (S) filling the sample loop (L_S) and going to waste (W). The aspiration rate of the sample and of the internal standard solution (R) are always 3.4 ml min^{-1}, except for system A where the sample aspiration rate is 9.2 ml min^{-1}. Lines *ay* and *by* are the synchronization lines (4 cm), M_C and M'_C are the mixing coils, ICP represents the spectrometer and D is the spectrophotometer set at 450 nm. In system A, the sample carrier stream (C_S), pumped at 3.2 ml min^{-1} is a 0.25 M $HClO_4$ solution, L_S = 300 cm and M_C = 40 cm. In systems B and C both sample and standard carrier streams are pumped at 1.6 ml min^{-1}, the sample and standard loops are 20 cm long and M_C = 140 cm. In system B, C_S and C_R are 0.5 M HCl solutions. In system C, C_S and C_R are replaced by the buffer solution; the second carrier stream (L'_S = 3.4 ml min^{-1}) is also the buffer solution, and the second sample loop (L'_S) is 5 cm. M'_C is 110 cm and the eriochrome cyanine R solution is used to simulate the sample. For details, see text.

ground correction was applied and standardizations with two points were always performed: the lower standards were zero for all elements and the concentrations of the higher standards are indicated in Table 1. Unless otherwise stated, the pre-burn time was 1 s.

For spectrophotometric measurements, a Varian model 634-S spectrophotometer equipped with a 178-OS Hellma flow cell (light path 10 mm, inner volume 80 μl) and connected to a Radiometer REC61 recorder with a REA 112 high-sensitivity unit was employed.

TABLE 1

Instrumental parameters for i.c.p.e.s. with the ICAP AtomComp and upper limits for calibration

Argon flow rates	Coolant 23 l min^{-1}; plasma 0 l min^{-1}; sample 0.5 l min^{-1}
Observation height	16 mm above load coil
Power input to plasma	Incident power 1.5 kW; reflected power < 5 W

Element	Wavelength (nm)	Higher standard for calibration (ppm)	Element	Wavelength (nm)	Higher standard for calibration (ppm)
Al	308.21	20	K	404.72	100
B	249.6 (2)[a]	10	Mg	279.07	100
Ca	317.9	100	Mn	257.6	10
Cd	228.8 (2)[a]	10	P	214.9 (2)[a]	10
Cu	324.7	10	Zn	213.8	10
Fe	259.9	10			

[a]Second order diffraction.

Reagents

All chemicals were of analytical grade and distilled-deionized water was always used. High-purity liquid argon was employed. The 0.3% (w/v) eriochrome cyanine R stock solution was prepared by dissolving 3 g of the dye in about 700 ml of water, the pH being adjusted to 2.9 with glacial acetic acid before diluting to 1 l with water. The 0.03% (w/v) eriochrome cyanine R working solution was prepared by suitable dilution of the stock solution with the buffer solution. This buffer solution, with pH of about 4.7, was 0.1 M in acetic acid and 0.1 M in sodium acetate.

Standards and samples

Spectrographically pure substances (Johnson, Matthey and Co., Specpure Reagents) were used for the preparation of the standard stock solutions. Mixed standards for plant and rock analyses were also 0.25 M in perchloric acid and 0.5 M in hydrochloric acid, respectively, so that they had acidities similar to those of the samples.

National Bureau of Standards Orchard Leaves (SRM 1571), used as the plant reference material, were mineralized by digestion with nitric and perchloric acids, as in earlier work [18].

Dolomite limestone samples from Maranhão, Brasil, were solubilized as follows. To 500 mg of a ground (60-mesh) air-dried sample, placed in a teflon beaker, 5 drops of water, 1 ml of concentrated nitric acid and 4 ml of 60% perchloric acid were added. The mixture was evaporated to dryness in a sand bath (ca. 120°C) and then 10 ml of 40% hydrofluoric acid were added. After drying again (105°C), the sample was dissolved in 0.5 M HCl solution and transferred to a volumetric flask. The final volume was adjusted to 100 ml with the same acid solution.

Flow diagrams

Figure 1A shows the flow diagram of the system designed for plant analysis, with the injector in the sampling position. The sample is aspirated to fill the sample loop, its excess being discarded. When the injector is switched to the alternative position, that of injection, the sample plug is intercalated into the continuously flowing sample carrier stream and a well defined and reproducible sample zone is established. This zone undergoes continuous dispersion while being directed towards the i.c.p. nebulizer and is then discarded. Since the sample loop is much longer than the mixing coil, the dispersion factor in this system tends to one and, at the central portion of the sample zone, a steady-state situation tends to be attained [10]. The system is adjusted so that only this portion of the sample zone, in which the signal-to-noise ratio is optimum, is measured. It is well known in flow injection analysis that, when very large sample volumes are employed, the sampling rate decreases sharply [10]. In this design, this draw-back is overcome by switching the injector back to the sampling position immediately after measurement so that the tailed portion of the sample zone, still inside the sample loop, is pushed by the next sample towards waste and not towards the spectrometer. The entire process can be regarded as a zone sampling process [7], where one portion of the sample zone is selected and processed while the other portion is discarded.

Considering the calcium and magnesium contents in the digests and the upper measurement limits of the spectrometer, sample dispersion was required for the limestone analyses. In flow injection systems where medium dispersion is attained, a transient signal is always measured. Since the presently available software in the PDP-8 computer is not programmed for the measurement of such a transient signal, and keeping in mind the possibility of differences in injection and measurement starting times, the internal standard technique became necessary. The flow injection system indicated in Fig. 1B was then designed in the merging zones configuration [4], which permits the reproducible addition of any species to the sample zone. When the injector rests in the position specified in Fig. 1B, both sample and standard are loaded into their corresponding loops before going to waste. After switching of the injector, the two established zones merge at confluence point y in a synchronized manner, perfect overlap between the two zones being attained [4]. Dispersion proceeds inside the mixing coil, and after a properly defined time interval, the transient signal is measured at the i.c.p. After the sample has been discarded, the injector is switched back to the position indicated in Fig. 1B, starting another cycle. In this system, only the measurement ratios are used for analytical purposes.

The system in Fig. 1C can be regarded as that of Fig. 1B with the inclusion of the zone sampling process [7]. In the situation presented in the figure, the sample loop and the internal standard loop are being loaded. When the injector is switched to the alternative position, both sample and standard volumes are introduced into their corresponding carrier streams and the

second sample loop is placed in the same path. The merging of the zones and dispersion of the sample proceeds as in system B. After a time interval (Δt), when the sample zone is passing through the second sample loop, the injector is switched back to the position indicated in Fig. 1C, starting a new cycle. This simple movement causes the introduction of a selected portion of the dispersed sample zone into the second sample carrier stream. This originates a second sample zone which is similarly processed and measured. Since the second sample loop is very small, acting as a point sampler, a Δt scan enables the entire dispersed zone to be stepwise transferred to the second carrier stream. This allows the detector to build an image of the axial sample distribution in the vicinity of point x which, in system B, corresponds to the f.i.a.—i.c.p. connection. The true axial sample distribution at the inlet of the i.c.p. nebulizer can then be estimated, regardless of the dispersion occurring after the zone sampling process and the characteristics of the detection unit.

Procedure

The effect of sample flow rate was investigated with the system in Fig. 1A, the injector not being used. The spectrometer was standardized as usual with pneumatic sample aspiration, the measurement time interval being 2 s. For this experiment, the sample (100 ppm Ca plus 100 ppm Mg in 0.5 M HCl) replaced the carrier stream, in an infinite volume configuration [16]. All measurements were made in duplicate. Flow rates of 0.42, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.6 ml min^{-1} were tested.

The performance of the i.c.p. as a detector for f.i.a. was evaluated with the systems B and C. The measured peak profile (Fig. 2) was achieved with system B, the sample consisting of 100 ppm Ca plus 100 ppm Mg in a 0.5 M HCl solution. The internal standard solution in this case was replaced by a 0.5 M HCl solution. The i.c.p. standardization was done with the standard and blank solutions in infinite volume configurations [16], the measurement time interval being 1 s. The difference (ΔI) between instants of sample injection and starting command for the i.c.p. was scanned from 0 to 30 s, in order to achieve the measured peak profile. The true sample zone distribution near the i.c.p. inlet (Fig. 2) was evaluated with the system C in a procedure similar to that already described [7]. The Δt values were scanned from 4 to 30 s. For this experiment, a spectrophotometric detector was used instead of the i.c.p. because an electronic command for the injector was necessary and the conditions of f.i.a.—i.c.p. operation had not been fully defined at this stage.

Effects of sample injected volume (25, 100 and 500 μl, corresponding to about 5, 20 and 100 cm sample loops) and mixing coil length (40 and 140 cm) were also studied.

System A was employed, with the 100 ppm Ca plus 100 ppm Mg standard, in 0.5 M HCl as a control. For each situation recalibration of the system and measurement of the peak profile were done as described above. System A

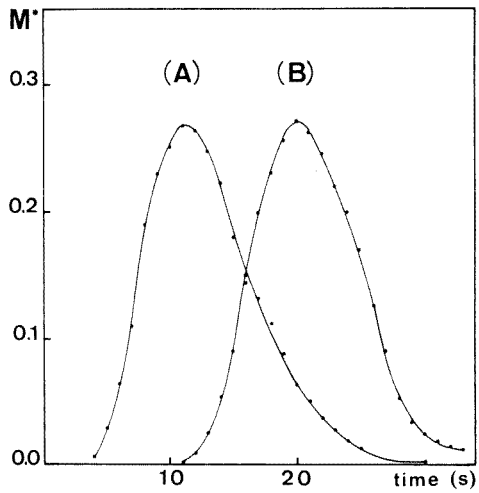

Fig. 2. The measured peak profile (A) and the estimate of the true sample zone distribution (B) near the inlet of the spectrometer. Abscissa corresponds to Δt or ΔI values and M^* is the ratio between a given measurement and that related to the infinite volume configuration [16]. The measured peak profile is based on calcium data.

was then applied to plant analysis. The measurement time interval was set at 10 s and the calibration of the system was made after injecting four standards (concentrations specified in Table 1) into the f.i.a.—i.c.p.e.s. system. The commands for starting the i.c.p. operation and the sample injection were simultaneous ($\Delta I = 0$). The precision of the measurements was evaluated in terms of relative standard deviations after a tenfold repetitive analysis of the sample. The accuracies were evaluated by comparing the f.i.a.—i.c.p.e.s. results with those obtained via normal operation of the i.c.p. with those reported by other authors and with the certified values. In addition, the precisions of the usual i.c.p.e.s. measurements were also estimated.

For the analysis of the limestone samples, system B was employed with a measurement time interval of 10 s. The ΔI value was also zero and a preburn time of 8 s became necessary. Cadmium was chosen as the internal standard based on the following considerations. First, its concentration in rocks is usually very low [19]. Secondly, the cadmium signal is practically unperturbed by other elements present in the digests. Preliminary tests indicated that successive measurements of 400 ppm of Fe, Al, Zr, Ti, Ca and Mg solutions had no effect in the cadmium line. Thirdly, the spectral interferences of cadmium on calcium and magnesium signals are negligible; even when a 1000 ppm cadmium solution was measured, its contribution to the calcium and magnesium lines (Table 1) was not observable. Finally, the analytical sensitivity in the atomic emission spectrometry of cadmium is high. In order to have gain and offset values for the cadmium standardization curve, similar to those related to calcium and magnesium, the concentration of the

internal standard solution was fixed as 20 ppm cadmium in 0.5 M HCl. Under such conditions, memory effects are not relevant.

The precisions of the measurements were evaluated by analysing a typical dolomitic sample ten times. Finally, the results of the combined system were compared with those obtained by normal i.c.p. spectrometry with pneumatic sample aspiration. In this latter situation, a tenfold manual dilution of the samples with a 0.5 M HCl solution became necessary.

RESULTS AND DISCUSSION

In agreement with earlier work [8], it was noticed that although the optimal sample flow rate depends on the element considered, the measured signal is little affected by changes in this flow rate in the 1.0—5.0 ml min^{-1} interval (Table 2). Also, fluctuation in the reflected power, which manifests itself by plasma extinction in extreme cases, became observable when the flow rate was increased beyond 5.0 ml min^{-1}. As a compromise between analytical sensitivity, plasma stability and sampling rate, the latter being rather dependent on the flow rate, a flow rate of 3.2 ml min^{-1} was chosen. This flow rate is higher than the pneumatic sample aspiration rate (ca. 1 ml min^{-1}) under standard conditions. It must be emphasized that in f.i.a.—i.c.p. systems for single component analysis where sensitivity is critical, the optimal flow rate must be defined prior to system design. For multi-element analysis, an optimized flow rate should be chosen, its value depending on the specific measurements required, but being as high as possible to minimize carryover.

A comparison of the measured peak shape with the estimate of the true axial sample zone distribution near the f.i.a.—i.c.p. connection (Fig. 2) makes it clear that the measured peak shape is much more dependent on the flow injection system than on the i.c.p. detector. In fact, the close similarities between half peak widths, peak heights and wash times [1] indicate that, in the flow injection system B, the i.c.p. behaves as a detector for which dead volume and response time effects are not relevant. This is an important practical result and perhaps is somewhat unexpected considering the large volume of the nebulizer.

TABLE 2

Effect of sample flow rate

Flow rate (ml min^{-1})	Relative emission[a]		Flow rate (ml min^{-1})	Relative emission[a]	
	Ca	Mg		Ca	Mg
0.42	88.3	43.1	3.0	106.8	97.2
1.0	109.2	85.8	4.0	102.0	102.1
1.6	108.1	95.5	5.0	105.5	101.0
2.0	109.5	94.3	6.6	97.1	83.9

[a]The intensity related to the pneumatic sample aspiration under standard conditions is 100.

Since the wash time at the 1% carryover level is less than 15 s (Fig. 2), it is evident that more than 240 samples could be analysed per hour with system B. This sampling rate was not achieved in this work, however, because the time required for the teletype to print results impaired the analytical frequency. The maximum rate that could be achieved with the present system was 100 samples per hour. Studies to overcome this problem are presently in progress. The i.c.p. could be a limiting factor in the sampling frequency in faster flow injection systems. This possibility was not investigated for two practical reasons. First, when very high sampling rates are achieved, the available time for measurement becomes too small, leading to loss in measurement precision. Also, it would become difficult to verify if the i.c.p. is actually accompanying the high-speed flow injection system because of the uncertainties in the very low ΔI values, which depended on manual operation.

In Fig. 2, the sample zone distribution near the f.i.a.—i.c.p. connection depends on Δt while the measured peak profile is ΔI-dependent. Since the ΔI value is equal to the Δt value minus a constant time interval required for transferring the operator starting command to the spectrometer (which includes the pre-burn time), the two curves in Fig. 2 are not superimposed. The measured peak profile can, therefore, be displaced leftwards by employing longer pre-burn times. It is good practice to adjust the pre-burn time so that the ΔI value is zero, i.e., the sample injection and starting command for the i.c.p. are simultaneous.

For the f.i.a.—i.c.p. system indicated in Fig. 1B, a measurement time interval of 10 s was chosen, which permits the measurement of more than 80% of the total peak area (Fig. 2), if its middle portion is selected. Figure 2 indicates that this situation is attained if a ΔI value of zero and a pre-burn time of 8 s are employed. Parallel experiments confirmed this: when pre-burn times of 2 and 10 s were employed, decreases in measured signal of 11% and 42%, relative to that using the 8 s pre-burn time, were observed.

The effects of mixing coil length and injected sample volume are summarized in Table 3. Data related to the 40-cm coil are approximate because the measurement precision deteriorated when this coil was employed. Sometimes, relative standard deviations of measurements in the range 10—20% were found. This happened mainly because the corresponding flow injection systems were faster, creating difficulties in defining the ΔI values. Negative ΔI values mean that the command for i.c.p. operation is done before sample injection. All data in Table 3 are in agreement with expected values [10].

The dispersion factor normally reflects the maximum concentration of the sample zone relative to the original sample concentration [7]. In the f.i.a.—i.c.p. system, the integrated signal represents an averaged measurement which is always lower than the maximum peak height. So, for the f.i.a.—i.c.p. system, the dispersion factor does not have the same importance as in other flow injection systems in which peak heights are considered for analytical purposes. In fact, as the measurement time interval increases, the f.i.a.—i.c.p.

TABLE 3

Effects of mixing coil length and injected volumes. Data refer to system A

Mixing coil length (cm)	Sample loop (cm)	Parameters of the measured peak			
		Half peak width (s)	Dispersion factor	Wash time (s)	ΔI_{max}[a] (s)
40	5	3	0.21	≈10	−4
	20	6	0.26	≈10	−2
	100	12	0.93	>10	4
140	5	10	0.09	12	8
	20	11	0.18	14	8
	100	13	0.78	16	13

[a]The time interval between the instant of sample injection and the measurement of the most concentrated portion of the sample zone.

dispersion factor becomes smaller even though the physical sample dispersion is the same.

Table 3 indicates that when the longer 140-cm mixing coil was used, the half peak width became less dependent on the injected sample volume. In f.i.a. systems with longer mixing coils (high sample dispersion), the sample loop can be replaced at will without the necessity of redefining the measurement time interval in the i.c.p.

Table 4 indicates the precision and accuracies of the proposed f.i.a.—i.c.p.e.s. method for plant analysis. It can be seen that, when the measurement is not a limiting factor in precision (i.e., when precisions indicated in Table 4 are better), the measurements obtained with the combined system tend to be more reproducible than those related to the normal i.c.p. spectrometry. This tendency was confirmed in the limestone analyses. The relative standard deviations of the calcium and magnesium measurements with the proposed system were 1.34% and 1.23%, while those related to the normal i.c.p. spectrometry were 3.30% and 1.76% respectively. It must be emphasized that these last two values do not include the effect of manual sample dilution in the reproducibility, since they are based on measurements performed in the same sample solution. The improvement in reproducibility could perhaps be explained as follows. In flow injection analysis, a continuous flow is always reaching the plasma torch and a nearly steady-state situation is attained, regardless of the presence or absence of the sample. In normal i.c.p. operation, with pneumatic sample aspiration, an air bubble is aspirated during the replacement of samples, which causes a sudden loss of the plasma steady-state situation. Also, the flow rate provided by a peristaltic pump is probably more stable than the pneumatic sample aspiration rate.

The f.i.a.—i.c.p. system in Fig. 1B is remarkably stable: after one hour of operation, the measured signals for one standard (100 ppm Ca plus

TABLE 4

Comparative results for NBS Orchard Leaves (SRM 1571). Data are given as % (K, Ca, Mg, P) or ppm (Fe, Al, Cu, Mn, Zn, B) of the elements in the plant samples. Numbers between brackets indicate the relative standard deviation

Element	F.i.a.—i.c.p.e.s.[a]	I.c.p.e.s.[b]	Certified values	Other values[c]		
				Ref. 20	Ref. 9	Ref. 21
K	1.48 ± 0.09 (6.0)	1.48 ± 0.04 (3.2)	1.47 ± 0.03	1.453	1.65 ± 0.08	1.60 ± 0.07
Ca	2.067 ± 0.076 (3.7)	2.146 ± 0.067 (3.1)	2.09 ± 0.03	2.084	2.10 ± 0.05	1.99 ± 0.07
Mg	0.586 ± 0.014 (1.4)	0.556 ± 0.018 (3.3)	0.62 ± 0.02	0.639	0.58 ± 0.02	0.62 ± 0.02
P	0.217 ± 0.004 (2.0)	0.206 ± 0.007 (3.4)	0.21 ± 0.01	0.193	0.189 ± 0.005	0.22 ± 0.005
Fe	334.3 ± 11.2 (3.3)	333.2 ± 7.9 (2.4)	300 ± 20	238.2	273 ± 18	183 ± 12
Al	225.1 ± 4.1 (1.8)	207.9 ± 9.0 (4.4)	—	201	—	234 ± 79
Cu	13.7 ± 0.2 (1.7)	13.3 ± 0.2 (1.8)	12 ± 1	13.01	14.2 ± 1.9	17 ± 4
Mn	93.8 ± 3.3 (3.6)	96.4 ± 2.5 (2.7)	91 ± 4	88.21	90.8 ± 2.2	101 ± 2
Zn	25.9 ± 1.0 (4.0)	25.5 ± 0.7 (2.9)	25 ± 3	25.74	27.9 ± 2.2	28 ± 5
B	32.1 ± 0.6 (1.8)	32.7 ± 0.7 (2.1)	33 ± 3	34.31	37 ± 10	41 ± 7

[a]Proposed method. [b]Normal i.c.p. spectrometry. [c]Other authors using i.c.p.e.s.

TABLE 5

Comparison of procedures for determination of calcium and magnesium in limestone samples. The percentages of the elements in the rocks are given

Sample	Ca		Mg	
	a	b	a	b
1	8.42	8.48	5.63	5.54
2	12.28	12.05	2.11	2.06
3	14.14	14.17	8.07	7.98
4	3.19	3.33	2.36	2.42
5	10.81	10.04	6.12	6.16
6	10.95	11.34	6.44	6.82
7	4.33	4.50	2.55	2.64
8	9.08	9.31	5.83	5.77
9	9.29	9.88	6.57	6.79
10	5.89	6.13	2.84	2.92

[a]Proposed method. [b]Normal i.c.p.e.s.

100 ppm Mg in 0.5 M HCl) underwent variations of only about 1% for calcium and 0.7% for magnesium. The linearities of the response curves in the 0—500 ppm range were verified for both calcium and magnesium, with calculated linear regression coefficients of 0.99991 and 0.99975 (N = 10), respectively. The results obtained for limestone with the f.i.a.—i.c.p. spectrometry compare well with those obtained by i.c.p. normal spectrometry (Table 5). Slight variations between results are probably caused by errors in the manual dilution of the samples, rather than differences in the measurement procedures.

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ATOMIC ABSORPTION SPECTROMETRY OF SELENIUM IN AN ARGON—HYDROGEN FLAME AFTER ELECTROCHEMICAL PRECONCENTRATION

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SUMMARY

Different methods of atomization of selenium from a platinum wire filament are described, including electrothermal heating of the filament and atomization in an argon—hydrogen flame. A combination of these approaches proved to give the best sensitivity and detection limit. The selenium was preconcentrated on the filament by electrolysis prior to the atomic absorption measurements to eliminate chemical interferences and improve the sensitivity of the method. The detection limit was 0.5 $\mu g\ l^{-1}$ for a 5-min electrolysis, and 0.1 $\mu g\ l^{-1}$ when the electrolysis time was 30 min.

The determination of selenium in the low-ppb range is impossible by direct flame atomic absorption spectrometry, owing to the poor sensitivity for this element. Instead, methods based on graphite furnace atomization and hydride generation are usually employed for determination of traces of selenium. However, for more complex matrices, atomization in a graphite furnace may lead to serious interferences, even if selenium is stabilized with a suitable metal [1—3]. Fewer interferences are encountered when the hydride generation technique is used, but even this technique suffers from interference, particularly in presence of certain elements which form volatile hydrides or stable selenides [4—7].

As an alternative to the above-mentioned techniques, an electrolytic preconcentration on a platinum wire, followed by atomization in an air—acetylene flame can be used [8, 9]. However, the air—acetylene flame gives rise to a high background absorption in the region of the selenium line and a significant improvement in detection limit was therefore expected if the low-absorption argon—hydrogen flame could be used instead. In addition, the electrolytic preconcentration step would eliminate the chemical interferences which are often encountered in this flame, because of its low temperature.

In this work different methods of atomization of selenium from the platinum wire filament were investigated, including atomization in an argon—hydrogen flame and electrothermal heating of the filament. A combination of these approaches proved to give the best sensitivity and detection limit.

EXPERIMENTAL

Equipment and solutions

A Perkin-Elmer 303 atomic absorption spectrometer and a Perkin-Elmer 159 recorder were used. The instrument was equipped with a deuterium background corrector, an electrodeless discharge lamp and a three-slot burner head. The selenium was measured at the resonance line 196.1 nm.

In most experiments an argon—hydrogen flame was used. Argon entered by the normal air inlet and hydrogen by the fuel-gas inlet, while the auxiliary inlet was shut. The flow rate of hydrogen was measured with a Tri-Flat Flowmeter (Fischer and Porter; tube 1/8-12-G-5/36, float SS-18). A quartz absorption tube with inner diameter 12 mm, outer diameter 15 mm, length 100 mm, and a circular hole midway along the wall, was placed in the optical path with the central axis 27.5 mm above the burner head. The tube rested on nickel supports. In some experiments the quartz tube was heated electrically by means of a Kanthal A wire coiled around the tube and connected to a transformer. The temperature in the tube was measured with a thermocouple.

The design of the filament electrode is shown in Fig. 1. The filament consisted of a spiral-wound 0.5-mm platinum wire, with a total length of 10 cm, which was spot-welded onto 1.0-mm platinum support wires. It was mounted in a holder similar to that used for the Delves cup technique. The filament was heated electrically; a transformer and a Variac were connected to the filament electrode via a microswitch, which was mounted on the Delves cup holder. The arrangement allowed the current to pass only when the filament was correctly positioned in the flame just below the hole in the absorption tube. The hole in the tube wall was made slightly larger than the size of the filament. The complete arrangement is shown in Fig. 2. The temperature of the filament was measured with an optical pyrometer.

The selenium was deposited on the filament by controlled potential electrolysis using a home-made potentiostat. The filament served as the working electrode, an Ag/AgCl/sat. KCl electrode (Metrohm EA427) was

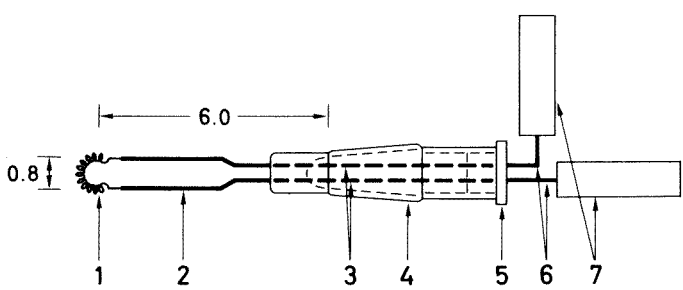

Fig. 1. Filament electrode (dimensions are in cm): 1, Pt filament, 0.5 mm wire; 2, Pt support wires, 1 mm; 3, Pt—Cu junction; 4, B 14/23 joint; 5, teflon stopper; 6, Cu wires, 1 mm; 7, banana jack.

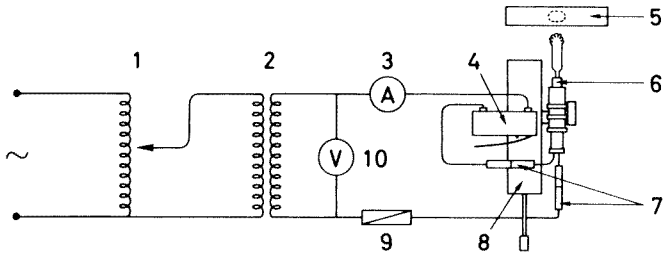

Fig. 2. Arrangement for electrothermal heating of the filament: 1, Variac; 2, transformer; 3, amperometer; 4, microswitch; 5, quartz absorption tube; 6, filament electrode; 7, banana jacks and plugs; 8, electrode holder; 9, 10-A fuse; 10, voltmeter.

used as the reference electrode, and the counter electrode was a platinum coil. A Metrohm EA880 vessel was used as electrolysis cell, and the solution was stirred with a constant-rate (500 rpm) magnetic stirrer during the electrolysis.

Selenium(IV) solutions were prepared by diluting analytical-grade selenous acid. The supporting electrolyte was usually 0.09 M (1:200) sulphuric acid (Suprapur, Merck).

Procedure

Electrolyze a 25-ml aliquot with efficient stirring for 5 min at −0.2 V vs. Ag/AgCl (or use another suitable potential). Remove the filament electrode from the cell, and rinse it with water and acetone before disconnecting the electrical circuit. Adjust the flow rates of the flame gases to 11 l min^{-1} for argon and 2.4 l min^{-1} for hydrogen, and ignite the flame. Let the filament dry for 30 s above the flame, ca. 10 cm above the burner (temperature 70–80°C). Place the filament electrode in the holder, and connect it to the transformer. Move the filament into its pre-adjusted position in the flame, directly below the hole in the absorption tube, and record the atomic absorption signal at 196.1 nm, as the microswitch triggers the electrothermal heating of the filament.

The heating power to the filament should be as high as possible, without risking melting of the filament. For the filament used in this work, the heating power was 41.2 W (4.05 V, 10.18 A).

RESULTS AND DISCUSSION

The determination of selenium by flame atomic absorption spectrometry after electrochemical preconcentration on a platinum wire filament has already been described [8, 9]. However, the air–acetylene flame used in the previous work had the disadvantage, in determinations of selenium, that it absorbed at wavelengths below 200 nm. The detection limit was restricted by this background absorption, which gave rise to a noisy baseline. The baseline was also shifted when the filament was moved into the flame,

although background correction was used [9]. The absorption peak obtained in the air—acetylene flame was non-symmetrical, with some tailing, and at high concentrations even double peaks could be observed. Owing to the relatively high temperature of the flame, the surface of the quartz tube was seen to deteriorate with time, and this gave rise to more pronounced tailing of the peak.

Alternative methods of atomization were therefore investigated. To avoid a high background absorption, the use of an argon—hydrogen flame was studied first. In contrast to the air—acetylene flame, it was found that the temperature of the argon—hydrogen flame was insufficient for direct atomization of selenium from the platinum filament. Electrical heating of the filament was therefore introduced, in addition to the flame atomization. With this arrangement, a well-defined symmetrical absorption peak with no tailing was obtained. The sensitivity was found to be twice as good as that of the air—acetylene flame atomization. Furthermore, background correction was no longer needed, because a very stable baseline with little drift and noise was obtained. The baseline did not shift when the filament was moved into the flame.

Effect of electrothermal heating

As already mentioned, no signal was obtained when the filament with selenium was moved directly into the argon—hydrogen flame (except at high concentrations of selenium, see below). Therefore, the filament was heated by passing an electric current through the wire, simultaneously with the flame atomization. The electrical heating was controlled by a microswitch, which allowed the current to pass only when the filament was correctly positioned in the flame just below the hole in the absorption tube. The microswitch ensured reproducible heating of the filament, protected it from being damaged by oxidation, and also prevented it becoming overheated and melting when it was removed from the flame.

The variation of the atomic absorption signal with the filament temperature was studied by recording the signal as a function of the electrical power applied to the filament. The results are shown in Fig. 3. It can be seen that the peak height increases markedly with the filament temperature. At the highest power used, the temperature was very close to the melting point of platinum; only a slight variation in the power would cause the filament to melt. Therefore, a somewhat lower power (41.2 W) was chosen as the standard condition for heating. The corresponding filament temperature was measured as 1680°C in absence of the flame.

Although the flame temperature was much lower than the temperature of the filament, the flame was still found to be essential for the atomization process to occur. Thus, no atomic absorption signal for selenium was obtained in absence of the flame, irrespective of the filament temperature, in contrast to the results previously obtained for cadmium [10]. The reason for this is probably that selenium forms polyatomic molecules in the colder vicinity of

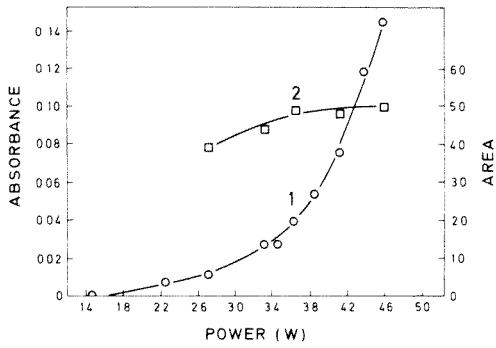

Fig. 3. Variation in peak height (curve 1) and peak area (curve 2) with the electrical power applied to the filament. Selenium(IV) (20 μg l^{-1}) in 0.09 M sulphuric acid, 5 min electrolysis time, deposition potential —0.8 V.

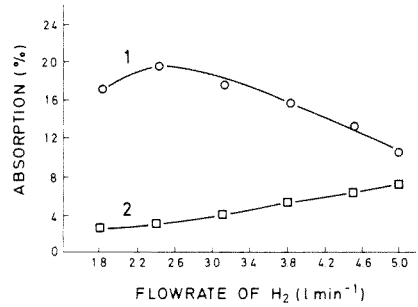

Fig. 4. Variation in peak height (curve 1) and flame absorption (curve 2) with the flow rate of hydrogen. Argon flow 11 l min^{-1}, 40 μg Se l^{-1} in 0.09 M sulphuric acid, 2 min electrolysis time, deposition potential —0.2 V.

the filament, in absence of the flame. From Fig. 3 it can be seen that the area of the atomic absorption signal was fairly constant for the higher powers applied, which indicates that the total amount of selenium atomized was nearly constant too, for this part of the curve. However, the peak width decreased markedly with increasing powers, indicating that the rate of atomization increases with the temperature.

The relatively high temperature needed for the atomization of selenium is explained by the formation of non-volatile platinum selenide at the filament surface. However, when larger amounts of selenium were deposited on the filament, some of the selenium was found to be present in an unstabilized form. This unstabilized selenium could be atomized in the argon—hydrogen flame, without electrothermal heating of the wire. Thus, when a solution containing 30 μg Se l^{-1} was electrolyzed for 5 min, it was found that 10% of the deposited selenium was lost within 30 s, when the filament was moved into the flame. Further, when selenium was deposited for 5 min from a 100 μg l^{-1} solution, the flame atomization resulted in multiple absorption peaks of selenium, in absence of electrothermal heating. The amounts of selenium deposited in these experiments were below that required for a monolayer coverage of the filament. However, the unstabilized selenium was probably deposited on active surface sites which were already covered with platinum selenide.

The use of a microswitch-controlled electrothermal heating of the wire ensured that both stabilized and unstabilized selenium were atomized simultaneously when the filament was moved into the flame. A single well-defined symmetrical absorption peak was then obtained, irrespective of the chemical form of the selenium present at the filament surface.

Effect of gas flow

The height of the atomic absorption signal was found to depend on the flow rate of the flame gases. Thus, the peak height increased when the flow rates were decreased below the values recommended in the instrumental manual. However, a minimum flow rate of 11 l min^{-1} for argon was needed in order to ensure a stable baseline. Atomic absorption signals were then recorded at different flow rates of hydrogen; the results are shown in Fig. 4. The maximum peak height was obtained at 2.4 l min^{-1}; a minimum hydrogen flow rate of 1.8 l min^{-1} was needed for the flame to burn.

From Fig. 4 it can also be seen that the background absorption of the flame increases with the flow rate of hydrogen, probably because of increased formation of water vapour. At a hydrogen flow of 2.4 l min^{-1} the background absorption amounts to ca. 3% only, which is low compared to the 12—13% absorption normally found for this type of flame at 196 nm [11]. The low absorption is explained by the low flow rates used, and the presence of the quartz absorption tube above the filament. In absence of the quartz tube the absorption was found to be ca. 8%.

Effects of the absorption tube

In addition to the positive effect upon the background absorption mentioned above, the quartz tube increases the residence time of the selenium vapour in the optical path. In agreement with this, the sensitivity obtained with the quartz tube was 5 times better than that observed in absence of the tube. The height of the quartz tube above the burner head was found to be of less importance. Maximum absorption was obtained when the tube was placed in the upper part of the flame, because of the higher temperature in this region. A distance of 27.5 mm from the burner head to the central axis of the tube was normally employed.

In contrast to the air—acetylene flame, the argon—hydrogen flame did not cause a visible deterioration of the quartz tube, even after prolonged use, probably because of the much lower flame temperature.

Electrically heated absorption tube

With the technique described above there will be a significant temperature gradient from the hot filament to the cooler flame surrounding it. An improved sensitivity would, therefore, be expected if a higher temperature could be ensured for the atomic cloud. For this reason, electrical heating of the absorption tube was tested in combination with the argon—hydrogen flame. With the particular set-up employed a maximum temperature of 1080°C could be reached within the heated tube. Using this temperature, a 50% increase in the peak height was observed, but this was obtained at the expense of a noisier baseline. Also a separate power supply was required for the heating, which was rather a slow process. Therefore, no practical use was made of the electrically heated absorption tube. However, these experiments indicate that selenium is more efficiently atomized when the temperature of the selenium vapour is increased.

A few experiments were carried out with the electrically heated tube, in absence of the argon—hydrogen flame. Only the filament heating was maintained, in addition to an argon flow through the tube, to minimize background absorption. No signal was obtained for selenium in these experiments, probably because of the low temperature of the atomic cloud.

The electrolysis step

Controlled potential electrolysis is very effective for separation and preconcentration when used in combination with atomic absorption spectrometry [8]. By adjusting the deposition potential, the efficiency of the separation of a given element from the matrix can be optimized. The potential needed for the reduction of selenium(IV) depends to a certain extent on the composition of the sample solution. In diluted sulphuric acid, the reduction of selenium starts at +0.6 V vs. Ag/AgCl, as can be seen from Fig. 5. A deposition potential of —0.2 V vs. Ag/AgCl was mostly used in this work. The evolution of hydrogen, which took place at more negative potentials, probably accounts for the minimum in the curve around —0.4 V. At this potential the gas bubbles did not leave the electrode, with the result that the electrode surface was partly blocked.

The amount of selenium deposited on the electrode was found to be a linear function of the electrolysis time for deposition times below 30 min. The result indicates that depletion of the solution during the electrolysis was insignificant, because otherwise a curved relationship would have been observed. In other words, the preconcentration step was nearly nondestructive, and repetitive analysis on the same aliquot was therefore possible. Normally a 5-min electrolysis was used, to keep the total analysis time to a minimum (8—9 min). Obviously, a significant improvement in sensitivity and detection limit will be obtained if a longer deposition time is used.

The peak height was found to depend on the rate of stirring of the solution. The size of the magnetic bar and the rotation speed of the stirrer both had a pronounced effect on the peak height. Optimum stirring was obtained by using a 2.0-cm stirring bar and a rotation speed of 500 rpm.

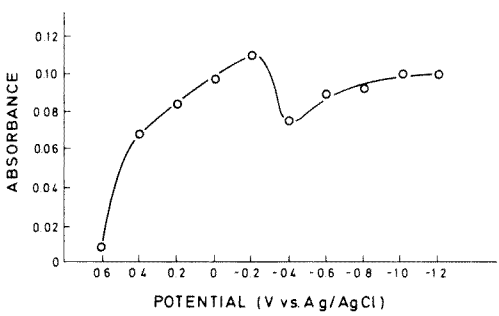

Fig. 5. Variation in peak height with deposition potential. Selenium(IV) (20 $\mu g\ l^{-1}$) in 0.09 M sulphuric acid, 5 min electrolysis time.

Sensitivity and interferences

The standard curve was found to be linear up to an absorbance of 0.18 (34% absorption). The curve was almost horizontal above an absorbance of 0.24 (43% absorption). Under optimized experimental conditions, the detection limit (signal-to-noise ratio 2:1) for a 5-min electrolysis time was found to be 0.5 $\mu g\ l^{-1}$. For a 30-min electrolysis, the corresponding value was 0.1 $\mu g\ l^{-1}$. The reciprocal sensitivity, expressed in $\mu g\ l^{-1}$ at 1%, was identical with these values. The values given above are a factor of 10 better than those obtained when the filament was heated in an air—acetylene flame [9]. The standard deviation of the method, including both the electrolysis and the atomization steps, was ca. 5%.

As already mentioned, the electrolytic preconcentration step eliminates most of the chemical interferences which may be encountered in the determination of selenium. Thus, a 1000-fold excess of iron and nickel proved to have no effect on the peak height. Only copper, which was deposited on the filament at −0.2 V, caused a 27% decrease in the signal, when present in a 1000-fold excess. Anions had no marked effect on the peak height. At −0.2 V even chloride did not interfere with the determination of selenium, because chlorine was only produced at the anode in insignificant amounts.

CONCLUSION

It has been shown that selenium(IV), after electrochemical preconcentration on a platinum wire filament, is best atomized in an argon—hydrogen flame with simultaneous electrothermal heating of the wire. A very low background is obtained in this way, because of the low-absorption flame used. The electrothermal heating of the wire, controlled by a microswitch, was found to be essential for the atomization process because of the low temperature of the argon—hydrogen flame and the formation of thermally stable platinum selenide at the filament surface. For maximum sensitivity, the filament should be heated to a temperature just below the melting point of platinum. No signal was observed with electrothermal atomization in absence of the flame.

The detection limit obtained for selenium, 0.5 $\mu g\ l^{-1}$ with a 5-min electrolysis, was a factor of 10 better than that found by atomization in an air—acetylene flame [9]. A further five-fold improvement in the detection limit was obtained for 30-min electrolysis times. A similar improvement in detection limit is to be expected for other elements which can be atomized at relatively low temperatures. The method should be particularly useful for the determination of trace elements in complex matrices. The determination of selenium in biological materials and in technical sulphuric acid will be the subject of later publications.

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INVESTIGATIONS OF REACTIONS INVOLVED IN ELECTROTHERMAL ATOMIC ABSORPTION PROCEDURES
Part 10. Factors Influencing the Determination of Arsenic

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SUMMARY

Factors of importance for the determination of arsenic by the graphite furnace technique are discussed with special reference to surface reactions. Detailed information with regard to atom formation was obtained by use of an on-line data acquisition system fast enough to monitor signals without distortion. The significance of the surface structure which comes into contact with the sample was assessed by application of the L'vov platform technique. Radioactive measurements showed that losses of arsenic occur even at temperatures as low as 120°C. The different behaviour of γ- and β-radiation was used to study the penetration of arsenic into the graphite. It is shown that arsenic can be stabilized up to 1800°C in standard graphite tubes if an auxiliary oxidizing agent is added. Conditions for stabilization with nickel and lanthanum are also discussed. Reported interference effects caused by chlorine and sulphur were examined experimentally and by use of high-temperature equilibrium calculations. Minimization of interference effects including optimal conditions for atomization are obtained by use of a stabilizing agent in combination with the platform technique.

The determination of arsenic by graphite-furnace atomic absorption spectrometry (g.f.a.a.s.) is associated with a number of difficulties. One of these originates from the fact that arsenic forms volatile compounds at low temperatures. It has been suggested that molecules like As_4, As_2 [1, 2] and AsO [3] are formed in graphite furnaces particularly at lower temperatures. By stabilizing arsenic with, for example, nickel [4], the formation of such volatile molecules is reduced. Arsenic is also known to form gaseous molecules with elements like chlorine and sulphur [5, 6]. Since these elements are frequently present in environmental samples, the determination of arsenic in such samples is rather problematic [7, 8] and for that reason separation techniques, especially extractions, have been suggested in order to eliminate the interference effects [9, 10].

Additional complications are caused by possible interactions of arsenic or arsenic compounds with the graphite. According to Nickel [11], who investi-

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gated the thermochemical behaviour of various elements in pressed graphite electrodes, arsenic does not form stable compounds (interlamellar compounds) with the graphite, and this finding is in agreement with the removal of atomic arsenic in graphite furnaces following a pure diffusion mechanism [12]. However, from several investigations it is apparent that graphite does interact with arsenic. L'vov and Pelieva [13] have found that arsenic is atomized at significantly lower temperatures (ΔT = 900°C) if a graphite tube is lined with a tantalum foil. These results indicate that the graphite is retarding the vaporization of arsenic. The existence of an arsenic compound with the general formula AsC_n has been proposed by L'vov who has calculated its heat of formation as 63 kcal mol^{-1} [14]. According to Rüdorf [15] and Henning [16], graphite forms salt-like compounds in the presence of oxygen acids according to

$$mC + 3\ H_nXO_4 \rightarrow C_mH_{n-1}XO_4 \times 2\ H_nXO_4 + H^+ + e^- \quad (1)$$

where X can be As, P, S or Se, n represents the number of hydrogen atoms in the molecule of acid, and m is the stage of the lamellar compound, i.e., the ratio of carbon layers to reactant layers. These compounds have no stoichiometric compositions and for the reaction of sulphuric acid with carbon, for example, m can approach 24. Kamegawa et al. [17] studied the adsorption of arsenite and arsenate ions on activated carbon. They found that only arsenate was absorbed on the graphite. Arsenite was not adsorbed but was easily oxidized by dissolved oxygen on the activated carbon. Carbon can be activated by sodium hydroxide treatment [18] or by steam [17]. This means that the graphite of the tubes used in a.a.s. would be active if aqueous solutions are analyzed. The presence of dissolved oxygen in graphite furnaces has recently been proposed by Salmon et al. [19]. Possible actions of graphite will be superimposed on other interference effects obtained.

The majority of papers dealing with arsenic determinations have described only effects caused by a number of matrix constituents. In the work described below, attempts were made to differentiate between the various parameters influencing the a.a.s. signals. The role of the graphite surface was investigated by employing the platform technique [14] which makes it possible to study the single effect of the surface in contact with the sample, independent of the atomizer material. An additional advantage inherent with the platform technique is that samples are vaporized into an environment of relatively high and constant temperature. This is advantageous for an effective decomposition of the arsenic species. Losses of arsenic during thermal pretreatment of the tube as well as the degree of penetration of arsenic compounds into the graphite, were monitored by using radioactive measurements. In addition, an on-line data acquisition system was used to obtain information from the shape of the arsenic signals [20].

EXPERIMENTAL

Instrumentation

Some of the experiments were made with a Perkin-Elmer atomic absorption spectrometer Model 372, provided with background correction and fitted with an HGA 74 furnace. The furnace was connected to a home-made power supply. Close temperature control of the graphite tube was achieved with an optical feed-back system, the principle of which was described earlier [21]. A Perkin-Elmer automatic sampler (Model AS-1) was used to dispense 20 μl of sample solutions. To the recorder output of the spectrometer a peak reader module was connected [22], providing simultaneous recording of the peak height and the peak area.

The remaining experiments were performed with a Varian—Techtron AA-6 atomic absorption spectrometer, provided with background correction and fitted with an HGA 74 furnace. In order to obtain a faster response time of the electronics, the value of the DAMP A time constant was altered from the original 260 ms to 10 ms, as described earlier [23]. To be able to study the analytical signals in more detail, a fast on-line data acquisition system was connected to the spectrometer [20]. After data processing, the atomic absorption as well as the temperature signals were plotted on a X-Y recorder.

The equipment for radioactive measurements consisted of a 45 × 50-mm NaI(Tl) well-type scintillation detector (Berthold, SZ 44/50-W-N) for γ-radiation or a methane-filled proportional counter (Frieseke and Hoepfner) for β-radiation connected to a high-voltage power supply (Berthold, LB 2210), a linear amplifier with integral discriminator (Berthold, LB 2220), and a scaler-timer unit (Berthold, BF 2270-1).

Temperature settings referring to the inner surface of the graphite tube were calibrated with a NiCr—Ni thermocouple (below 1000°C) and with an optical pyrometer (Keller Spezialtechnik Pyro Werk GmbH, Model PB06AF3) or a PtRh—Pt thermocouple for higher temperatures. The instrumental parameters are summarized in Table 1. Background correction was used for experiments with sodium sulphate.

TABLE 1

Instrumental parameters

	Time (s)	Temp. (°C)	Heating rate (°C s^{-1})
Drying	25[a]; 40[b]	100[a]; 120[b]	5
Ashing	25[a]; 35[b]	500	50
Atomization[c]	6[d]; 14[e]	2550	1900[d]; 1600[e]
Cleaning	3	Max	—
Wavelength (nm)		193.7	
Spectral bandwidth (nm)		0.7	
Lamp source		EDL (8 W)[d]; EDL (8 W)[e]	
Argon flow (l min^{-1})		0.25 internal; 1.6 external	

[a]Without platform. [b]With platform. [c]Temperatures given for standard tubes; for glassy carbon tubes the same instrumental settings were used. [d]Varian AA-6. [e]Perkin-Elmer 372.

Reagents and materials

A 1000 μg ml^{-1} stock solution of arsenic was prepared from arsenic trioxide (p.a., Riedel—de Haën) and acidified to 0.1 M with hydrochloric acid. Standard solutions were prepared weekly by dilution with distilled water and were stored in acid-washed polyethylene bottles. The radioactive stock solution of ^{74}As used contained 0.2 μg As ml^{-1} ($t_{1/2}$ = 18 d) in 0.04 M HCl in the form of carrier-free arsenic acid (Radiochemical Centre, Amersham). A 0.2 ng As ml^{-1} radioactive standard solution was prepared by dilution with distilled water and was used in all experiments. A 5% (w/w) stock solution of nickel was made from nickel nitrate and a 1.6% (w/w) solution of lanthanum from lanthanum nitrate. All gases used were of SR-grade purity, and the chemicals were of analytical grade.

Glassy carbon (Tokai Lux Ore and Chemical, Tokyo) tubes manufactured in this department were used in some experiments; in all others, standard graphite tubes (Perkin-Elmer) were used.

Determinations with the platform technique

Platforms of rectangular shape were manufactured from standard or glassy carbon tubes. The platforms were 8 mm long, 5 mm wide and 1 mm thick; in order to minimize heating by conduction from the furnace walls, cuts were made along the lateral edges of the platform so that only 1 mm in each corner was in contact with the wall. The platform was installed in the middle of the tube and the autosampler was used to pipette the solutions. The platforms were cleaned before use by heating three times at 2550°C for 10 s.

Procedure for radioactive measurements

Standard ^{74}As solution (20 μl) was injected on a platform installed in the middle of the tube and was thermally treated ("ashed") at different temperatures, 90 s at each temperature. After each such 90-s ashing step, the platform as well as the tube were removed and the amount of ^{74}As left was measured. Care was taken in positioning the graphite parts in a reproducible way in the detectors in order to ensure a similar geometry for all counts.

When γ-radiation was measured, relative losses of ^{74}As at different temperatures were compared with the undried droplet. However, when γ- and β-radiation were both measured, relative losses of ^{74}As were compared with the amount of ^{74}As left after a drying step at 120°C for 60 s.

Measurements of molecular absorption by As_2

The As_2 absorption was measured by using the emission lines of a nickel hollow-cathode lamp at 225.386 and 227.021 nm. Absorption bands for As_2 at 225.40 and 227.96 nm have been reported by Pearse and Gaydon [24]. Arsenic (20 μg) was vaporized at isothermal conditions from a tungsten wire [25] or from graphite pieces [26]. All values reported have been corrected for the absorbance caused by blank solutions.

TABLE 2

Species considered in the equilibrium calculations

Gaseous: As_2, As, $AsCl_3$, AsH_3, AsS, Ar, C, CH_2, CCl_2, CO_2, CO, CS, COCl, HCO, Cl_2, Cl, HCl, H_2, H, H_2O, H_2S, O_2, O, SO, SO_2, S_2, S
Condensed: As, $AsCl_3$, As_2O_3, As_2S_3, C

High-temperature equilibrium calculations

The calculations were performed as described in Part 1 [27]. The elements and compounds considered are given in Table 2. For most arsenic compounds, data are available only up to 1200 K [5], and extrapolated values were therefore used for higher temperatures.

RESULTS AND DISCUSSION

Characterization of surface effects

Signals obtained for glassy carbon and ordinary graphite tubes are shown in Fig. 1. It should be emphasized that all results were registered using an on-line data acquisition system [20] fast enough to monitor the signals without distortion. As can be seen, the appearance temperature for arsenic is 1100°C for both tubes. Furthermore, the peak maxima coincide. In two aspects, the signals differ greatly depending on the type of graphite. First,

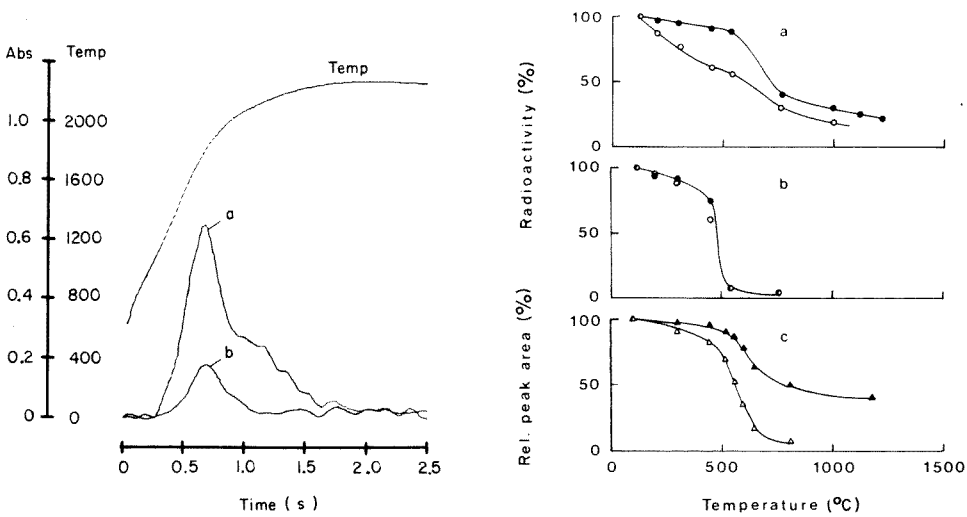


Fig. 1. Smoothed signal transients for 20 μl of 0.2 mg As l^{-1} in water: (a) ordinary graphite tube; (b) glassy carbon tube.

Fig. 2. Ashing curves for arsenic using platforms and tubes of ordinary graphite [a (•, ○); c (▲)] and glassy carbon [b (•, ○); c (△)]. (a, b) The relative amounts of ^{74}As left when β (○) and γ (•) radiation was measured. (c) The atomic absorbance signals for 20 μl of 1 mg As l^{-1} solution obtained after pretreatment at the temperature shown.

the free atom concentration is much lower in the glassy carbon tube. Secondly, the tailing of the signal which takes place in the ordinary graphite tube (a) indicates that some retention process is involved in the dissipation of the atoms. During these experiments, it was noticed that the sensitivity increased with the age of the tube as well as with larger contact area between graphite and sample solution. However, these problems could be almost overcome by use of the platform technique [14].

The reason for the discrepancy in efficiency of atom formation between glassy carbon and ordinary graphite was further investigated by radioactive measurements. The upper curve of Fig. 2(a) shows the relative amounts of ^{74}As left on an ordinary graphite platform after pretreatment at various temperatures when γ-radiation was measured. The results indicate that losses of analyte occur at temperatures much lower than the appearance temperature. The lower curve of Fig. 2(a) was obtained by measuring β-radiation, which is shielded by graphite. This means that the difference in the relative activity of the two curves is a measure of the fraction of ^{74}As which had penetrated into the graphite. The relative amounts of ^{74}As left using glassy carbon (see Fig. 2b) show that this material is less efficient in retaining this element and penetration is not observed. The ashing curves for atomic absorption measurements given in Fig. 2(c) are in line with the results presented in the upper diagrams of the figure. The different abilities of glassy carbon compared to ordinary graphite in stabilizing arsenic can be used to explain the larger signals obtained with ordinary graphite, since for glassy carbon a larger fraction of the analyte is likely to be lost as molecules before a sufficiently high temperature is reached for their decomposition. The differences in γ- and β-radiation (Fig. 2a) indicate that compounds between graphite and arsenic have been formed.

These observations are in agreement with the findings of Henning [16], who reported that arsenic acid forms electrolytic lamellar compounds with graphite in the presence of oxygen and hydrogen (see eqn. 1). At low temperatures, oxygen is chemisorbed on graphite and forms carbon—oxygen complexes providing active sites for attracting arsenic. Hydrogen must be present in order to stabilize the graphite—arsenate compounds created. The formation of active sites is catalyzed by water and this means that the surface of ordinary graphite tubes should provide conditions for intercalation if water-containing samples are analyzed. The tenacity with which water is held on the graphite has been investigated earlier [28]. However, water as well as adsorbed oxygen are removed stepwise from the graphite with increase of the temperature [29] which means that interlamellar arsenic compounds become more unstable. The optimum temperature for the adsorption of oxygen is reported to be 500°C [19]. It is interesting to note that at this temperature the relative difference between γ- and β-radiation measurements (Fig. 2a) is greatest.

As can be seen from Fig. 2(a) and (c), a small fraction of arsenic is not removed from the ordinary graphite platform even at temperatures above

1200°C. From a.a.s. measurements, it was noticed that significant amounts of arsenic were still left on the platform after atomization at 2550°C. For that reason a burnout step at maximum temperature had to be employed. It is unlikely that the described mechanism for the retention of interlamellar compounds has significance at such high temperatures. However, it is known [30] that electrolytic lamellar compounds can be converted to rather stable residual lamellar compounds which are formed at crystal imperfections of the graphite where the reactant can be strongly bound by free valencies. Such a change is likely to be the reason for the retention of arsenic at high temperatures.

The existence of such thermally stable graphite—arsenate compounds was investigated further by using radioactive measurements; the results are given in Table 3. At 1400°C there is still 16.7% ^{74}As left on the platform (ordinary graphite). As also can be seen from Table 3, arsenic is evaporated from the platform and partially retained on the tube wall. It should be noted that the total amount of arsenic left when ordinary graphite is used after pretreatment at 1400°C is as much as 45%. Therefore it is evident that, to a certain degree, adsorption and re-evaporation processes must be involved during the atomization sequence.

The absence of retention of the evaporated arsenic on the glassy carbon tube shows that the conditions for compound formation are not fulfilled on such inactive surfaces. The results given in Table 3 and Fig. 2 make it possible to explain the somewhat drawn-out signal shape obtained with ordinary graphite tubes. The nature of the graphite surface is important for the formation of arsenic atoms in two respects: first, arsenic is stabilized on the surface where the sample has been applied; secondly, during ashing and atomization the evaporated arsenic or arsenic species can react again with the graphite surface. A quantitative measure of these two types of arsenic retention can be established from the results given in Table 4, because glassy

TABLE 3

The relative amounts of ^{74}As left after pretreatment for 90 s at different platform temperatures[a]

	Undried sample	Temperature (°C)				
		120	540	790	1120	1400
Glassy carbon						
Platform	100	68.9	60.9	8.6	6.7	<2
Tube	<2	<2	<2	6.3	<2	<2
Ordinary carbon						
Platform	100	90.5	71.0	42.0	30.0	16.7
Tube	<2	7.6	15.2	35.4	42.7	28.0

[a]Platform temperatures were measured with a NiCr—Ni thermocouple. Aliquots (20 μl) of 0.2 μg As l^{-1} solution were used.

TABLE 4

The effect of graphite surface on the signal of arsenic[a]

	Peak height (A)		Peak area (A × s)	
	Ordinary tube	Glassy carbon tube	Ordinary tube	Glassy carbon tube
Ordinary platform	0.551 ± 0.012	0.636 ± 0.003	1.071 ± 0.028	1.086 ± 0.010
Glassy carbon platform	0.351 ± 0.018	0.436 ± 0.012	0.511 ± 0.029	0.500 ± 0.017

[a]Aliquots (20 μl) of 0.2 mg As l^{-1} solution were used. The results are the means of 5—6 determinations with standard deviations.

carbon was found not to react with the evaporated arsenic species (see Table 3). As can be seen, the nature of the graphite surface on which the sample is applied is the most crucial factor. Glassy carbon and ordinary graphite represent two extremes with regard to their reactivity. In separate experiments, it was found that even smaller alterations in graphite properties bring about changes in the arsenic signals.

Condensed phase interference effects

As can be concluded from the above results, the number of active sites on the graphite available for reaction with arsenic strongly affects the measured absorbance signal. The availability of active sites is influenced by the surface properties of graphite as well as by the presence of other species in the atomizer. The species might change the surface characteristics by interaction with the graphite, or react in a similar way as arsenate, or create available active sites. In practical work it is difficult to distinguish clearly between the different ways in which the number of available active sites might be changed because the different mechanisms are interrelated. For example, nitric acid is known to increase the distance between the graphite layers and at the same time it creates more available active sites. In consequence, higher arsenic signals are obtained in the presence of this acid [31]. Another example comprises the lower atomic absorption signals obtained in the presence of phosphoric acid. This signal depression can be explained by the results given in Fig. 3; ^{74}As is removed to a greater extent compared to water if phosphoric acid is present. The reason for this might be that the chemically similar arsenic and phosphorus are competitors (see eqn. 1) so that the formation of interlamellar arsenic compounds with graphite is prevented. The difference between the lower curves, which should be a measure of the amount of arsenic incorporated in the graphite, compares favourably with the results shown in Fig. 2(a). In some experiments with potential gas phase interferences (see below), it was found that the effects caused by nitrogen should be related to the condensed rather than to the gaseous phase. As can be seen in Table 5, which represents results obtained with the same graphite

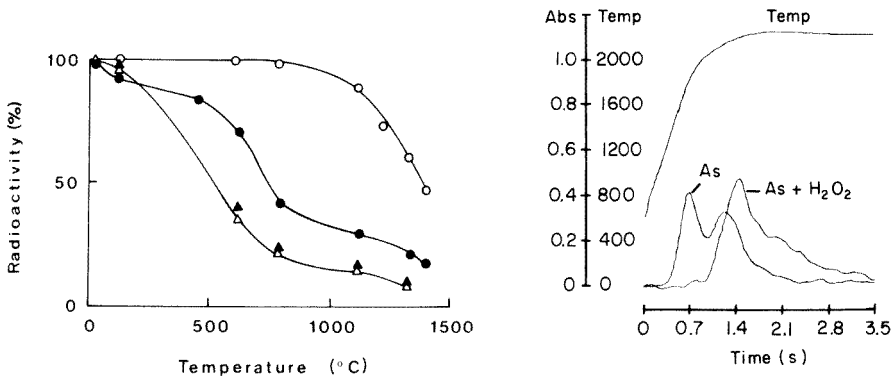


Fig. 3. Relative losses of ^{74}As as a function of temperature: (•) in water; (△) in H_3PO_4 (0.03%); (▲) in H_3PO_4 and La (3.2 μg); (○) in H_3PO_4 and Ni (10 μg).

Fig. 4. Smoothed signal transients for 10 μl of 0.2 mg As l^{-1} in H_2O and in 15% H_2O_2.

tube but with the two different types of platforms, interference effects were obtained only when the ordinary graphite platform was used. It can therefore be concluded that the formation of AsN(g), as proposed by L'vov and Pelieva [32], is not likely to be the reason for the observed interference effect in the presence of excess of nitrogen.

Stabilization of arsenic with oxidizing agents

As mentioned above, arsenic can be stabilized by graphite provided that an auxiliary oxidizing agent is present. A separate study of this is illustrated in Figs. 4 and 5. The former shows the transient signal of arsenic in water as well as in the presence of hydrogen peroxide. In aqueous solution, arsenic atoms are already formed at 1200°C, but much higher temperatures are required for complete atomization of the analyte. In the presence of hydrogen peroxide, the arsenic signal does not commence until a tube wall temperature of about 1800°C is reached, which means that almost ideal conditions for atom formation are attained. This observation is in line with results from radioactive measurements shown in Fig. 5. Other oxidizing agents like nitric acid, potassium permanganate or gaseous oxygen added to

TABLE 5

Dependence of the relative absorbance signals on the type of inert gas[a]

	Peak height (%)		Peak area (%)	
Platform	Ar	N_2	Ar	N_2
Glassy carbon	100	97.8	100	102.9
Ordinary	100	82.6	100	87.7

[a]An ordinary graphite tube and 20 μl of 0.2 mg As l^{-1} solutions were used.

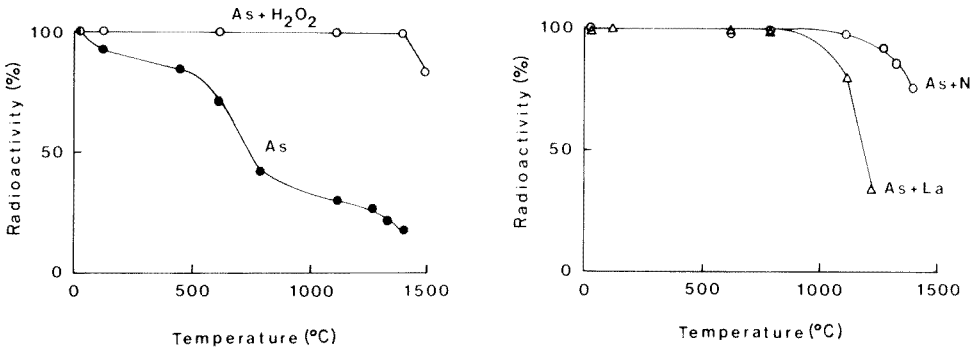


Fig. 5. Relative losses of ^{74}As in the presence of H_2O and 15% H_2O_2 as a function of temperature.

Fig. 6. Relative losses of ^{74}As in the presence of 3.2 μg of La and 10 μg of Ni, respectively, at different platform temperatures.

the purge gas caused similar effects. For example, in the presence of 0.3% nitric acid, the peak absorbance signal increased by 25%. The oxidizing agents were not effective in combination with glassy carbon.

Stabilization of arsenic with nickel and lanthanum

Another type of stabilization is caused by nickel or lanthanum. Table 6 shows that these elements are almost equally efficient in increasing the sensitivity for arsenic. However, with lanthanum, losses of arsenic begin at around 900°C (Fig. 6). This observation is in line with the signal transients presented in Fig. 7. The results given in Table 7 show that, in the presence of nickel, arsenic is retained independently of the type of graphite.

As lower analyte signals are obtained in the presence of phosphoric acid (see above), the effect of phosphoric acid on the stability gained in the presence of nickel or lanthanum was investigated. As can be seen in Fig. 3, phosphoric acid neutralizes the stabilizing effect of lanthanum, which means that compounds of arsenate are likely to be washed free by this acid. It should be observed that nickel additions can be used to eliminate the effects caused by phosphoric acid (compare Figs. 3 and 6).

TABLE 6

Relative increase of absorbance values for 2 ng of arsenic caused by the addition of 10 μg of nickel or 3.2 μg of lanthanum when a modified Varian—Techtron AA6 was used

	Peak height (%)	Peak area (%)
As + Ni	241	127
As + La	244	119
As	100	100

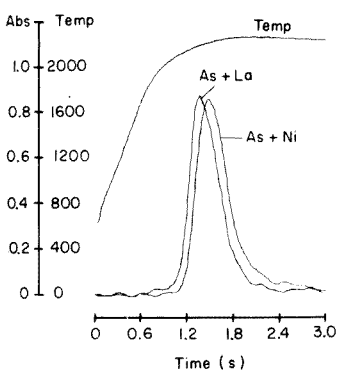
TABLE 7

Relative amounts of ^{74}As left in the presence of nickel when different platform temperatures and materials are used[a]

	Undried sample	Temperature (°C)				
		120	540	750	1120	1400
Glassy carbon						
platform	100	98.5	98.2	98.3	96.5	57.0
tube	2	2	2	2	2	2
Ordinary carbon						
platform	100	99.4	99.7	96.3	98.0	74.9
tube	2	2	2	2	2	7.0

[a]Platform temperatures were measured with a NiCr—Ni thermocouple. Aliquots (20 μl) of solutions containing 0.2 μg As l^{-1} and 500 mg Ni l^{-1} were used.

From these observations, it can be concluded that lanthanum and nickel stabilize arsenic by different mechanisms. In the presence of nickel, intermetallic compounds with arsenic, e.g., $Ni(AsO_3)_2 \cdot NiO$ [33], are likely to be formed. In contrast, lanthanum seems to stabilize the oxidizing centers on graphite and in consequence, the created interlamellar compounds are also stabilized.



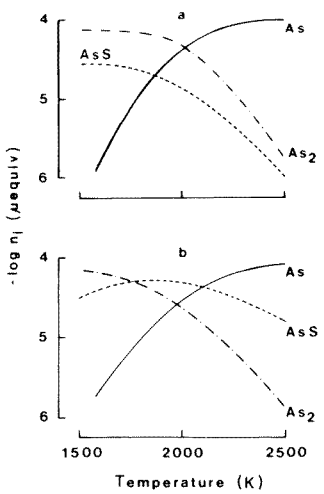


Fig. 7. Smoothed signal transients for 2 ng of As in the presence of La (3.2 μg) or Ni (10 μg).

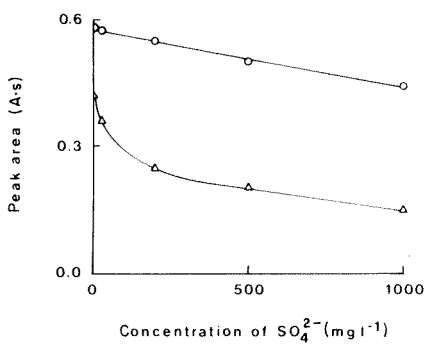
Fig. 8. Calculated distribution of arsenic species as a function of temperature for (a) $P_{O_2} = 10^{-20}$ atm and (b) $P_{O_2} = 10^{-12}$ atm. The input amounts used were: $S_2 = 5 \times 10^{-2}$ μmol, As = 10^{-4} μmol, H_2 = 0.2 μmol.

Gas-phase interference effects

Owing to the uncertainty of thermodynamic data for most of the gaseous arsenic compounds, it is impossible to obtain a survey of the gaseous arsenic system by use of high-temperature equilibrium calculations. However, the influence of some individual elements on the formation of arsenic atoms was examined. Sulphur and chlorine are known to form stable volatile compounds with arsenic, and some calculations including these elements were therefore done.

It was found that $AsCl_3(g)$ was the main arsenic compound for temperatures up to 2000 K, if chlorine is present in excess over arsenic and hydrogen. However, in the presence of excess of hydrogen the equilibrium partial pressure of chlorine will not be large enough at this temperature to cause the formation of significant amounts of $AsCl_3(g)$. Signal depressions of 4 ng of arsenic in the presence of 0.2% hydrochloric acid were found to be 23 and 49% for ordinary and glassy carbon platforms, respectively. As has been shown, the formation of hydrogen through the reaction between carbon and adsorbed water is larger in ordinary graphite tubes than in glassy carbon; this explains the difference obtained in the interference effects. Results from the measurements of the relative losses of ^{74}As in the presence and absence of 0.2% hydrochloric acid were identical. This indicates that the interference effects should be related to the gas phase.

Figure 8 shows the influence of sulphur on the distribution of arsenic species for two partial pressures of oxygen. As can be seen the interferences can be minimized by increasing the temperature of the environment into which the sample is vaporized. For such conditions the partial pressure of oxygen will be lower because of more complete reaction between the carbon surface and oxygen. The formation of AsS(g) will therefore be reduced (compare Fig. 8a and b). Vaporization at higher temperatures is possible with the platform technique. Figure 9 shows a comparison of interference



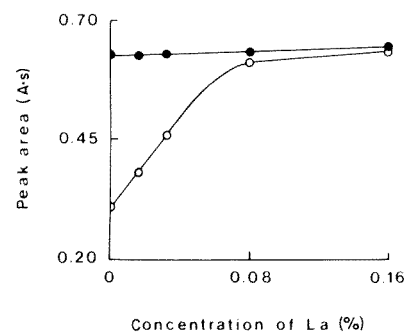


Fig. 9. Absorbance signals for 4 ng of As in the presence of different amounts of sodium sulphate measured with (○) and without (△) the platform.

Fig. 10. Absorbance signals for 4 ng of As in the presence of different amounts of La: (●) without sulphate; (○) with sulphate (500 mg l^{-1}).

effects caused by sodium sulphate with and without the platform. The platform clearly minimizes the signal depression, which is in accordance with theoretical predictions. Another way to prevent the formation of gaseous arsenic sulphide is to hinder simultaneous vaporization of arsenic and sulphur. This can be achieved by adding lanthanum, which is effective in stabilizing sulphur by forming lanthanum sulphide [34], as shown in Fig. 10.

The significance of the calculations was further investigated by utilizing molecular absorption spectrometry. The relative absorbance of diatomic arsenic was measured for different tube temperatures. The samples were vaporized from graphite or tungsten surfaces by introducing loaded graphite pieces or a tungsten wire into an ordinary graphite tube kept at constant temperature. Figure 11 shows the signals obtained for samples introduced by the tungsten wire. The peak area absorbance values were corrected for the variation in diffusion rate with temperature. The signals decreased at temperatures above 1700°C which compares favourably with the theoretical predictions given in Fig. 8(a). However, when samples were introduced on graphite pieces, diatomic arsenic was not formed in significant amounts. One reason for this might be that the system is far from an equilibrium state under these conditions or that all species which can influence the mass balance constraints have not been considered in the calculations.

If the diatomic arsenic is excluded from the calculations, the calculated ratio of AsS(g)/As(g) will be changed only slightly, e.g., by 11% at 1500 K. This explains the relatively good correlation between experimental and theoretical results for arsenic in the presence of sulphur as discussed above.

Conclusions

The atomizer material as well as the nature and size of the contact area with the sample are critical parameters in the determination of arsenic with graphite furnaces. Stabilizers like nickel or lanthanum should be added to the samples in order to minimize losses of arsenic compounds in the furnace.

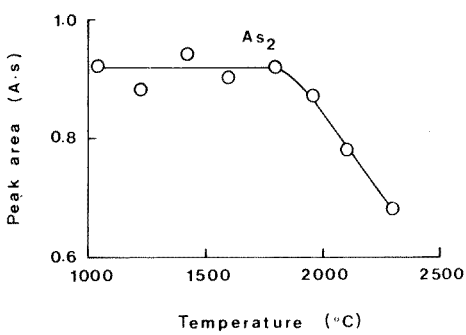


Fig. 11. Peak area absorbance of diatomic arsenic as a function of temperature. Samples were introduced by means of a tungsten wire after the preset tube temperature had been attained. All values are corrected for the change of the diffusion coefficient with temperature.

Optimum conditions are obtained by using the platform technique in combination with suitable stabilizing agents. Surface reactions similar to those involved in the detection of arsenic might also be significant for elements like selenium, phosphorus and sulphur, which also are known to form electrolytic interlamellar compounds with graphite.

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SPECTRAL INTERFERENCES FROM PHOSPHATE MATRICES IN THE DETERMINATION OF ARSENIC, ANTIMONY, SELENIUM AND TELLURIUM BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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SUMMARY

The spectral interferences of phosphorus species originating from the thermal decomposition of calcium phosphate on Sb, As, Se and Te resonance lines, and the influence of increasing amounts of Ce, Ni, W, Pd, Pt, Zr and other elements on non-correctable signals generated by calcium phosphate and on selenium and phosphorus sensitivity have been studied. The results indicate that spectral interference is caused by P_2 absorption and that the extent of interference depends on the wavelength and the spectral band width. The generation of P_2 is masked by large amounts of all the tested reagents. There is a significant reduction in selenium sensitivity in the presence of high concentrations of Ce, Pd and Pt while no decrease in sensitivity is caused by the presence of even 1% nickel and tungsten solutions. All the reagents tested provided enhanced phosphorus sensitivity, thorium being the best.

Recently some authors have called attention to the overcompensation effect connected with simultaneous background correction when a continuous deuterium arc source is used in electrothermal atomic absorption spectrometry (e.a.a.s.) [1—3]. With a deuterium arc source this spectral interference occurs from the matrices because the discontinuous absorption profile of the matrix is close enough to cause direct spectral overlap of the analytical resonance line.

Phosphorus is one of the most difficult elements to determine by e.a.a.s. [4, 5]. The occurrence of primary resonance lines in the vacuum ultraviolet region necessitates the use of a complicated vacuum spectrometer. The sensitivity obtained from the 213.5/213.6-nm doublet appears to be unsatisfactory for many applications. However, the addition of reagents such as lanthanum, barium, calcium, cerium, magnesium, yttrium and iron is reported to increase the sensitivity to a significant extent [4].

This study describes the spectral interferences of phosphates, human serum and whole blood on elements with resonance lines below 220 nm and the influence of some selected metal reagents on the sensitivity for phosphorus.

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EXPERIMENTAL

Apparatus, reagents and standard solutions

A Perkin-Elmer model 5000 atomic absorption spectrometer equipped with a deuterium arc source, a Perkin-Elmer model HGA 500 graphite furnace, an AS-40 automatic sampler and PRS-10 printer were used. Analytical signals were recorded on a Perkin-Elmer model 56 recorder. Antimony, arsenic, phosphorus, selenium and tellurium Perkin-Elmer electrodeless discharge lamps were operated according to the manufacturer's instructions.

Graphite platforms were fabricated as reported by Slavin and Manning [6].

The reagents employed were of analytical grade. Commercially available 1000 ppm standard solutions (BDH) of the following elements were used: aluminium, barium, cadmium, calcium, chromium, copper, iron, lithium, magnesium, manganese, nickel and selenium as nitrates. The 1000 ppm standard solutions of other elements were obtained by making appropriate solutions of cerium(IV) sulphate, caesium chloride, cobalt chloride, lanthanum oxide (in hydrochloric acid) molybdenum (in nitric acid), palladium chloride, platinum chloride, thorium nitrate, sodium tungstate, vanadium (in nitric acid) and zirconium nitrate. The standard graphite tubes used throughout this work were purged with argon (99.99%).

Procedures

Influence of phosphate matrix, human serum and whole blood on antimony, arsenic, selenium and tellurium resonance lines. A 20-μl portion of the sample was dispensed into the graphite tube, dried at 110°C (ramp 10 s, hold 15 s) and ashed at 450°C (ramp 10 s, hold 5 s), 600°C (ramp 5 s, hold 5 s) and 1200°C (ramp 10 s, hold 20 s). The residue was atomized at 2200°C (maximum power) for 6 s (internal gas flow 50 ml min^{-1}), with subsequent cleaning of the tube at 2800°C for 2 s. The non-atomic absorption signals were recorded at spectral band widths of 2.0, 0.7 and 0.2 nm.

Influence of selected metal reagents on the interfering signals generated by calcium phosphate. Primary and secondary selenium resonance lines were selected to study the effect of increasing amounts of different metal reagents on the interfering signals. Aliquots (20 μl) of aqueous calcium phosphate standard (550 μg P ml^{-1}) with and without added metal reagent, were dispensed into the tube, dried, ashed and atomized as described above.

Effect of selected metal reagents on the phosphorus sensitivity. Aliquots (20 μl) of aqueous standard (15.5 μg PO_4 ml^{-1}), with and without added metal reagent (0.1%) were dispensed into the tube, dried at 110°C (ramp 15 s, hold 25 s), ashed at 1200°C (ramp 10 s, hold 20 s) and atomized at 2700°C for 6 s using the maximum power mode (internal gas flow 20 ml min^{-1}). The phosphorus doublet at 213.5/213.6 nm with a spectral band width of 0.7 nm was used to measure the analytical peak heights. New standard graphite tubes were used for each reagent tested, for reasons described elsewhere [7].

RESULTS AND DISCUSSION

In a previous study, an unexplained spectral interference arising from biological matrices at the 204.0-nm secondary selenium resonance line was reported [8]. When biological fluids such as whole blood and urine were atomized after ashing at 1200°C in the absence of stabilizing reagents, large negative absorption signals were recorded at the 196.0-nm selenium primary resonance line. This overcompensation effect of the deuterium arc background corrector has been explained as being due to the presence of several iron absorption lines within the spectral band width [3]. These signals, however, were still present to a considerable extent when fluids low in iron such as serum and urine were atomized. With identical analytical parameters, the same matrices gave uncorrectable positive signals at the 204.0-nm selenium line. During these experiments, it was found that calcium phosphate, which is present at high concentration in body fluids, gave the same type of interference. When phosphoric acid and calcium chloride were mixed in the graphite tube the signals recorded during the atomization were similar to those obtained from calcium phosphate alone while individual atomization of each reagent had no effect on the base line (Fig. 1).

On the basis of these findings calcium phosphate was selected for further investigation of its possible interference on other elements with resonance lines in the far-ultraviolet region. The effects of calcium phosphate on the determinations of selenium, arsenic, antimony and tellurium are listed in Table 1. In the 190—250-nm region, both sodium and, especially, calcium phosphate give a considerable non-specific absorption (Fig. 2). The actual non-specific absorption of calcium phosphate did not exceed the capacity of the background corrector and the uncorrectable signals should not be explained by its capacity.

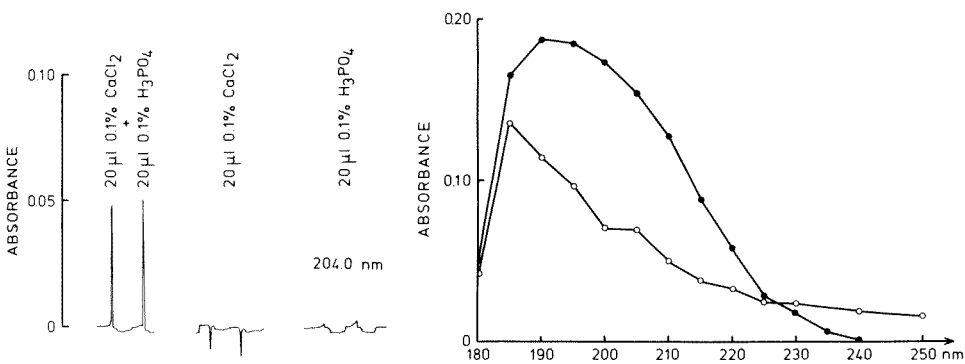

Fig. 1. Uncorrectable signals recorded at 204.0 nm from calcium phosphate matrix (ashing 1200°C, atomization 2200°C, maximum power mode).

Fig. 2. Non-specific absorption from atomization of 20-µl aliquots of solutions of (•) calcium phosphate and (○) sodium phosphate (550 µg PO_4 l^{-1}) between 180 and 250 nm. Ashing 250°C; atomization 2200°C; maximum power mode.

TABLE 1

Uncorrectable absorption signals from atomization of 20 μl of a $Ca_3(PO_4)_2$ soln. (550 μg PO_4 l^{-1}) (ashing and atomization temperatures were 1200 and 2200°C, respectively)

Element	Wavelength (nm)	Absorbance			Relative sensitivity
		Spectral band width (nm)			
		2.0	0.7	0.2	
Se	196.0	−0.051	−0.047	−0.037	1.0
	204.0	+0.059	+0.045	+0.047	3.0
	206.3	+0.018	−0.029	−0.029	11.0
	207.5	+0.055	−0.020	−0.023	35.0
As	189.0	−0.044	−0.044	−0.056	1.0
	193.7	−0.078	−0.069	−0.070	0.5
	197.2	−0.046	−0.058	−0.049	2.0
Sb	206.2	−0.031	−0.019		1.0
	217.5	+0.011	+0.009		1.5
	231.1	−0.004	−0.004		2.1
Te	214.2	+0.024	+0.021	−0.029	1.0
	225.9	+0.027	+0.021	+0.009	15.0

Molecular absorption commonly consists of a continuum on which fine structure is superimposed. The background measurement may be faulty if the non-specific absorption is due to line-rich electronic excitation spectra. This cannot be concluded from measurements obtained with atomic absorption instruments normally used. High-temperature equilibrium calculations and experimental work on the thermal decomposition of calcium phosphate in graphite tubes by Frech et al. [9, 10] showed that the most stable phosphorus species were gaseous P_2, PO and PO_2, of which P_2 is the major species in the range 1200—2300°C. PO, PO^+ and P_2 have been shown to have line-rich electronic excitation spectra in the far-ultraviolet region [11].

L'vov et al. [12] have recently described the use of a graphite platform placed inside the graphite tube. This arrangement makes it possible to obtain nearly isothermal conditions during the atomization step. When ordinary graphite platforms were used here, the effects of phosphate matrices on the base line were found to increase. This suggests that the spectral interference is caused by P_2 rather than PO because an increase in the atomization temperature has been shown to decrease the PO/P_2 ratio [10]. Since phosphates are resistant to thermal decomposition ($Ca_3(PO_4)_2$, m.p. 1670°C), the following reactions are predicted to take place in the presence of carbon at high temperature [13]

$$Ca_3(PO_4)_2 + 5C \xrightarrow[1385°C]{\nabla} 3CaO + P_2 + 5CO$$

$$Ca_3(PO_4)_2 + 8C \xrightarrow[1780°C]{\nabla} 3Ca + P_2 + 8CO$$

During the present work it became apparent that addition of large amounts of nickel to samples containing phosphates suppressed the uncorrectable non-specific absorption signals. Hence systematic studies were undertaken to determine the influence of matrix modification reagents used in the determination of selenium on the spectral interferences. Among all the reagents tested for thermal stabilization of selenium [7], only cerium, nickel, palladium, platinum, tungsten and zirconium were able to depress the uncorrectable non-specific signals significantly. The effects of these reagents are shown in Fig. 3. The influence of added metal on the interfering signals was almost identical at both resonance lines tested. Although the interfering signals present at 204 nm were completely suppressed by addition of 0.6% nickel or platinum, their influence was slightly altered at 196 nm. The possibility of the presence of iron as an impurity in the solutions was evaluated as this may produce overcompensated signals only at the 196-nm selenium line. The iron contents were found to be less than 1 $\mu g\ ml^{-1}$ in the reagents and did not have any effect on the baseline. The suppression of the interfering signals at low zirconium concentrations was followed by an enhancement at higher concentrations. This may be due to alteration in the physical nature of the graphite tube by refractory carbide formation. Similar behaviour was observed with increasing concentrations of tungsten. The interfering signals were completely suppressed by addition of 1% tungsten or zirconium solutions when unused graphite tubes were employed. The degree of supression decreased gradually with increasing number of firings, again probably because of carbide formation.

Although the mechanism of the matrix modification by metal reagents for suppression of the signals from phosphates that cannot be compensated is not entirely clear, it may be suggested that the formation of PO and P_2 species during atomization of the modified phosphate matrices is strongly decreased.

If large amounts of reagents are added to the samples it may be reasonable to expect an influence on the analyte absorbance. The results described for selenium in Fig. 4 show that all reagents which suppress the uncorrectable absorption signals enhance the selenium sensitivity when present at low concentrations. Cerium, which increased the sensitivity very greatly at low

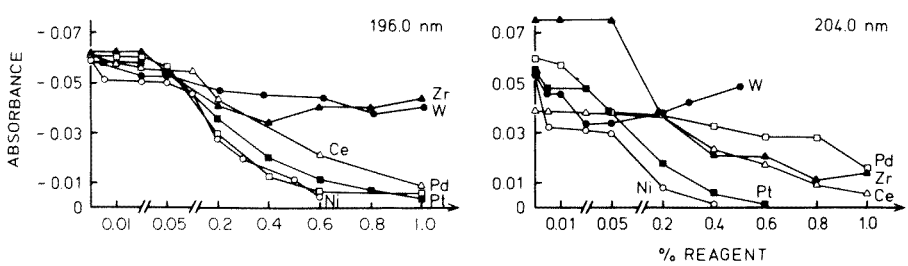

Fig. 3. The influence of selected metal ions on the uncorrectable signals generated by 20 μl of calcium phosphate solution (550 $\mu g\ l^{-1}$).

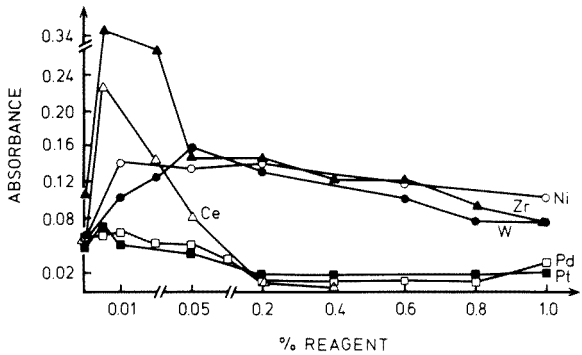

Fig. 4. The effect of selected metal ions on the absorbance for selenium (1.0 μg Se ml^{-1}).

concentrations, caused complete suppression of the selenium signal at concentrations higher than 0.4%. Although nickel, tungsten and zirconium had a suppressive effect at high concentrations, the selenium sensitivities were still higher than those obtained from pure selenium standard solutions. The results given in Fig. 5 show that by addition of suitable amounts of nickel, the uncorrectable absorption signals are strongly suppressed. The overcompensation which is still present at 196 nm for whole blood is due to the presence of iron. The sensitivity at the 204-nm line is not suitable for the direct determination of selenium in whole blood.

Enhancement of phosphorus sensitivity

The effect of metal reagents on the atomization products of phosphates and subsequent reduction of uncorrectable absorption signals may be explained

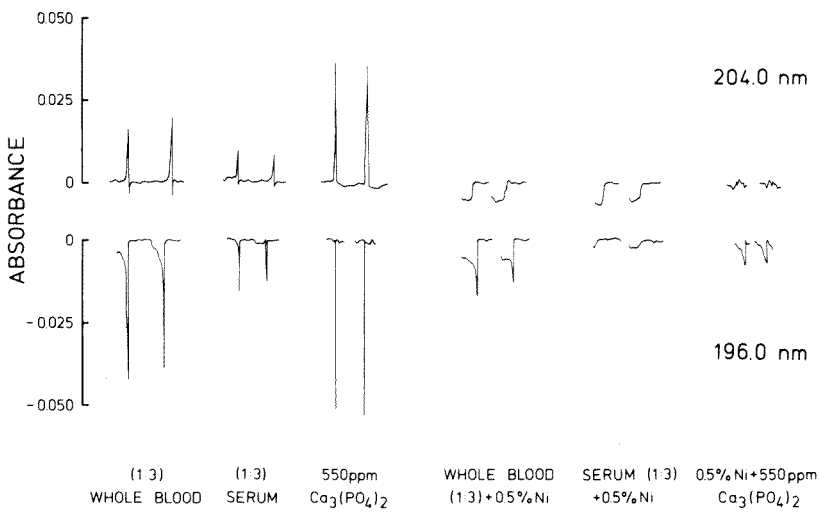

Fig. 5. The influence of nickel on the uncorrectable signals at the 196.0- and 204.0-nm selenium resonance lines; 20-μl injections in each case.

TABLE 2

Enhancement of phosphorus sensitivity in presence of selected metal ions

Metal ion (0.1%)	Sensitivity[a]	Metal ion (0.1%)	Sensitivity[a]	Metal ion (0.1%)	Sensitivity[a]
Al	+++	Cs	+++	Mo	+
Ba	+++	Cu	+	Ni	++
Ca	+	Fe	+++	Pd	++
Ce	+++	La	++	Th	++++
Cd	+++	Li	+++	W	+
Co	+++	Mg	++	V	++
Cr	++	Mn	++	Zr	+++

[a]Sensitivity range (ng P/0.0044 absorbance): ++++, 1—5; +++, 6—10; ++, 11—15; +, 16—20. Without matrix modification, 20—200 ng P/0.0044 absorbance.

by an increase of phosphorus atoms in the gas phase. The results listed in Table 2 show that all reagents tested had a significant enhancing influence on the phosphorus sensitivity. During this work large variations in phosphorus sensitivity from pure standard solution were found with different graphite tubes (20—194 ng P/0.0044 absorbance). In contrast, the phosphorus sensitivity in the presence of the metal reagents was more reproducible. As is apparent from Table 2, matrix modification with thorium may provide the best phosphorus sensitivity. The use of aluminium cannot be recommended because of an intense overcompensation effect at the wavelength employed (P_2, PO). It is interesting to note that there are many other metal ions which provide even better sensitivity than calcium and lanthanum which are frequently used in the e.a.a.s. determination of phosphorus.

The most effective reagents for suppression of the uncorrectable absorption signals, nickel and tungsten, showed a relatively poor tendency to increase the concentration of atomic phosphorus during the atomization step compared with the other metals tested.

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CHEMICAL SPECIATION OF CHROMIUM IN SEA WATER
Part 1. Effect of Naturally Occurring Organic Materials on the Complex Formation of Chromium(III)

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SUMMARY

The effect of naturally occurring organic materials on the coprecipitation behaviour of trivalent chromium is studied to estimate the relative abundance of organic-bound chromium in sea water. Some amino acids and polyhydric organic acids can bind chromium(III) under the conditions prevalent in natural sea water. Some of the chromium(III) in sea water therefore probably occurs in organic species.

The chemical speciation of chromium in sea water has been examined by sample analysis and theoretical calculations for many years as an interesting problem in marine geochemistry [1—7]. However, the analytical results concerning the relative abundance of the trivalent and hexavalent species have not always been consistent, although thermodynamic calculations suggest that chromate should be the predominant species in the natural oxygenated environment [8]. In past research, analyses have been based on the supposition that the major chromium species are inorganic forms of Cr(III) or Cr(VI), such as $Cr(OH)_2 \cdot 4H_2O^+$ and CrO_4^{2-} [8]. Since there is a fair amount of organic material in real sea water which is capable of binding Cr(III), such as organic acids, amino acids and humic acid [9], the inconsistency of past results may be due not merely to the peculiarities of the analytical techniques used by different workers, but also to the fact that the presence of organic species was not taken into account.

In the present paper, the effect of naturally occurring organic materials on the coprecipitation behaviour of chromium(III) is described in detail, in order to check the ability of these materials to bind Cr(III) in sea water. It is shown that some of the organic materials are able to form complexes with Cr(III) under the conditions prevalent in natural sea water.

EXPERIMENTAL

Apparatus and reagents

A Nippon Jarrell-Ash AA-8200 atomic absorption apparatus equipped with a FLA 100 electrothermal atomizer was used for determinations of chromium. In the tracer technique using ^{51}Cr, a Packard Auto-Gamma 5100 single-channel pulse-height analyzer was used. For pH measurements a Hitachi-Horiba M-5 pH meter was used.

Chromium-51 solutions were obtained from New England Nuclear through the Japan Isotope Association in the form of Na_2CrO_4 and $CrCl_3$; the radiochemical purities were better than 99%. Mineral acids and aqueous ammonia were ultra-pure analytical reagents further purified by distillation. All other chemicals were of analytical-reagent grade and were checked for contamination with chromium. Iron(III) chloride and other reagents containing a significant amount of chromium were purified by solvent extraction or by other appropriate methods.

Hydrated iron(III) oxide was prepared by precipitation from an iron(III) chloride solution with aqueous ammonia, and washed copiously three times with distilled water.

Procedures

Test solutions were prepared as follows. Definite amounts of Cr(III) (labelled with ^{51}Cr when a tracer technique was used), organic materials and buffer solution were added to three different solutions: (i) sea water filtered through a 0.45-μm Millipore filter; (ii) artificial sea water prepared as described by Lyman and Fleming [10] and pretreated in the same manner as sea water; and (iii) aqueous solutions including some of the sea salts equivalent to sea water.

A portion (50 ml) of each test solution was kept in a constant temperature bath at 25°C for 72 h (unless otherwise indicated). Then freshly prepared hydrated iron(III) oxide (equivalent to 5 mg Fe) was added to the solution. After it had stood for 3 h, the precipitate was filtered through a Millipore filter. When a tracer technique was used, the precipitate was dissolved in diluted hydrochloric acid solution, followed by dilution to 50 ml. An aliquot of this solution was used for γ-activity measurement together with the supernatant liquid. When electrothermal atomic absorption spectrometry was applied, the precipitate was dissolved in a 7 M hydrochloric acid solution and iron(III) was removed by extraction with 4-methylpentan-2-one. The separated aqueous phase was then evaporated to dryness, and the residue was dissolved in 5 ml of diluted nitric acid solution. The resulting solution was used for atomic absorption spectrometry.

RESULTS AND DISCUSSION

From all previously published data, it is concluded that sea water contains about 10^{-8} M dissolved chromium. Meanwhile, it is generally known that a fair amount of organic materials capable of binding metal ions is present in sea water. For example, the total amount of organic acids (such as citric acid) may be around 3×10^{-6} M in a coastal area [9]. If equilibrium is assumed to exist between Cr(III) and these organic materials in sea water, it is possible to calculate the theoretical relative amount of organically bound chromium. In the following calculation, charges of ions are neglected to simplify the equations. The overall formation constant of hydrolyzed Cr(III) ions, which are considered to be the dominant inorganic Cr(III) species in sea water, is given as $[Cr(OH)_2][H]^2/[Cr] = 10^{-9.5}$ [11]. When the formation constant of an organic complex, CrL (L is some organic ligand) is expressed as $K = [CrL]/[Cr][L]$, and the pH of sea water is taken as 8.1, the relative amounts of $Cr(OH)_2$ and CrL can be expressed by $\log [Cr(OH)_2]/[CrL] = 6.7 - (\log K + \log [L])$. If it is assumed not unreasonably, that $[L] = 10^{-6}$ M, then the value of $\log K$ should be larger than 12.5 in order to satisfy the condition $[CrL] > [Cr(OH)_2]$. Although it is not possible to compare this calculated value with a large number of examples, owing to lack of suitable formation constant data for Cr(III), the $\log K$ value for the Cr(III) 1-phenylbiguanide complex is reported to be 12.02 [12], which is close to the calculated value. Accordingly, it can safely be stated that the existence of organically bound chromium is theoretically possible.

Shown in Fig. 1 is the standard coprecipitation behaviour of Cr(III) with hydrated iron(III) oxide. In sea water and artificial sea water, Cr(III) coprecipitates quantitatively in the pH range above 5.5 whereas in pure aqueous solution with simple pH adjustment, the coprecipitation percentage decreases suddenly above pH 9.5, probably because an anionic hydroxo complex of Cr(III) is formed. Because the concentration of Cr(III) was fixed at 2×10^{-6} M in this experiment, the effect of coexisting materials was not significant compared with the case of 10^{-8} M described later. The further examinations were designed on the basis of this finding.

In Fig. 2, the relationship between the percentage coprecipitation of Cr(III) and the storage time (between preparation of a test solution and its treatment with hydrated iron(III) oxide) is shown for the various test solutions under the conditions $[Cr(III)] = 10^{-8}$ M, 25°C and pH 8.1. The percentage coprecipitation decreases remarkably with the passage of time in the presence of organic acids, such as citric acid and ascorbic acid, compared with solutions without organic acids. This fact suggests that the hydrolyzed ion, $[Cr(OH)_2 \cdot 4H_2O]^+$, changes gradually to organic complexes which do not coprecipitate with hydrated iron(III) oxide because Cr(III) is very inert in the ligand exchange reaction at room temperature. Since the effect of coexisting materials is considerable when the test solution was stored for more than 72 h (Fig. 2), the storage time of the test solutions was fixed at

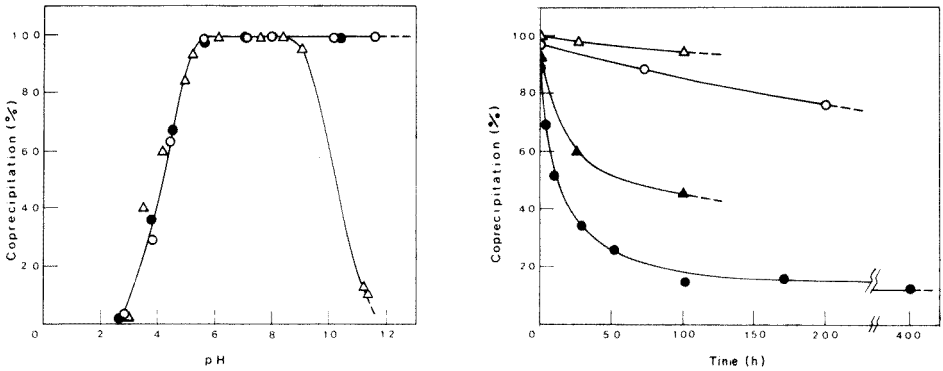

Fig. 1. Effect of pH on the coprecipitation of 2×10^{-6} M Cr(III) with hydrated iron(III) oxide. The storage time before treatment with hydrated iron(III) oxide was 24 h. Solutions: (○) sea water; (●) artificial sea water; (△) pure water with pH adjusted with minimum amounts of sodium hydroxide and perchloric acid.

Fig. 2. Effect of storage time on the coprecipitation of 10^{-8} M Cr(III) in sea water at 25°C in the presence of organic acids: (△) sea water (pH 8.1); (○) 0.02 M borate buffer in sea water (pH 8.1); (▲) 0.02 M borate buffer + 5×10^{-6} M citric acid in sea water (pH 8.1); (●) 0.02 M borate buffer + 5×10^{-6} M ascorbic acid (pH 8.5).

72 h in further experiments. Although inorganic anions, such as borate affected the coprecipitation somewhat (Fig. 2), only the effect of organic materials was examined in the present study because most of the common sea salts did not appreciably affect the coprecipitation.

The effects of various organic materials on the coprecipitation of Cr(III) are shown as a function of pH in Fig. 3. No appreciable decrease of coprecipitation was observed over the whole pH range in the presence of monobasic higher fatty acids, such as stearic acid, lauric acid and oleic acid (Fig. 3A). Therefore it is assumed that such acids cannot form complexes with Cr(III) in sea water. The coprecipitation decreased only slightly in the neutral pH range in the presence of amino acids, such as glycine, aspartic acid, lysine and cystine (Fig. 3B), but it decreased remarkably around pH 8 in the presence of histidine which is an active ligand (Fig. 3C). The fact that the mixture of L-histidine and L-aspartic acid is more effective than either of them separately is probably due to the formation of a mixed ligand complex. In the presence of humic acid, which was obtained commercially and used by assuming its mean molecular weight to be 400, coprecipitation seemed to decrease slightly (Fig. 3C). However, it was observed that some Cr(III) was connected with the deposited humic acid because the amount of humic acid added (2 mg l^{-1}) may greatly exceed its solubility in sea water. Therefore, it is considered that humic acid acts mainly in the solid phase as a ligand for Cr(III). As shown in Fig. 3D, the coprecipitation decreased very significantly over the pH range 7—10 in the presence of polybasic organic acids, such as citric acid, malic acid and ascorbic acid.

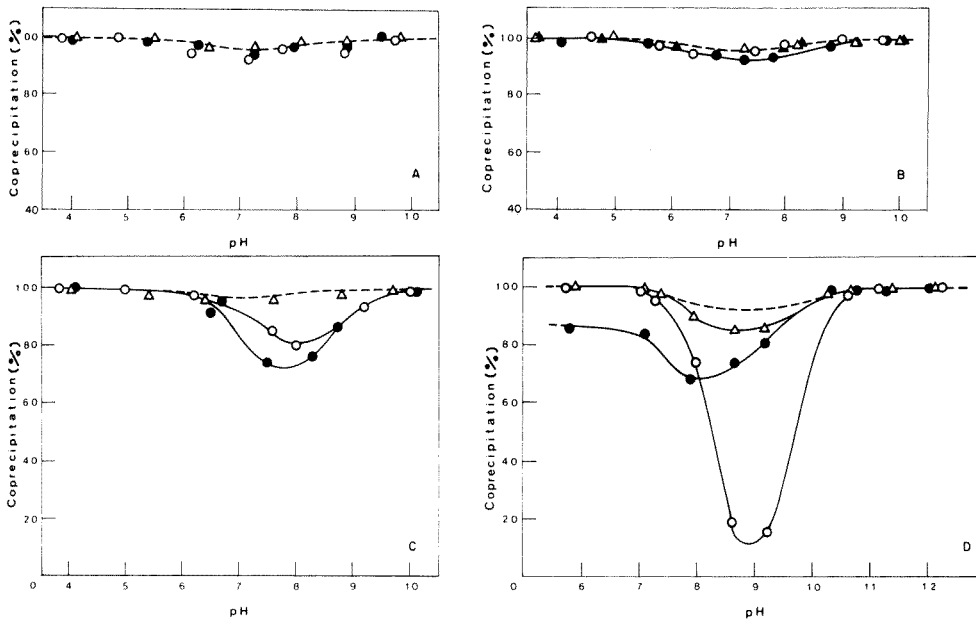

Fig. 3. Relationship between coprecipitation of Cr(III) and pH in the presence of various organic acids ([Cr] = 10^{-8} M, 25°C, concentration of acids = 5×10^{-6} M). A, Fatty acids: (○) stearic acid; (●) lauric acid; (△) oleic acid; (---) sea water only. B, Amino acids: (○) glycine; (●) aspartic acid; (△) lysine; (▲) cystine; (---) sea water only. C, Amino acids and humic acid: (○) histidine; (●) histidine + aspartic acid; (△) humic acid; (---) sea water only. D, Polyhydric organic acids: (○) ascorbic acid; (●) citric acid; (△) malic acid; (---) sea water only.

In Fig. 4 are shown the effects of some sea salts on the complex formation of Cr(III) with ascorbic acid in order to explain why the decrease of coprecipitation appeared only around pH 8 with many organic materials. In an aqueous solution including no salts other than minimum amounts of sodium hydroxide or perchloric acid necessary to adjust the pH, the coprecipitation was less than 20% over the whole pH range, whereas it increased in the acidic range when sodium chloride equivalent to sea water was added to the aqueous solution. Further, it also increased in the alkaline range when calcium chloride and magnesium chloride were added, as well as in sea water. Accordingly, it may be concluded that complex formation of Cr(III) with organic materials in sea water is affected by chloride ion in the acidic range and by magnesium and calcium ions in the alkaline range.

In addition, the coprecipitation behaviour of previously synthesized Cr(III) complexes with citric acid and ascorbic acid was examined to ascertain whether or not the decrease of coprecipitation was caused by complex formation. As shown in Fig. 5, both complexes coprecipitated hardly at all over the wide pH range in sea water, just as expected.

In conclusion, it is probable that chromium(III) is highly stabilized and

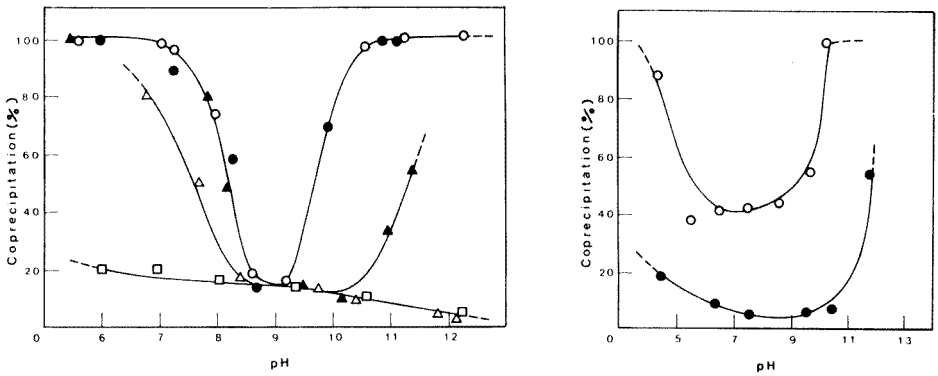

Fig. 4. Effect of sea salts on the coprecipitation of 10^{-8} M Cr(III) in the presence of 5×10^{-6} M ascorbic acid at 25°C: (○) sea water; (●) $MgCl_2$ + NaCl (equivalent to sea water); (▲) $CaCl_2$ + NaCl (equivalent to sea water); (△) NaCl (equivalent to sea water); (□) distilled water with pH adjusted with minimum amounts of acid or alkali.

Fig. 5. Relationship between coprecipitation of organic Cr(III) complexes and pH in sea water (concentration of complexes = 2×10^{-6} M, 25°C): (○) Cr(III) ascorbate; (●) Cr(III) citrate.

solubilized in sea water by complex formation with organic materials such as amino acids and polybasic organic acids under the conditions normally prevailing in sea water.

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DETERMINATION OF NITRO AROMATIC, NITRAMINE, AND NITRATE ESTER EXPLOSIVE COMPOUNDS IN EXPLOSIVE MIXTURES AND GUNSHOT RESIDUE BY LIQUID CHROMATOGRAPHY AND REDUCTIVE ELECTROCHEMICAL DETECTION

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SUMMARY

Reductive and oxidative electrochemical detection with liquid chromatography is applied to the determination of nitro aromatics, nitrate esters, nitramines, and diphenylamines in military explosives and single and double base smokeless gunpowders. A sensitive and highly selective method is presented for the detection of organic "gunshot residue" on the hand of individuals who have discharged a weapon. The detection limits at S/N = 3 are of the order of 0.5, 1, 2, and 0.3 picomol for nitro aromatic, nitramine and nitrate ester explosive compounds, and diphenylamines, respectively.

Detection of explosive substances has been the subject of many investigations. Law enforcement officials and forensic chemists have traditionally examined post-blast debris in search of explosive material and gunshot residue. The latter is useful in connection with suicides, and to ascertain if a suspect has fired a gun or just handled a weapon which was discharged. Environmental chemists and toxicologists are concerned about the possible health and ecological hazards caused by long- and short-term exposure to explosive substances. These concerns have been strengthened by reports of the toxic and mutagenic properties of various explosive substances and nitroaromatic compounds in marine organisms [1], laboratory animals [2], and bacteria [3—5]. Traces of ethyleneglycoldinitrate (EGDN) explosive [6] and trinitrotoluene (TNT) metabolites [7] have been found in the biological fluids of workers associated with the production of explosives. Relevant toxicology studies have been carried out [8—10].

Nitrate esters are widely used to decrease the frequency and severity of angina attacks, and to decrease heart workload and myocardial consumption of oxygen in patients suffering from various cardiovascular ailments. Polynitrate esters are rapidly decomposed in plasma [11], and plasma levels of these drugs in patients undergoing treatment are usually less than 10 ng ml^{-1}

[12]; there is a definite need for improved techniques for monitoring these drugs in biological fluids.

Detection of explosive material in debris obtained from the site of explosion, contaminated ground water, and gunshot residue is difficult because of the thermal instability of explosive compounds. Chemical tests, infrared spectroscopy, piezoelectric quartz crystal detection, mass spectrometry, polarography, negative ion plasma chromatography, and thin-layer chromatography have been used. Gas chromatography with flame ionization, electron capture, or thermal conductivity detection has also been explored for this purpose. Applications of some of these methods to determination of explosives have been reviewed [13—15]. The use of gas chromatographic methods is limited because of thermal instability of many explosive compounds and other problems [14, 15].

The current methods for detection of gunshot residue are based on the detection of elevated levels of metals such as antimony, lead, barium, and copper [16, 17] and by microscopic evaluation of particles obtained from suspects' hands [18, 19]. Very often, evaluation of metal residue is inconclusive in criminal investigations because amounts of antimony, lead, and barium are below certain threshold levels. These minimum amounts are usually considered necessary because elevated levels of metals are not unique to gunshot residue [19]. A new approach for the detection of gunshot residue is based on the detection of unburned gunpowder flakes and volatile organic components of gunpowders [20]. Flakes of partially burned smokeless powder have been found on the hands of suicide victims and in laboratory tests.

Liquid chromatography is ideally suited for the separation of thermally unstable and non-volatile explosive compounds. Liquid chromatography with ultraviolet [21, 22], thermal energy analysis (t.e.a.) [23], off-line chemical ionization mass spectrometry (c.i.m.s.) [24], and electron capture detection (e.c.d.) [25] schemes have been used for the determination of explosive compounds. Liquid chromatography with ultraviolet detection (l.c.—u.v.) methods usually offer adequate detection limits for nitro aromatic compounds, but routine detection of less than several nanograms of nitrate esters and nitramines is difficult. Liquid chromatography with e.c.d., c.i.m.s., and t.e.a. detection is a new entry in this field and shows promise for the future.

In the last five years, oxidative mode electrochemical detection in liquid chromatography has become widely accepted for solving many problems of clinical, pharmaceutical, and environmental interest. Several reviews have been published on the advantages resulting from the combination of liquid chromatography with electrochemical detection (l.c.—e.c.) [26—28]. Progress in reductive mode l.c.—e.c. has been slow because of problems associated with dissolved oxygen, metal impurities, and the lack of reliable electrodes [27, 28]. Recent technological advances in detector design and the availability of more suitable electrode materials has generated a renewed interest in this technique [27, 29—34].

This paper describes the application of reductive mode l.c.—e.c. using glassy carbon and amalgamated gold electrodes to quantify explosive compounds in military explosives and smokeless gunpowders, and the development of a highly sensitive and selective method for the detection of nitroglycerin, 2,4-dinitrotoluene (2,4-DNT), and diphenylamine (DPA) in gunshot residue.

EXPERIMENTAL

Apparatus

A model LC-304T (Bioanalytical Systems, Purdue Research Park, West Lafayette, IN) equipped with a LC-300 solvent delivery system, a Rheodyne 70-10 fixed volume (20 μl) rotary sample injection valve, LC-22 temperature controller, a LC-23A column heating compartment, a LC-4A electronic controller, a LC-19 accessories package, and an oxygen removal apparatus [34] was used in all l.c. separations. An Altex fixed wavelength detector (254 nm) model 153 was used in l.c.—u.v. experiments. The l.c. columns were 25 × 0.46 cm C_{18} and C_8 Biophase with 5-μm packing (Bioanalytical Systems). A Spectra-Physics Minigrator Model 23000-011 was used to quantify l.c. peak areas. Cyclic voltammetry experiments were performed with a Bioanalytical Systems Model CV-1B controller using mercury film (Au/Hg) and glassy carbon (g.c.) electrodes.

The cyclic voltammetry and thin-layer mercury film electrodes were prepared by placing enough triple-distilled mercury on the highly polished gold surface to cover the entire surface. After 2—3 min, the excess of mercury was removed from the electrode surface using a computer card. Microfilters (MF-1) with 0.2-μm nitrocellulose filters (RC58; Bioanalytical Systems) were used to filter gunshot residue sample solutions.

Chemicals

Spectro-grade 1-propanol, triple-distilled mercury, reagent-grade anhydrous sodium acetate, and monochloroacetic acid (all from Fisher Scientific) were used as purchased. Reagent-quality absolute ethanol was obtained from U.S. Industrial Chemicals Company. All solvents for l.c. were filtered through 0.22-μm Millipore filters.

Nitroglycerin and isosorbide dinitrate were obtained as a mixture of lactose and nitrate ester (10% and 25% w/w, respectively; Purdue University Pharmacy) and were used without further purification. Samples of explosive compounds, gunpowders, and mixtures of commercial and military explosives were gifts from the Bureau of Alcohol, Tobacco, and Firearms, Department of Treasury, Cincinnati, OH, and SEMO Crime Laboratory, Southeastern Missouri State University, Cape Girardeau, MO, and were used as received.

Determination of number of electrons

Hydrodynamic voltammograms were obtained by repeated injections of standards while varying the potential of the thin-layer amperometric detector. The number of electrons involved in the reduction of explosive compounds under hydrodynamic conditions (in the thin-layer cell after chromatographic separation) were estimated using the normalized function n_{eff}, which can be derived from Faraday's law. The expression is $n_{eff} = Q/FN_{inj} = nf$, where Q is the number of coulombs passed during the reduction process, F is the Faraday constant (9.65×10^4 coulombs/equivalent), N_{inj} is the number of moles of analyte injected, n is the number of electrons involved in the electrochemical reaction (equivalents/mole), and f is the fraction of molecules injected onto the l.c. column which reacts at the electrode surface. The n_{eff} values of nitro aromatic explosives were determined with the detector potentials set at the plateau of their respective hydrodynamic voltammograms. In case of nitramines and nitrate esters, which exhibit complex waveforms on Au/Hg and g.c. electrodes, n_{eff} values were measured at potentials between —0.9 and —1.2 V vs. Ag/AgCl. The n values were then determined by a comparison of n_{eff} values of explosive compounds with n_{eff} values of *p*-nitroaniline ($n = 6$, at pH 1—9 [35]) and *p*-nitrophenol ($n = 4$ for the first reduction wave at pH 5 [36]).

Determination of explosives and smokeless gunpowders

Samples of explosive and smokeless powders were dissolved in acetone (50—300 μg ml^{-1}) and then diluted (10—100 fold) with the mobile phase prior to injection onto the column. All solutions were kept in amber glassware to prevent photodecomposition (especially important for nitramines and nitrate esters). A 0.22 caliber revolver, Western Long Rifle Extra Power 0.22 Rim Fire and 0.22 Remington Long Rifle cartridges were used in all gunshot residue experiments.

After the revolver had been fired, the back of the firing hand was swabbed with cotton dipped in acetone. The cotton swab was placed in a brown glass screwcap vial to minimize photodecomposition of nitroglycerin. The swab was washed with 20% ethanol in water solution. After the ethanol solution was filtered through a 0.2-μm nitrocellulose filter, a 20-μl volume of the deoxygenated solution was injected onto the column.

The same procedure was used to obtain a "blank" sample before the revolver was fired.

RESULTS AND DISCUSSION

Oxidation—reduction chemistry of explosive compounds

Most explosives can be classified into one of several groups represented by nitro compounds, nitric acid esters, nitramines, salts of perchloric, nitric, and chloric acids, azides and other miscellaneous compounds capable of producing an explosion, and mixtures of explosives from the above groups.

Representatives of common commercial and military explosive compounds suitable for trace determinations using reductive mode l.c.—e.c. are listed in Fig. 1 along with their common abbreviations.

The initial characterization of the electrochemical reactivity of explosive compounds was peformed using cyclic voltammetry (c.v.). Results obtained on a mercury film (Au/Hg) electrode are summarized in Fig. 2. Symbols used in tabulation of c.v. information are described elsewhere [33]. Cyclic voltammetry can be used to obtain a rapid evaluation of redox properties and selection of proper operating potentials for electrochemical detectors. As a general guide, the potential of an electrochemical detector is set 50—100 mV more negative than E_{pc} (reduction peak potential) of the analyte most difficult to reduce. The range of reduction potentials of explosives is very wide; however, they fall into several categories according to their structures. As expected, trinitro aromatic compounds such as tetryl, TNT, and

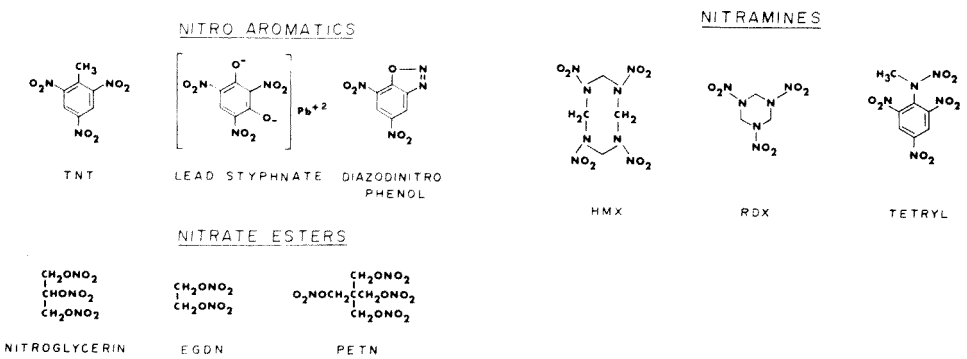


Fig. 1. Representative compounds found in military and commercial explosives and propellants which are suitable for reductive l.c.—e.c.

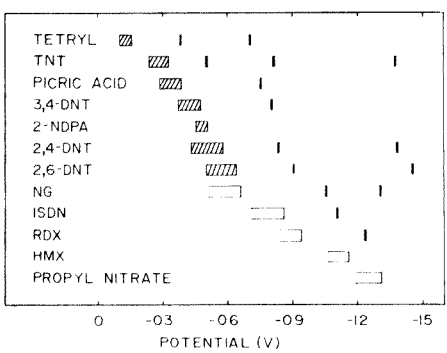


Fig. 2. Cyclic voltammetric data summary for selected explosive compounds. Supporting electrolyte was 0.06 M monochloroacetic acid, 0.44 M sodium acetate, 0.001 M EDTA, in 15% (v/v) 1-propanol, pH 3.5. Scan rate 300 mV s^{-1}. Concentrations of explosives were between 100 and 200 $\mu g\ ml^{-1}$.

picric acid are more easily reduced on a gold/mercury electrode than dinitro and mononitro compounds. Polynitrate esters and nitramines are more difficult to reduce. The reduction potential for 2-nitrodiphenylamine was lower than for 2,4-DNT or 2,6-DNT. The suitability of glassy carbon electrodes was also investigated. The reduction potentials of all explosive compounds studied occurred at more negative potentials on glassy carbon than on mercury film electrodes. The difference was typically between 100 to 250 mV, depending on the specific compound. The peak potentials were reproducible to ±20 mV on the Au/Hg electrode and ±40 mV on the glassy carbon electrode. The uncertainty of peak potentials on the Au/Hg electrode was due to variation in the thickness and age of the mercury layer. (Values of E_{pc} are lower on a pure mercury surface than on the amalgamated gold surface.) Poorer reproducibility of peak potentials and peak currents on glassy carbon was caused by irreproducible pretreatment of the electrode surface which is characteristic of glassy carbon electrodes [37]. The cyclic voltammograms of nitrate esters and nitramines were poorly defined, usually resulting in broad shoulders as illustrated by the cyclic voltammogram of RDX (Fig. 3A).

The redox properties of explosives under hydrodynamic conditions on both Au/Hg and glassy carbon were similar as illustrated in Fig. 4. Large background currents (>1 μA) and lack of long-term stability of g.c. electrodes at potentials necessary for the reduction of nitrate esters and nitramines [28, 32] prevent detection of trace amounts of these explosives. Nonetheless, on a day-to-day basis, greater convenience of a glassy carbon electrode makes it preferable over a Au/Hg electrode for the detection of nitro aromatic explosives.

The redox chemistry of nitro aromatic compounds has attracted considerable attention. The nitro group is an excellent electron acceptor. Its redox properties are strongly influenced by the nature and the positions of other functional groups. Electrochemical studies on a variety of mono-

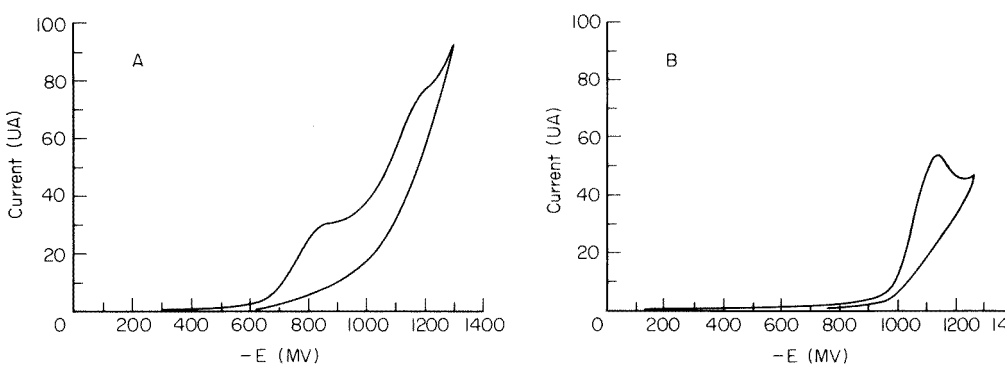


Fig. 3. Cyclic voltammogram of RDX (A) and *N*-nitrosodipropylamine (B) on a gold/mercury electrode. Conditions as in Fig. 2.

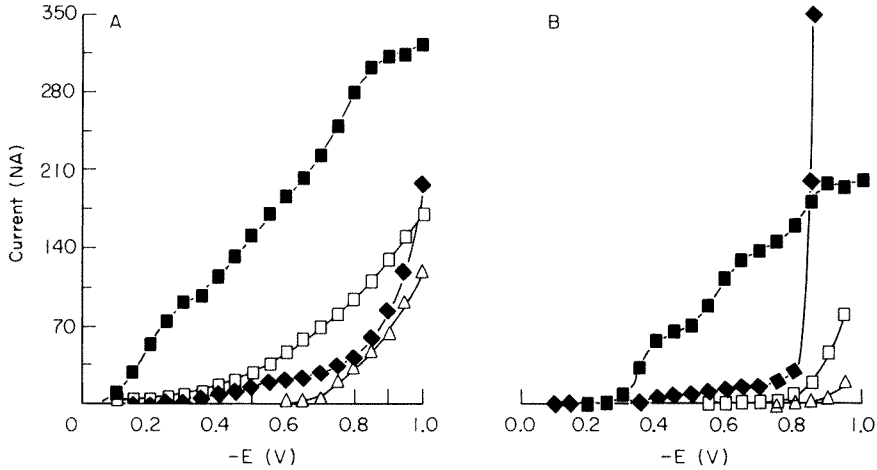
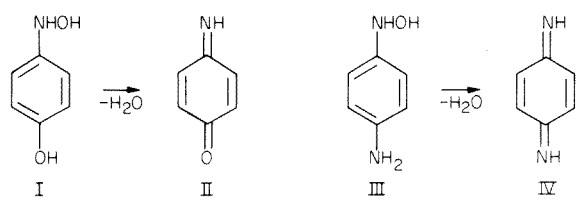


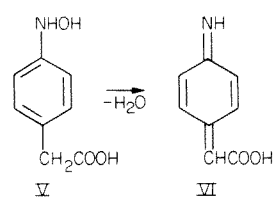
Fig. 4. Hydrodynamic voltammograms of RDX (△), TNT (▪), nitroglycerin (□), and background current (♦) on gold/mercury (A) and glassy carbon (B) electrodes. Conditions: Biophase C_8 reverse phase column; 0.05 M monochloroacetic acid, 0.037 M sodium acetate, 0.001 M EDTA, 18% (v/v) 1-propanol, 5% (v/v) ethanol, pH 3.5; 2.0 ml min^{-1}.

and polynitro aromatic compounds have shown repeatedly that the reduction of the nitro aromatic group usually occurs in two steps to form an amine via the hydroxylamine.

Compounds such as *p*- and *o*-nitroaniline and *p*- and *o*-nitrophenols, which are capable of rapid dehydration of hydroxylamines to form quinoid forms (II and IV), undergo a single $6e^-$ reduction process, because the quinoid forms are easier to reduce than the parent nitro compounds



Recently, Raghavan et al. [38] showed that nitrophenylacetic acid is also reduced via a $6e^-$ process in aqueous solutions (pH 2—12), presumably as the result of the formation of quinoid intermediate (VI)



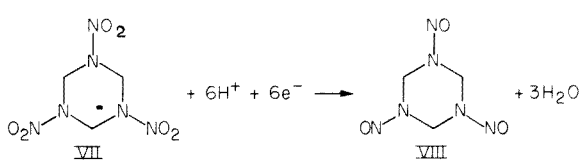
The reduction processes of polynitro aromatic compounds are much more complex and several reviews have been published [39, 40]. The mechanism depends on the number of nitro groups, and their relative positions on the rings, the nature of other substituents on the aromatic system, and the pH. Typically, *m*-polynitro isomers with or without any alkyl substituents undergo sequential $4e^-$ reduction of each nitro group followed by a partial or complete reduction of the resulting hydroxylamine groups depending on the pH. Tetryl, TNT, 2,4-DNT, and 2,6-DNT are reduced in this fashion in both static solution and flowing streams as illustrated in Fig. 2 and by the hydrodynamic voltammogram of TNT in Fig. 4. Picric acid and 3,4-DNT are reduced similarly to the *o*- and *p*-isomers by an initial $4e^-$ or $6e^-$ reduction of the first nitro group followed by a complete or a partial reduction of the remaining groups. Hydrodynamic results on Au/Hg at pH 3.5 indicate a partial reduction ($4e^-$) of the first nitro group in 3,4-DNT followed by a complete reduction of the second nitro group ($\approx 10e^-$ total) while picric acid undergoes a complete reduction of two nitro groups ($\approx 15e^-$). Pearson observed 16-electron reduction of picric acid [36] and 17-electron reduction of a related compound, styphnic acid [41]. It is interesting to note that picric acid was reduced in two steps indicating that two nitro groups are reduced simultaneously.

Cyclic voltammetric data for nitrate esters are summarized in Fig. 2. A stepwise reduction of each nitrate group is apparent with the reduction potentials being dependent on the number of nitrate groups present. The nitrate ester group is reduced via a $2e^-$ process which releases nitrite ion and alcohol [42]

$$RO{-}NO_2 + 2e^- + H_2O \rightarrow ROH + OH^- + NO_2^-$$

The hydrodynamic voltammogram of nitroglycerin on a Au/Hg electrode in Fig. 4 is complex, while on glassy carbon only the beginning of the first reduction wave is observed. Coulometric evaluation of chromatographic peaks of nitroglycerin (n_{eff} values) indicates $4e^-$ and $6e^-$ reduction of nitroglycerin at -0.90 V and -1.20 V, respectively.

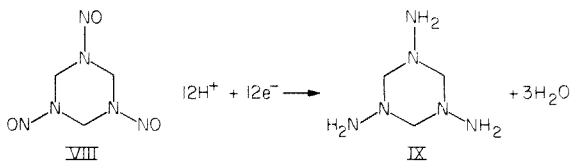
While there is a great wealth of information on the redox properties of nitro aromatic and nitrate esters, the opposite is true for nitramines. Lewis [43] observed a single $6e^-$ reduction wave for RDX (VII) in an acetone solution of alkaline sulfite to form VIII



VII VIII

Laviron and Fournari [44] reported a $6e^-$ reduction of simple nitramines derived from secondary amines. In acidic media, the reduction always proceeded via formation of the *N*-nitroso intermediate which was reduced

at more negative potentials. Hydrodynamic voltammograms of RDX at pH 3.5 on Au/Hg and glassy carbon in Fig. 4 are featureless; however, a comparison of the n_{eff} values obtained at −0.9 to −1.0 V with those of *p*-nitrophenol and *p*-nitroaniline indicates a $6e^-$ reduction of RDX in the potential region where the detector was usually operated for simultaneous detection of nitro aromatic, nitrate ester, and nitramine explosives. The cyclic voltammogram of RDX in Fig. 3A reveals a two-step reduction process. The second reduction peak occurs in the same potential region where *N*-dipropylnitrosamine (DPN) is reduced. Thus, the second reduction peak of RDX probably corresponds to a $12e^-$ reduction of VIII to form IX



VIII IX

Because the reduction of VIII occurs near the solvent breakdown, it was not possible to obtain more detailed information about the reduction mechanism either by cyclic voltammetry or hydrodynamic voltammetry with the mercury film amperometric detector. It is also possible that VIII is reduced via a stepwise, $4e^-$ reduction of each nitroso group in the same manner as reported for alkyl nitrosamines [45].

Liquid chromatography

Figure 5 illustrates the isocratic separation of three classes of explosive compounds using a ternary solvent with a nonpolar stationary phase. Under these chromatographic conditions, the separation of three DNT isomers could not be achieved unless the concentration of propanol or the column temperature were lowered at the expense of increasing the time needed for a complete separation. With the conditions adjusted to a 15-min separation, two out of three DNT isomers were resolved. The effects of propanol and dioxane on the capacity factors are illustrated in Fig. 6. Propanol was approximately 2.5 times stronger as an eluent than dioxane.

In propanol/ethanol ternary mixtures with pH 3.5 buffer, tetryl and TNT eluted ahead of DNT derivatives, but in dioxane/buffer mobile phases, the elution order was scrambled. Trinitrotoluene eluted after DNT isomers, and tetryl eluted with 2,4- and 2,6-DNT isomers. Dioxane/buffer mobile phases provided better separation of DNT isomers than propanol-containing mobile phases. Better overall separation of all three classes of explosive compounds was achieved with a propanol/ethanol/buffer mobile phase system when the separation time was kept below 15 min. It is interesting to note that the separation of tetryl and TNT in the propanol/ethanol/buffer system is improved with increasing concentration of propanol, while the opposite effect was observed in dioxane/buffer mobile phase systems.

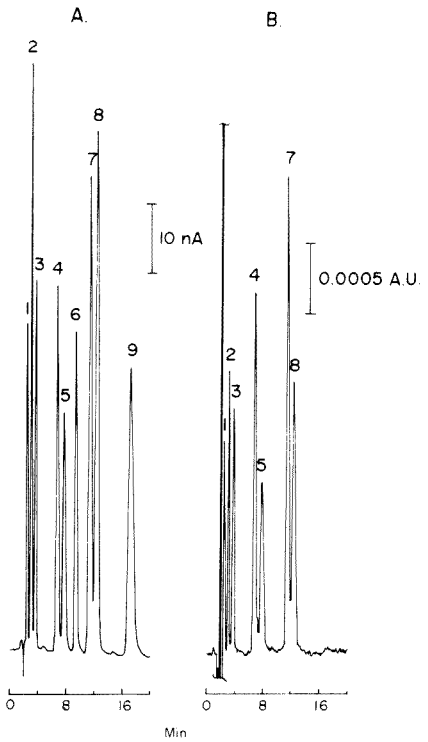


Fig. 5. Comparison of electrochemical and ultraviolet detectors for a synthetic mixture of explosives. (A) E.c. detector, E = —1.00V vs. Ag/AgCl; (B) u.v. detector at 254 nm. The mixture contained 34.5 ng HMX (1), 14.3 ng picric acid (2), 23.2 ng RDX (3), 30.0 ng tetryl (4), 12.1 ng TNT (5), 46.5 ng nitroglycerin (6), 29.6 ng 2,4-DNT (7), 34.0 ng 2,6-DNT (8), and 81.7 ng PETN (9) in a 20-μl injection. Conditions: Biophase C_{18} column, 0.02 M monochloroacetic acid, 0.0147 M sodium acetate, 0.001 M EDTA, 5% (v/v) ethanol, 17% (v/v) 1-propanol, pH 3.5, at 1.7 ml min^{-1}.

Liquid chromatography with ultraviolet absorption detection usually offers adequate sensitivity for nitro aromatic compounds, but the determination of several nanograms of nitrate esters is difficult as illustrated in Fig. 5. The detection limits and the capacity factors of explosives studied under the same chromatographic conditions as in Fig. 5 are summarized in Table 1. For nitroglycerin, the Au/Hg amperometric detector gave almost five hundred times lower detection limits at S/N = 3 than the u.v. detector at 254 nm. Detection limits of nitrate esters by u.v. can be improved by using shorter wavelengths. Crouthamel and Dorsch [46] reported detection limits of 30 ng of nitroglycerin at 200 nm with 60:40 methanol/water mobile phase, while Prime and Krebs [22] obtained a detection limit of 3 ng for ethyleneglycoldinitrate at 200 nm with a (1 + 1) mixture of acetonitrile and water. These detection limits are still not competitive with the limits obtained with the Au/Hg amperometric detector and the detector selectivity is very

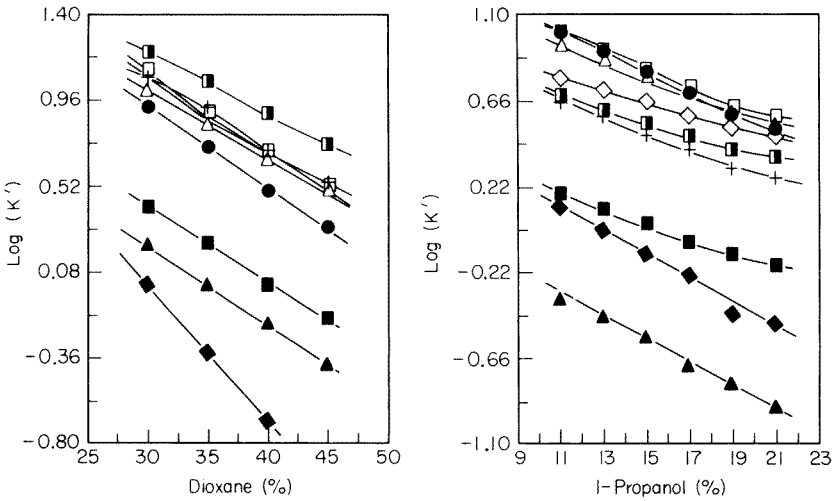


Fig. 6. The effect of dioxane and 1-propanol, present in the mobile phase, on the capacity factors of explosive compounds separated on a Biophase C_{18} reverse-phase column at 1.7 ml min^{-1}. (▲) HMX; (◆) PIC; (■) RDX; (+) TET; (▣) TNT; (◇) nitroglycerin; (●) 2,4-DNT; (□) 2,6-DNT; (△) 3,4-DNT.

TABLE 1

Summary of liquid chromatographic results on a C_{18} nonpolar stationary phase (Conditions as in Fig. 5)

Compound	k'	Detection limits (ng) at S/N = 3	
		U.v.	E.c. at −0.1 V
HMX	0.21	1.5	0.29
Picric acid	0.58	0.47	0.065
RDX	0.88	0.88	0.17
Tetryl	2.58	0.77	0.21
TNT	3.04	0.65	0.14
Nitroglycerin	3.96	160	0.38
2,4-DNT	4.96	0.57	0.16
2,6-DNT	5.00	1.2	0.17
3,4-DNT	5.50	1.3	0.15
PETN	7.90	—	0.40

poor at 200 nm. The l.c.—e.c. detection limit of nitro aromatic explosives can be improved by a factor of about three when the detector potential is lowered to −0.7 to −0.8 V and by optimization of the chromatographic conditions. Only a slight improvement in the detection limits of nitrate esters was observed by similar changes, while detection limits for nitramines were much worse. Detection limits may also be improved by decreasing

the area of the working electrode because higher signal-to-noise ratios are obtained [47].

Chromatograms of common explosive mixtures are illustrated in Fig. 7. No interference from electroinactive components was observed and the detection limits at S/N = 3 of COMP B, COMP C, C-4 and Flex X explosives were 1, 2.5, 0.6, and 3.5 ng, respectively.

Determination of propellants in gunshot residue

A g.c.—m.s. study of 33 common smokeless gunpowders completed by Mach et al. [48] revealed the presence of five major volatile components: nitroglycerin, 2,4-dinitrotoluene (2,4-DNT), diphenylamine (DPA), dibutylphthalate (DBP), and ethyl centralite (1,3-diethyl-1,3-diphenylurea). Initial effort in this work was focused on the determination of easily reduced components such as nitroglycerin (NG) and 2,4-DNT, and the minor components

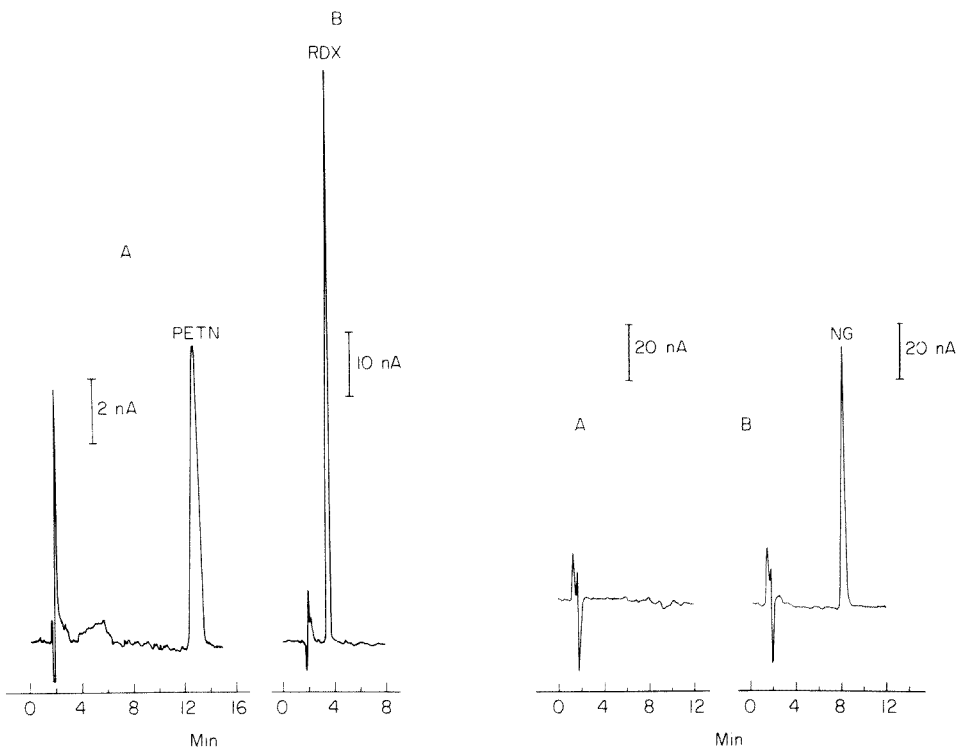


Fig. 7. Chromatogram of 50.7 ng of Flex-X (A) and 75.3 ng of C-4 (B) military explosives. Chromatographic conditions as in Fig. 4 with a Au/Hg electrode set at —0.85 V vs. Ag/AgCl.

Fig. 8. Chromatogram of a gunshot residue obtained before (A) and after (B) firing three 0.22-caliber Remington Long Rifle cartridges. Nitroglycerin (NG) peak corresponds to 81.9 ng injected. Conditions as in Fig. 4 with Au/Hg electrode set at —1.05V vs. Ag/AgCl.

2-nitrodiphenylamine (2-NDPA), 4-nitrodiphenylamine (4-NDPA), 2,6-DNT, and mononitroglycerin (MNG). The results of determinations on seven smokeless gunpowders are summarized in Table 2. Minor components (MNG and 2,6-DNT) found in gunpowders [48] are probably impurities of the major components or can be formed by a reaction of DPA with decomposition products of nitrocellulose and nitroglycerin during storage (2-NDPA and 4-NDPA). Mononitroglycerin and 2,6-DNT were not detected in the sampled gunpowders (at the 0.05% level), and therefore, their detection below this level was not attempted because their concentration in the gunshot residue would be too small to be of any practical value.

In the preliminary examination of gunshot residue, several shots were fired using a 0.22-caliber revolver. After the revolver had been fired, the residue was collected from the back of the firing hand with a cotton swab dipped in acetone. Only nitroglycerin was detected when Remington cartridges were used (Fig. 8) and both nitroglycerin and 2,4-DNT were detected after Western cartridges had been discharged (Fig. 9A).

It is expected that unburned flakes of gunpowder in gunshot residue usually retain their original composition of organic constituents [16]. Similar results were obtained in our studies as illustrated in Fig. 9. The levels of nitroglycerin detected varied depending on the person firing the weapon, the number of shots fired, the time after firing and the swabbed area. An attempt was made to bypass the problems of variation in the collection efficiency and levels of gunshot residue deposits among different personnel firing a revolver. The back of the firing hand was swabbed after each gun discharge for several shots in a row. The gunshot residue was detected only after the first swabbing even if 2—3 shots were fired after the initial swabbing. The loss of residue retention on the back of the firing

TABLE 2

Concentration of electroactive components in smokeless gunpowders quantified by l.c.—e.c. (Conditions as in Fig. 10)

Powder	Composition (g/100g)				
	NG[a]	2,4-DNT[a]	DPA[b]	2-NDPA[b]	4-NDPA[b]
DuPont Hi Skor[c]	26.7	1.44	0.61	0.055	0.062
Hogdon Top Mark[c]	23.4	0.18	0.66	0.15	0.081
Winchester Western Smokeless Powder[c]	10.3	0.42	0.62	0.098	0.079
Hercules Blue Dot[c]	20.3	—	0.58	—	—
Powder from Western Long Rifle[c] Extra Power .22 Rim Fire Cartridge	19.0	0.32	0.32	—	0.040
Powder from .22 Remington Long[c] Rifle Cartridge	28.6	—	0.13	—	—
DuPont 4198[d]	—	5.09	0.52	—	0.099
DuPont IMR 4227[d]	—	6.19	0.56	0.057	0.10

[a]Reductive l.c.—e.c. [b]Oxidative l.c.—e.c. [c]Double base. [d]Single base.

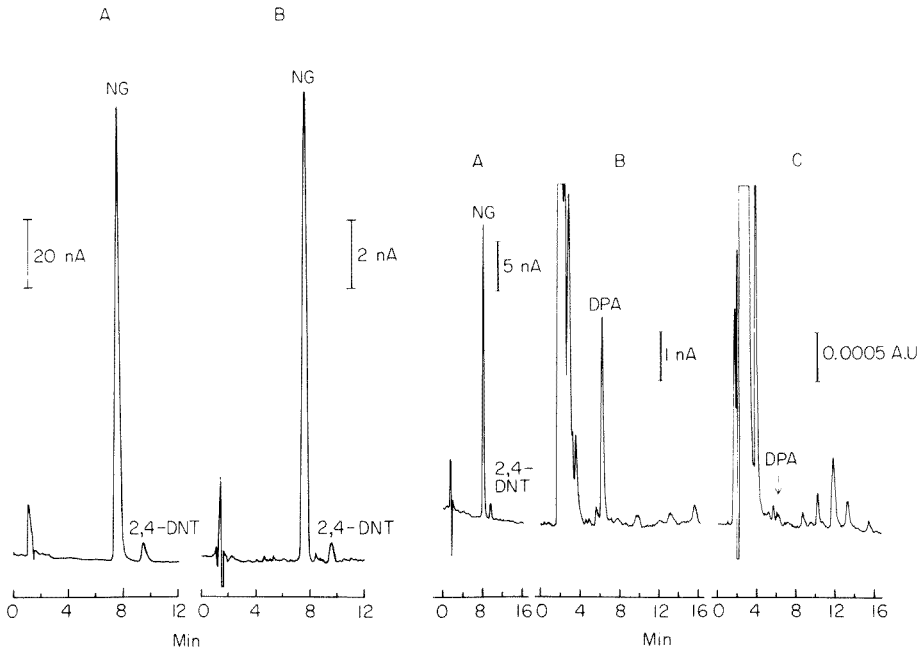


Fig. 9. Chromatograms of (A) swabbed sample after firing three shots using 0.22-caliber Western Long Rifle Extra Power Rim cartridge, and (B) 97 ng of gunpowder from 0.22-caliber Western Cartridge. Conditions as in Fig. 5 except at 2.2 ml min^{-1}.

Fig. 10. Comparison of e.c. and u.v. detectors for a gunshot residue. (A) Reductive mode e.c. detector, Au/Hg electrode at —1.00 V; flow rate 2 ml min^{-1}; other conditions as in Fig. 5. (B) Oxidative mode e.c. detection. (C) U.v. detection at 254 nm. Conditions: C_{18} Biophase reverse-phase column, 0.2 M H_3PO_4, 75% (v/v) methanol, pH 2.3 at 1.3 ml min^{-1}.

hand is probably due to removal of the lipid layer during swabbing with acetone. If sufficient time was allowed for the re-establishment of the lipid layer, residue was detected again.

To determine the stability of the gunshot residue to both chemical and physical interferences, several different persons fired the revolver and each hand was swabbed at different time intervals after discharging the weapon. Times ranged from 0—1 h. Immediately after firing, nitroglycerin levels were presumably fairly high because of visible unburned powder flakes on the skin. Later, as movement dislodged some particulate matter, the amounts decreased to lower but still detectable levels (700—800 ng), even though persons who fired the weapon were allowed to continue in all normal daily routines except washing their hands.

Detection of gunshot residue based on the use of reductive l.c.—e.c. to detect nitroglycerin on the back of the firing hand is, of course, applicable only if the cartridges used contain nitroglycerin. However, both types of smokeless gunpowder contain an aromatic amine, DPA. Aromatic amines

TABLE 3

Detection limits of diphenylamines

Compound	$E_{1/2}$[a] (V)	Detection limits (ng) at S/N = 3	
		Oxidative l.c.—e.c.[b]	l.c.—u.v.[b]
Diphenylamine	+0.97	0.039	1.36
2-Nitrodiphenylamine	+1.08	0.082	0.91
4-Nitrodiphenylamine	+1.05	0.070	0.92

[a] $E_{1/2}$ from hydrodynamic voltammograms on a glassy carbon electrode. [b] Conditions as in Fig. 10C.

are easily oxidized on carbon electrodes as reported in recent applications on the utility of oxidative l.c.—e.c. for the determination of pesticide residue [33].

The determination of DPA in gunshot residue is more difficult than the determination of nitroglycerin because the concentration of DPA in smokeless powders is much smaller than that of nitroglycerin (Table 2). In addition, oxidative mode chromatograms (Fig. 10B) are more complex than the reductive mode chromatograms because of interference from phenolic compounds which are present in the lipid layer on the skin, especially if handcream lotions that contain parabens [49] have been used. The determination of DPA in the gunshot residue is illustrated in Fig. 10B, C. Figure 10 also illustrates the relative merits (sensitivity and selectivity) of reductive and oxidative electrochemical detectors relative to the u.v. detector. Detection limits for the DPAs are listed in Table 3.

This method provides a simple, rapid, and inexpensive tool for determining components in explosives and gunshot residues. An advantage of this approach over the current metal residue methods is the lack of false positive results because there are no known environmental sources of nitroglycerin. During the course of this study, no easily reducible molecules were detected in the gunshot residue except gunpowder components. Detection of nitroglycerin and other components of smokeless powder is therefore indicative of firing a weapon or handling nitroglycerin-based explosives.

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QUANTITATIVE ENRICHMENT OF TRACE LEVELS OF IONS BY ELECTRODIALYSIS

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SUMMARY

The effect of ionic strength on the rate of ion transport across ion-exchange membranes under either constant potential or constant current conditions in electrodialysis precludes direct application of this method to quantitative enrichment studies. Addition of an excess of electrolyte to a sample to normalize the ionic strength permits either dialysis mode to be employed. The rate of ion transport into a receiver electrolyte becomes directly proportional to concentration over at least 2.5 orders-of-magnitude. The concentration range for which linear enrichment is achieved for cations extends down to the 10^{-7} M level with 30-min dialyses into a mixed 0.1 M $MgCl_2$—0.1 M HCl receiver. Alternatively, an internal standard approach can be used; however, the difficulty in matching the behaviour of membrane transport numbers as a function of ionic strength can limit the application of this approach.

Electrodialysis is performed by passing current through a sample that is separated from the electrode chambers by anion- and cation-exchange membranes [1, 2]. The properties of electrodialysis make it potentially applicable to quantification of trace ions in samples which require a prior separation and/or enrichment step. Simultaneous transport of anionic and cationic components into separate receiver chambers is a fundamental characteristic of the method. Multicomponent determinations are thus facilitated. The receivers are aqueous electrolytes which are well-suited for subsequent assay by most instrumental techniques.

Despite these properties, electrodialysis has not been utilized extensively as an ion separation and enrichment method. In a series of papers, Tsunakawa [3] employed exhaustive electrodialysis as a separation method. This approach is time-consuming and subject to error from uncertainty in definition of exhaustion. Because the membrane transport numbers are not unity, that point is actually a steady state, the position of which depends upon the sample composition.

Kinetic methods offer an alternative to equilibrium methods that can overcome the above-mentioned limitations. One goal of the present paper is to develop electrodialysis techniques that use the total analyte ion transport

during prescribed dialysis times as a basis for quantification. This approach constitutes a two-point, fixed-time kinetic method [4].

A second goal is to compare electrodialysis to Donnan dialysis as an analytical tool. It has been demonstrated that the latter is a useful enrichment and matrix normalization technique for the determination of trace levels of cations [5, 6] and anions [7—9]. Because electrodialysis is applicable to high ionic strength samples, it complements Donnan dialysis as a separation method. It was considered that electrodialysis may yield greater enrichment efficiency because of the ability to control the transport rate electronically. It is shown below that advantages that may accrue from the increase in transport rates that are attainable by electrodialysis are partially offset by increased difficulty in the quantification.

EXPERIMENTAL

Reagents and equipment

Reagent-grade chemicals were used throughout. The water was purified by passing distilled water through a series of ion-exchange cartridges (Research-Universal-Adsorber; Cole Parmer Instrument Company).

A conventional five-chamber electrodialysis cell [1, 2] was constructed from plexiglas. Each chamber had a 3.6-cm^2 cross-sectional area and a 1.4-cm width. The outer components contained 1-cm^2 platinum foil electrodes. The chambers were separated by alternating anion- and cation-exchange membranes across the 3.6-cm^2 gaps. Each contained a 0.5-cm i.d. tubular plexiglas inlet through which the receiver electrolyte (5 ml) could be transferred.

The central (sample) chamber was fitted with a pair of tubes so that the sample could be continuously cycled with a Manostat Varistaltic pump operated at 0.5 l min^{-1}. Sample volumes were typically 500 ml.

The dialyses were performed for 30 min unless otherwise stated. Sample cations were dialyzed into the chamber that was isolated from the sample by a cation-exchange membrane and from the platinum cathode by an anion-exchange membrane. The anion- and cation-exchange membranes were types P1025 and P1010, respectively (RAI Research Corporation, Hauppauge, New York). They were pretreated as previously reported [10].

The constant potential electrodialysis experiments were done with an operational amplifier potentiostat of convention design [11]. High-voltage operational amplifiers (Teledyne Philbrick Model 1032) were used with Teledyne Philbrick 2217 power supplies. Any convenient high-voltage source (0—100 V) could be used. The constant current experiments were done with either a Keithley Model 225 constant current source or an operational amplifier galvanostat [11]. In the latter case, the dialysis cell was put into the negative feedback loop of the operational amplifier so that the cell resistance did not influence the dialysis current.

Procedures

The cations were quantified by flame atomic absorption spectrometry with a Varian Model 475 instrument. Solutions in the receiver chamber were either aspirated directly after dialysis or were first diluted to 10 ml with additional electrolyte. Direct aspiration was appropriate only when an internal standard was used to compensate for volume changes that occur during dialysis. An enrichment factor was computed as the ratio of the analyte concentration in the 5-ml receiver after dialysis to the concentration in the initial sample.

Temperature studies and membrane area studies were done with a three-chamber cell. The sample (250 ml) was placed in a jacketed beaker. The receiver-electrolysis chambers were glass cylinders with an open end of each covered by an ion exchange membrane [7]. An O-ring and teflon tape held the membrane in place. Temperature was controlled with a Forma Temp Jr. refrigerated and heated bath and circulator. Glass cylinders of various diameters were used for the receiver compartments in the membrane area studies. The three-chamber cells were only used with non-electroactive test species.

RESULTS AND DISCUSSION

Constant potential electrodialysis (c.p.e.d.)

An ideal electrodialysis experiment can be performed by applying a constant potential to a cell that consists of a solution of a single salt separated from the electrodes by ion-exchange membranes with transference numbers of unity under all conditions. In this case, the dialysis current, and hence the ion flux, would be directly proportional to the sample concentration. If the dialysis time were so short that the change in the sample conductance was negligible, the total ions dialyzed during that time would be directly proportional to the sample concentration. The two-point, fixed-time kinetic model can be applied by correlating the concentration of the test ion in the receiver electrolyte after dialysis for a prescribed time to the sample concentration. Ideally, linear calibration curves should be obtained. The data are therefore analyzed by the linear least-squares method using

$$y = (a \pm s_a)x + (b \pm s_b) \quad (1)$$

where x is the initial molar concentration of the sample and y is the concentration of the test ion in the receiver after dialysis. The standard error of estimate, s_{yx}, and the correlation coefficient, r, are also computed. The slope, a, is the enrichment factor.

In practice, the ideal behaviour can be observed on single component samples over narrow concentration ranges. For example, twelve lithium chloride samples in the 2×10^{-5}—1.4×10^{-4} M range were dialyzed at 8.5 V for 30 min. The following fit to eqn. (1) was obtained: a, 0.72 ± 0.02; b, $(2.9 \pm 0.2) \times 10^{-6}$ M; s_{yx}, 3.5×10^{-6} M; r, 0.995.

When the experiment was repeated with samples in the range 1×10^{-5}—

1×10^{-3} M LiCl, a good fit to eqn. (1) was not obtained. The intercept increased to $(4.1 \pm 1) \times 10^{-5}$ M, and r decreased to 0.98. Two factors may cause the departure from the ideal model. Proton from water-splitting at the membrane/sample interface [12, 13] carries a greater fraction of the current at lower concentrations. Also, the transport numbers of ion-exchange membranes depend upon the dialysis current and the sample ionic strength and composition [14—16]. This interpretation is supported by noting the effect of addition of KCl on the c.p.e.d. of sodium ions (Table 1). The results indicate that sodium ions are more mobile than potassium ions in the membrane phase. Otherwise, the enrichment factor for sodium ion would have been decreased by having the greater dialysis current at higher ionic strengths offset by potassium carrying a larger fraction of the current through the cation exchange membrane.

The above suggests that if the sample ionic strength, composition, and conductance were normalized by addition of an excess of an electrolyte, a two-point, fixed-time kinetic model could be employed over a wide range. This hypothesis was tested by including 0.01 M NaCl in a set of fourteen copper(II) samples in the range 1×10^{-6}—1×10^{-4} M. At an applied potential of 7.2 V and with a 0.2 M $MgCl_2$ receiver, the fit to eqn. (1) was: a, 5.04 ± 0.07; b, $(-3 \pm 3) \times 10^{-6}$ M; s_{yx}, 6.9×10^{-6} M, r, 0.9996. These results demonstrate that c.p.e.d. may be used for quantitative enrichment of ions by using ionic strength normalization in conjunction with calibration curves prepared at a prescribed dialysis time. With receiver volumes much less than the sample volumes, enrichment factors comparable to those obtained by solvent extraction can be attained. Moreover, the eventual analytical determination is performed in a controlled medium, the composition of which is independent of that of the sample. The simple combination of ionic strength normalization and a calibration curve is not a very practical approach to quantitative electrodialysis, however. The uncertainty of the initial sample ionic strength except when investigating aqueous samples with low total dissolved solids would often make it necessary to employ an internal standard or standard addition method for quantification with or without ionic strength normalization. The standard addition method is of limited analytical utility with electrodialysis because of the possible non-

TABLE 1

Effect of ionic strength of the 1×10^{-5} M Na^+ sample solution on the c.p.e.d. transport rate
(Conditions: 24 V; 30-min dialyses; 800-ml samples; 5-ml receivers.)

Ionic strength[a]	2.6×10^{-4}	3.6×10^{-4}	1.3×10^{-3}
Enrichment factor[b]	10.4	10.8	14.2

[a]KCl added to the analyte. [b]Concentration of analyte in receiver/initial sample concentration.

linearity of the working curves when ionic strength normalization is not used.

Ideally, the use of an internal standard would eliminate the need for ionic strength normalization. The criteria for selection of an internal standard ion for electrodialysis are the typical ones for chemical determinations. In addition, it must be considered that the transference numbers of ions in an ion-exchange membrane depend upon the dialysis current and the sample ionic strength and composition [14—16]; these dependencies vary from ion to ion. The analyte and internal standard must be matched in terms of the effect of the sample on the transport numbers in order to eliminate the effect of ionic strength.

Table 2 contains data which demonstrate the capability of the internal standard method to compensate for ionic strength variation. The selectivity factor is less variant when the receiver ionic strength is increased because the contribution of Donnan dialysis, which is independent of sample ionic strength over a wide range [7], to the net transport is greater. No attempt was made in the present study to identify ideal analyte/internal standard pairs.

Constant current electrodialysis (c.c.e.d.)

In an ideal cell, c.c.e.d. would not be applicable to quantitative ion enrichments unless exhaustive dialysis were employed. The total ion transported during any time increment would be proportional to the current but independent of sample concentration. In practice, the dialysis rate is an empirical function of concentration (Fig. 1) because of the contribution of proton from water-splitting [12, 13] to the total cation transport. The lower the sample concentration, the greater the fraction of the current carried by protons. It follows that with mixed samples, an increase of the ionic strength will decrease the analyte enrichment factor (Table 3).

As in the case of c.p.e.d., normalization of sample ionic strength leads to direct proportionality between the quantity of ion transported during a

TABLE 2

Quantification of electrodialysis by the internal standard method[a]

Sample ionic strength	M KCl, receiver	Enrichment factor		Selectivity factor[b]
		Na	Li	
2.6×10^{-4}	1×10^{-3}	9.4	8.2	0.88
1.3×10^{-3}	1×10^{-3}	16.6	12.7	0.77
2.6×10^{-4}	1×10^{-1}	14.6	13.1	0.89
1.3×10^{-3}	1×10^{-1}	16.3	14.3	0.88

[a]Samples: 1.0×10^{-5} M Na^+, 2.5×10^{-4} M Li^+, plus KCl to adjust ionic strength; dialysis times, 30 min. [b]Ratio of enrichment factors for Li and Na.

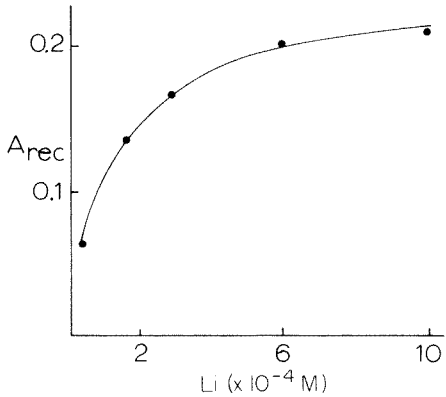

Fig. 1. Calibration curve for constant current electrodialysis with varying ionic strength standards. Samples, LiCl; receiver, 0.01 mM KCl; dialysis time, 30 min; current, 1.0 mA.

TABLE 3

Effect of ionic strength of the 1×10^{-4} M Na^+ sample solution on the c.c.e.d. transport rate
(Conditions: 1.0 mA; 30-min dialyses; 200-ml samples; 5-ml receivers. The receiver electrolytes were 0.05 M KCl.)

Ionic strength[a]	1.0×10^{-4}	2.0×10^{-4}	1.1×10^{-3}
Enrichment factor[b]	13.0	9.9	3.5

[a]KCl added to the analyte. [b]See footnote b, Table 1.

prescribed time and sample concentration, so that the two-point fixed-time kinetic model can be used. For example, a fit to eqn. (1) of 24-points in the range 1×10^{-6}—1×10^{-4} M Cu(II) with 0.01 M NaCl in each sample yielded the following with a 9.35 mA applied current: a, 4.46 ± 0.03; b, $(6 \pm 1) \times 10^{-6}$ M; $s_{y.x}$, 2.5×10^{-6} M; r, 0.9999.

The results do not suggest any advantage of the constant current mode; however, it can be predicted that c.c.e.d. would be more precise. The current and hence the dialysis rate, is controlled by the galvanostat and therefore not a function of the membrane and solution resistances. Also c.c.e.d. should not be influenced by the membrane areas and the temperature. The results that are summarized in Table 4 support this prediction. In practice, electrodialysis would be used in experimental designs where the membrane area would not be constant. For example, several cells would typically be used in parallel in the determination of ions in a batch of samples. The unavoidable variation in membrane area about the nominal value and of the sample temperature would be detrimental to the precision of the c.p.e.d. method.

TABLE 4

Effect of temperature and area on electrodialysis of lithium ions
(Conditions: dialysis time, 30 min; receiver, 0.05 mM KCl, sample, 0.1 mM LiCl.)

Constant current			Constant potential		
Signal (mA)	Variable and value	Enrichment factor	Signal (V)	Variable and value	Enrichment factor
0.3	*T*, 30°	1.88	35	*T*, 30°	1.35
0.3	*T*, 60°	1.82	35	*T*, 60°	2.02
1.0	*A*, 1.6 cm^2	2.32	25	*A*, 1.6 cm^2	2.81
1.0	*A*, 2.4 cm^2	2.35	25	*A*, 2.4 cm^2	4.53

Applications

For applications of electrodialysis to the enrichment of trace level samples, an important consideration is to maximize the enrichment factors. The effects of the magnitude of the applied electrical signal and of the composition and concentration of the receiver electrolyte were examined. The receiver electrolyte can influence the enrichment factor by partially controlling the current in c.p.e.d. and by causing Donnan dialysis. In addition, the affinity of the receiver cation for the sulfonate sites on the membrane seemingly affects the transport process in electrodialysis in a manner similar to the case of Donnan dialysis [5]. A series of experiments was performed with copper(II) samples and various receiver electrolytes. The c.c.e.d. mode (9.35 mA) was used to eliminate the effect of resistance on the dialysis rate. The sample and receiver volumes were 800 and 5 ml, respectively. The copper concentrations which resulted in 1% absorption with various receivers were 1×10^{-5} M (0.1 M KCl receiver), 2×10^{-6} M (0.1 M H_3PO_4 receiver), and 3×10^{-7} M (0.2 M $MgCl_2$—0.05 M HCl mixture as a receiver). The comparable 1% absorption value was 1×10^{-6} M Cu(II) for measurements without enrichment.

The $MgCl_2$—HCl receiver also yielded more reproducible enrichments. The copper(II) concentration which yields an absorbance of twice the uncertainty in that value after dialysis was used as the detection limit. This parameter was primarily established by the variation in the membrane transport from trial to trial rather than by uncertainty in the atomic absorption step. With a 0.2 M $MgCl_2$—0.05 M HCl receiver, the detection limit was 5×10^{-7} M Cu(II); with 0.1 M KCl, it was 1×10^{-5} M Cu(II); and with a 0.1 M H_3PO_4 receiver, it was 4×10^{-6} M Cu(II). That magnesium(II) has a higher affinity for sulfonate than does copper(II) apparently minimizes hold-up of the latter on the membrane and allows it to be transported across the membrane more rapidly. Similar to Donnan dialysis [4, 6], the optimum electrodialysis receiver electrolyte is an acidified magnesium(II) solution. Addition of aluminum(III) that is used for Donnan dialysis [4] was not found to be necessary for electrodialysis.

A study of the effect of the magnitude of the applied signal on the enrichment factor was made. The sensitivity was nearly proportional to the current. For example, c.c.e.d. yielded enrichment factors for lithium of 1.57 and 2.66 for 0.40 mA and 0.80 mA, respectively. With sodium samples at 0.50 and 1.00 mA currents, the respective enrichment factors were 3.25 and 6.25. The highest current used was 99.9 mA. For this study, seven copper(II) chloride samples in the range 1×10^{-6}—1×10^{-5} M, with 0.01 M NaCl added to each, were used. The receiver electrolytes were 0.2 M $MgCl_2$—0.05 M HCl mixtures. The sample and receiver volumes were 800 ml and 5 ml, respectively. The following fit to eqn. (1) was obtained: a (enrichment factor), 15.1 ± 0.2; b, $(1.4 \pm 0.1) \times 10^{-6}$ M; s_{yx}, 2.1×10^{-7} M; r, 0.9999. These values demonstrate that greater enrichment factors occur at higher current and that the magnesium chloride receiver yields greater precision. Without an applied signal, only Donnan dialysis occurs. Under comparable conditions (but without added sodium chloride), an enrichment factor of 6.92 was attained.

To test for possible matrix effects on the dialysis rate, electrodialysis was applied to a lake water sample. The test ion was copper(II), and nickel(II) was included as an internal standard. The original sample did not contain detectable levels of these ions, so a series of spiked samples was used. The water was filtered with Whatman no. 42 paper and Gelman 0.45-μm sterilized membranes. Seven samples were prepared to contain 1×10^{-5} M $NiCl_2$ and 5×10^{-7}—2×10^{-5} M $CuCl_2$. The c.c.e.d. mode at 40.0 mA for 30 min was used with sample volumes of 750 ml and 5-ml portions of 0.1 M $MgCl_2$—0.01 M HCl were used in the receiver. A fit of the internal standard working curve to eqn. (1), but with y equal to [Cu(II)]/1×10^{-5} after dialysis, yielded the following: a, $(7.44 \pm 0.07) \times 10^4$; b, $(2.4 \pm 0.6) \times 10^{-3}$; r, 0.9994, s_{yx}, 1.89×10^{-2}. Without considering the internal standard, the following fit to eqn. (1), with y equal to the copper(II) in the receiver after dialysis, was obtained: a, 14.1 ± 0.3; b, $2 \pm 2 \times 10^{-6}$ M; r, 0.998; s_{yx}, 6.6×10^{-6} M. Thus a 10^{-6} M Cu(II) sample is enriched to $(1.4 \pm 0.7) \times 10^{-5}$ M if the internal standard correction is not made. With the internal standard, the related y-axis reading is 7.4 ± 1.2; therefore, the precision is significantly improved by the inclusion of the internal standard. Further, without the internal standard, the uncertainty of the ionic strength makes the slope a function of the nature of the sample.

When the experiment was repeated without an internal standard but with 0.05 M NaCl added to all solutions, a slope within 5% of that with laboratory standards prepared in distilled water was obtained. The potential value of ionic strength normalization is thus demonstrated. Also, it can be concluded that no short term deleterious effects of the lake water matrix on the transport properties of the membranes are evident.

The present study has demonstrated that electrodialysis can be employed for ion enrichment. In comparison to Donnan dialysis, higher enrichment factors are attained, but the quantification procedure is not as straight-

forward. Internal standard and/or ionic strength normalization methods are required in order to obtain reasonable precision. At concentrations below 1×10^{-5} M, an internal standard should be used. Electrodialysis is advantageous with high ionic strength samples such as those which result when sample digestion is required. Considering its reportedly successful application to de-salting biological samples, electrodialysis may be applicable to quantitative separation of ions from physiological fluids using the general approaches developed herein.

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ELECTROCHEMISTRY AT CARBON FIBERS
Part 1. Characteristics of the Mercury Film Carbon Fiber Electrode in Differential Pulse Anodic Stripping Voltammetry

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SUMMARY

Carbon fibers are proposed as a support electrode for a mercury film electrode. The response of these electrodes is evaluated for use in differential pulse anodic stripping voltammetry. The mercury film is deposited in situ in aqueous solution and used to quantify cadmium in solutions of cadmium salts and organocadmium compounds in the 1—10 $\mu g\ l^{-1}$ (ppb) concentration range. The good resolution and extremely low background current obtained allow a limit of detection at 0.04 μg Cd l^{-1}.

Carbon fibers have recently received increasing attention as electrodes in voltammetry [1, 2]. Their small size, in combination with good electrical properties, has suggested various uses. It has been shown previously [3] that mercury film electrodes give increased resolution compared to the hanging mercury drop electrode (h.m.d.e.) and that carbon fiber electrodes exhibit a very small background current [2]; it seemed desirable to combine these two properties.

In this study, low-modulus carbon fibers were used as the support for a mercury film electrode used in differential pulse anodic stripping voltammetry (d.p.a.s.v.). Carbon fibers with no mercury film showed linearity and reproducibility in the mg l^{-1} (ppm) range, but not at the lower concentration range desired. The electrochemical properties to be discussed are potential window, useful concentration range, reproducibility, resolution, and limit of detection.

EXPERIMENTAL

Electrodes

The working electrodes were low-modulus, 8-μm diameter carbon fibers from polyacrylonitrile (Hercules). Single fibers were mounted, using silver conducting paint (G.C. Electronics 22-246), onto a 20-gauge wire (Belden 8529-100) stripped of insulation 1 cm back from the end (Fig. 1a). After a brief time, allowing the paint to dry, epoxy cement was applied to the bare wire. The remaining insulation was moved down the wire until it was even

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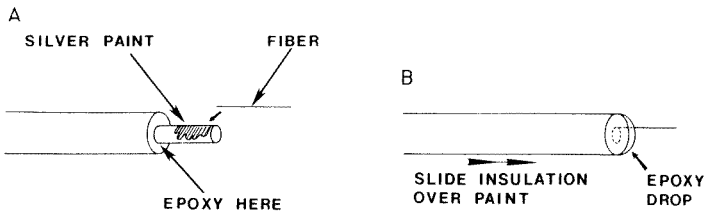

Fig. 1. Schematic representation of electrode preparation. (A) Attachment of fiber to wire; (B) application of insulation.

with the end of the wire (Fig. 1b). The fiber protruded from the "bead" formed by the epoxy. After curing overnight, this "bead" effectively insulated the wire and the silver paint from electrical contact with the solution. The fiber was then cut to a length of 0.5 cm and was ready for use. The epoxy and insulation exhibited no observable electrochemical affect, even after exposure to solution for many days. The reference electrode was Ag/AgCl (Microelectrodes MI-401); the auxiliary electrode was a platinum wire. All potentials reported are vs. Ag/AgCl.

Reagents

A buffer consisting of 10% (v/v) 95% ethanol/doubly distilled water, 0.1 M in potassium phosphate (Baker) at pH 7.3 was used in all measurements. The buffer was purged with nitrogen and pre-electrolyzed over a mercury pool held at −1.5 V for 96 h. Cadmium acetate and other salts were reagent grade or better (Fisher Scientific Company). The model compound used for this study was 4-cadmium (acetate)-estriol (4-CdE3) prepared in this laboratory [4] from estriol (Sigma, Grade III).

Procedure

All d.p.a.s.v. was performed using a PAR model 174A Polarographic Analyzer in combination with a PAR model 315 Automated Electroanalysis Controller. All solutions consisted of 10 ml of buffer, 0.075 ml of 100 ppm mercury(II) acetate solution (750 ppb in Hg(II)) and appropriate additions of analyte from 1 ppm stock solutions. Solutions were purged with nitrogen for 15 min and were held under a nitrogen atmosphere throughout the measurement step. A conditioning potential of +0.18 V was applied to the working electrode for 90 s and a deposition potential of −1.3 V was held for 150 s with stirring and 30 s without stirring, immediately after which an anodic scan was begun at a rate of 5 mV s^{-1}.

RESULTS AND DISCUSSION

The low-modulus carbon fibers chosen for the investigation of the new "micro" mercury film electrode exhibited the same low background current as high-modulus fibers, but were much less likely to break during mounting,

purging, or stirring operations. Their small size allowed samples as small as 10 μl to be monitored. The potential range of these electrodes, under the conditions described above, was found to be −1.4 to +0.2 V. There was a cathodic shift of peak potentials compared to the same analyte at a h.m.d.e.

Standard curve

Voltammograms were recorded on a set of 4-CdE3 solutions ranging in concentration from 1 to 10 ppb. A linear response of peak current was obtained. The response line had a slope of 5.3 ± 0.12 nA per ppb 4-CdE3, y-intercept of 0.1 ± 0.62 nA, $S_{y,x}$ = 0.73 nA, and correlation coefficient of 0.9993. The response was actually linear up to 100 ppb, except that the peak often split into two separate peaks at intermediate concentrations (20—50 ppb). This splitting is known to occur over narrow concentration ranges in other carbon and metal electrode systems [5, 6]. A preferential surface site mechanism, while the most probable explanation, has not yet been proven for this system. Further experiments are currently under way to determine the morphology and site speciation of low-modulus carbon fibers.

The response was also found to vary linearly with deposition time. For the data in Fig. 2, the regression equation of peak current vs. time was i_p = (17.40 ± 0.35 nA min^{-1})$t_{(min)}$ − (5.53 ± 0.68 nA) with $S_{y,x}$ = 0.73 nA and

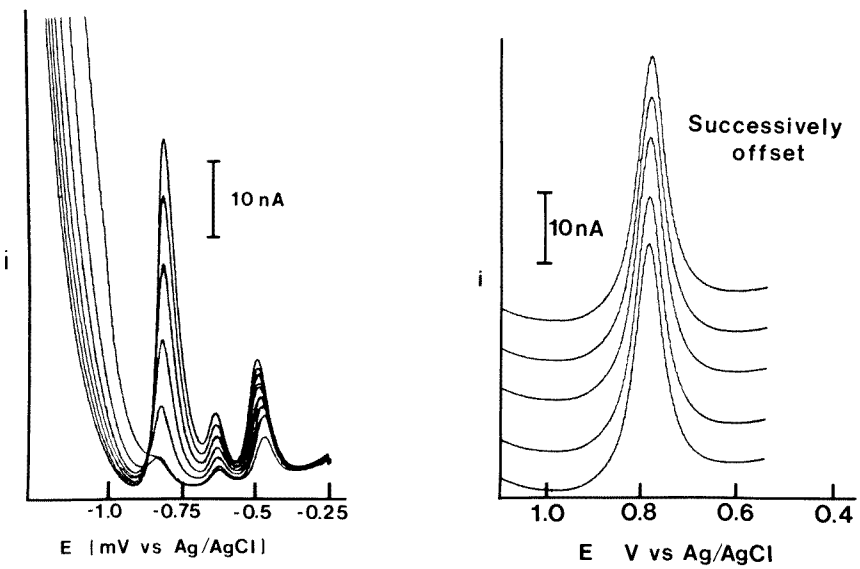

Fig. 2. Variation of peak height with deposition time at −0.8 V for 9.0 ppb 4-CdE3; conditions as described in text except for deposition time. Deposition times: 30, 60, 90, 120, 150, 180 s, bottom to top.

Fig. 3. Repetitive runs on solutions with the same concentration (7.0 ppb 4-Cd3). Each trace shown is offset and is the second run in each new solution processed through the entire procedure described in text.

$r = 0.9992$. There was no unstirred deposition time for this study. In the case of the sub-parts per billion concentrations of analyte, longer deposition times can readily be used. The other stripping peaks in Fig. 2 are for lead (ca. −0.6 V) and copper (ca. −0.4 V). The 4-CdE3 solution was not pre-electrolyzed because it showed negligible background of cadmium. It is apparent that under the conditions used here, the response is not linear with copper concentration.

Reproducibility and limit of detection

A two-factor simplex optimization [7] was performed to find optimal potentials for conditioning and deposition. Although all concentrations were related only to peak height, the response optimized was peak height divided by peak width at half height, for reasons of resolution. The conditioning potential (+0.18 V) was found to be the most critical factor. Variations of ±0.02 V led to considerable lack of reproducibility.

Replicate voltammograms were recorded on five different 7.0 ppb 4-CdE3 solutions (Fig. 3). The first voltammogram in each new solution was not usable, but all runs thereafter were indistinguishable. Results based on the second run of each solution gave a concentration of 7.09 ± 0.13 ppb at a 95% confidence level. It was found that the concentration of mercury(II) acetate could vary widely without influencing the current response for 4-CdE3. The 4-CdE3 peak height remains constant for mercury concentrations from 300 to 750 ppb.

The limit of detection was estimated by measuring the peak current for cadmium in five aliquots of a blank solution using the standard procedure. The blank was equivalent to 0.075 ± 0.0144 ppb. The minimum detectable difference between a mean sample and mean blank reading ($i_s - i_p$) was then calculated to be equivalent to 0.04 ppb at the 95% confidence level.

Resolution

Cadmium, lead, and copper all yielded anodic dissolution peaks in the potential range studied. The peaks were sharper, and therefore better resolved, at a fiber-based electrode than at a h.m.d.e., as expected. Each peak was shifted by the same amount from the h.m.d.e. peak potential. Because the volume of the mercury on the fibers was very small, the problem of intermetallic compound formation could have been serious. To investigate the possibility, a set of 10 ppb 4-CdE3 solutions was prepared with copper concentrations varying from 1.0 ppb to 1.0 ppm. The peak current for 4-CdE3 remained constant and complete resolution was retained for all solutions. The electrode proved to be far less sensitive for copper under these conditions.

It can be concluded that mercury film carbon fiber electrodes from low modulus fibers are cheaply and easily made, have long lifetimes, and possess excellent resolution and reproducibility. The stability and size of these electrodes suggest a variety of uses. A minimum of care in handling is sufficient, in contrast to the care necessary for use of a h.m.d.e.

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THE COULOMETRIC GENERATION OF MANGANESE(II) FROM A MANGANESE ELECTRODE: The Titration of EDTA

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SUMMARY

The anodic behavior of a manganese electrode is discussed. Manganese(II) can be generated with greater than 95% current efficiency over a limited current density range in neutral aqueous and methanolic chloride media. The current efficiency for the generation of manganese(II) as a function of current density, supporting electrolyte, solvent, pH, and generation time is given. Electrogenerated manganese(II) is useful in the coulometric titration of 4.460×10^{-6}—2.214×10^{-5} mol of EDTA in 1.0 M sodium chloride buffered at pH 5.5. The end-point can be detected amperometrically by monitoring the anodic current from the oxidation of manganese(II) at platinum. The titration error is about 1% in replicate determinations. Permanganate can be generated in aqueous sodium hydroxide media but the current efficiencies are less than 20% and irreproducible.

Some corrosion and anodic dissolution studies of manganese and its alloys in various media have been performed for the purpose of industrial synthesis [1—3] and to investigate reaction kinetics [4—7]. Melendez and Brenet [7] observed the formation of manganese(II), MnO_2, and permanganate in phosphate media depending on the pH and the potential at which the manganese electrode was held. Zaretskii et al. [1] obtained a yield of permanganate of about 40% when currents greater than 20 A dm^{-2} were passed through a ferromanganese anode immersed in aqueous 4 M sodium hydroxide. In a process to refine manganese from a ferromanganese anode, they observed yields of manganese(II) of up to 80% in acidic sulfate media and up to 100% in chloride media using currents ranging from 200 A dm^{-2} to 1000 A dm^{-2}. Giraitis et al. [2] have produced manganese(II) cyclopentadienyl compounds by applying 20—30 V between a manganese anode and a copper cathode in tetrahydrofuran solutions containing sodium cyclopentadienyl. Kharabadze [3] synthesized tri- and tetra-valent sulfates of manganese by passing high currents through a manganese anode in concentrated sulfuric acid media.

Several metals have been investigated as coulometric titrant sources [8—18] and it is surprising that no such studies have been made on manganese in view of the above literature citations, and the potential utility of manganese

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ions for quantitative applications. The present paper demonstrates that manganese is a suitable electrode for coulometric generation of manganese(II) in aqueous and nonaqueous chloride media despite some self-dissolution. The electrogenerated manganese(II) can be utilized to titrate various substances such as EDTA.

EXPERIMENTAL

Apparatus

The constant-current source was a Sargent Model IV coulometric current source standardized against a Leeds-Northrup 10-ohm precision resistor using a Sargent potentiometer.

Potential—current curves were obtained with the coulometric current source and a Fluka Model 8120A multimeter. A Sargent—Welch polarograph was used to measure indicator currents in the titration of EDTA with electrogenerated manganese(II).

Electrochemical studies were performed in a Metrohm Model EA880 universal titration vessel. A Sargent saturated calomel electrode (s.c.e.) was used as the reference electrode. Sargent platinum flag electrodes of about 1 cm^2 were used as counter and indicator electrodes. The counter and reference electrodes were isolated from the solution by fine-porosity isolation tubes. All solutions were purged with prepurified nitrogen prior to electrochemical studies. Solutions were stirred with a magnetic stirrer.

Materials

All chemicals were reagent grade and deionized water was used to prepare aqueous solutions.

Disodium (ethylenedinitrilo)tetraacetate dihydrate (Baker Analyzed Reagent; 100.0%) was used to prepare EDTA solutions. These solutions were standardized with calcium carbonate.

Manganese electrodes were fabricated by sealing polished rectangular manganese plates (Alfa Products, 99.9% purity) into alligator clips with Pyseal (Fisher Scientific Company, Catalog No. C-228). The final exposed areas were about 2 cm^2. Electrodes were conditioned between generations by sanding with successively finer grades of emery paper.

Procedures

Titration conditions. Titrations with coulometrically generated manganese(II) were performed in solutions containing 1.00 mol l^{-1} sodium chloride buffered at pH 5.5 with acetate buffer. The end-point of the titration was detected by applying a potential of +0.90 V (vs. s.c.e.) to a platinum electrode and measuring the resulting current.

Determination of current efficiency. The current efficiency was obtained by an EDTA titration [19]. Corrections for self-dissolution were made where noted. The normal self-dissolution of manganese as a function of time

in various media was determined by immersing manganese electrodes (approximately 2 cm^2) in the stirred and purged (deoxygenated) solutions of interest. The amount of manganese(II) that entered the solution was found by EDTA titration.

RESULTS AND DISCUSSION

Current efficiency studies

Aqueous chloride media. In the present work, studies showed that chloride solutions were satisfactory media for the generation of manganese(II). Figure 1 represents the potential—current behavior of manganese in aqueous sodium chloride medium. The anodic limit of manganese is quite negative which indicates that manganese readily dissolves anodically.

The Pourbaix diagram for manganese [20] shows that it was likely that manganese(II) was being generated at these negative potentials (see Fig. 1). The addition of 1.0 M sodium hydroxide to the above solutions yielded a white gelatinous precipitate, which turned brown on exposure to air; this supports the conclusion that manganese(II) is present.

Figure 2 shows the current efficiency for generation into aqueous sodium

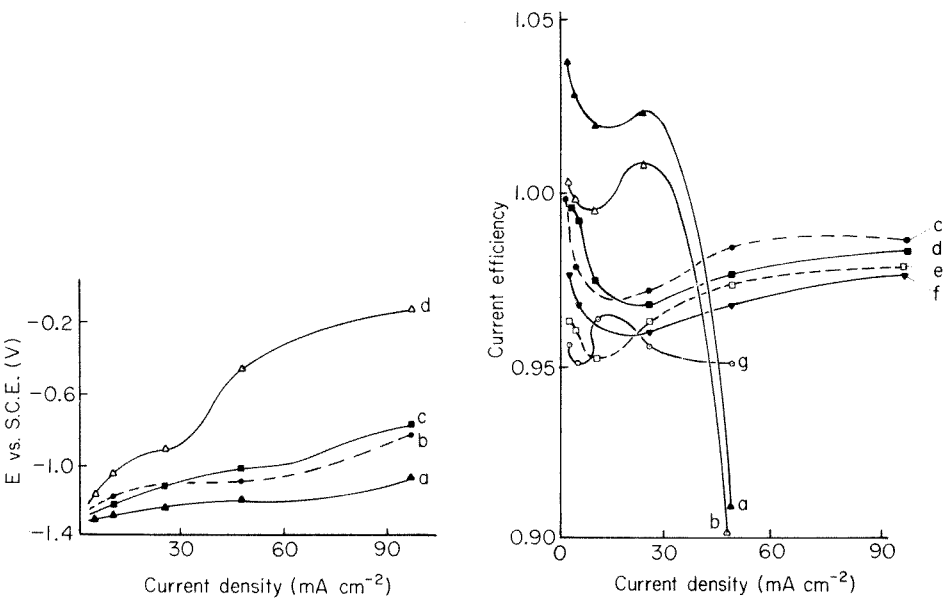

Fig. 1. Potential of a manganese anode as a function of current density: (a) 2.5 M NaCl; (b) 1.0 M NaCl; (c) 0.5 M NaCl; (d) 0.1 M NaCl.

Fig. 2. Current efficiency as a function of current density for the generation of manganese(II) from a manganese anode in NaCl solutions: (a) methanolic 1.0 M LiCl; (b) methanolic 1.0 M LiCl with data corrected for normal self-dissolution; (c) 2.5 M NaCl; (d) 1.0 M NaCl; (e) 1.0 M NaCl with data corrected for normal self-dissolution; (f) 0.5 M NaCl; (g) 0.1 M NaCl.

chloride solutions calculated for a two-electron oxidation of manganese. A comparison of curves (d) and (e) indicates that the contribution of self-dissolution to the current efficiency was small.

The purged electrolysis solutions were clear and colorless during generation but rapidly turned brown when exposed to air, presumably because of MnO_2 formation. A thin brown film (probably MnO_2) was observed to form on the manganese electrode during generation. However, no other colored species were observed to form at the manganese—solution interface during electrogeneration, and the thin brown film adhered to the electrode surface.

In addition, a small amount of gas discharge was noted at the manganese anode. The gas was possibly hydrogen formed by the reduction of water at the negative potentials the manganese anode acquired during generation. Further evidence for the reduction of water according to $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ was the fact that the pH of the electrolysis solutions increased during generation, even though the cathodic compartment was isolated.

It was concluded from a consideration of the Pourbaix diagram, simple chemical tests, visual observations, and current efficiencies close to 1.00 for a 2-electron oxidation of manganese, that manganese(II) was the principal manganese species entering solution.

The current efficiencies were similar at most sodium chloride concentrations and slightly less than 100%. In 0.1 M NaCl at high current density, the current efficiency decreased rapidly because of power lost to IR heating.

It should be noted that despite the side-reactions which occurred during the generation of manganese(II), the current efficiencies were reproducible. The current efficiencies for five 24.50-C electrolyses at 19.30 mA in 1.0 M sodium chloride were 0.9743, 0.9779, 0.9725, 0.9717, and 0.9752. These results were typical.

Methanolic chloride media. The current—potential behavior of manganese in methanolic 1.0 M lithium chloride was similar to that in aqueous 1.0 M sodium chloride.

Figure 2 shows the current efficiency (curve a), and current efficiency corrected for normal self-dissolution (b), for generations in 1.0 M lithium chloride for the 2-electron oxidation of manganese. These generations were also reproducible at a given current density. The greater than 100% current efficiencies can be accounted for by self-dissolution. The current efficiencies decreased at higher current densities because of IR heating.

The current efficiency for generation into the methanolic medium is greater than its aqueous analog. This is not surprising in view of the fact that no gas was noted at the manganese anode as in the aqueous solutions, and a thinner deposit formed on the manganese in the methanolic chloride medium. Furthermore, the slightly greater complexing power of chloride ion in methanol [21] for manganese(II) over the aqueous case should lead to higher current efficiencies in the methanolic medium.

It is obvious that manganese(II) can be generated successfully into methanolic chloride media.

Effects of pH. Figure 3 (curve a) illustrates the effect of pH on current efficiency in aqueous 1.0 M NaCl solutions. These data were obtained by passing 48.20 mA for 514 s, and were very reproducible at a given current density. In the relatively flat region between pH 4 and pH 7, the current efficiency varied from 0.980 to 0.950. The high current efficiency values at the lower pH range can be attributed to self-dissolution. The low current efficiencies at the higher pH levels were the result of the formation of a passive $Mn(OH)_2$ coat.

Effect of generation time. Figure 3 (curve b) shows that the current efficiency for the generation (current density: 48.20 mA cm^{-2}) of manganese(II) in aqueous 1.0 M sodium chloride (pH 6.3) declines slowly as a function of time in a nonlinear manner. This decrease in current efficiency can be explained by the buildup of an oxide coat on the manganese surface (see below) which retards electron transfer. Therefore, the relationship between current efficiency and generation time should not be overlooked in applications.

Titration of EDTA

Hernandez Mendez et al. [22] titrated hexacyanoferrate(III) with a manganese(II)—cyanide complex generated by reduction of Mn(III) in 0.09—1.5 M potassium cyanide. The titration of EDTA was used to demonstrate the applicability of the electrogenerated manganese(II) in the present system. It was found necessary to purge and blanket the analyte solution with nitrogen to prevent the formation of MnO_2. Cathode isolation was essential to prevent the precipitation of $Mn(OH)_2$.

At the end-point, the current initially rose sharply, because of the oxidation of manganese(II) to MnO_2 and after a short period of time the current decreased because a coat of MnO_2 formed on the platinum indicating electrode. It should be noted that this method is an adaptation of that of

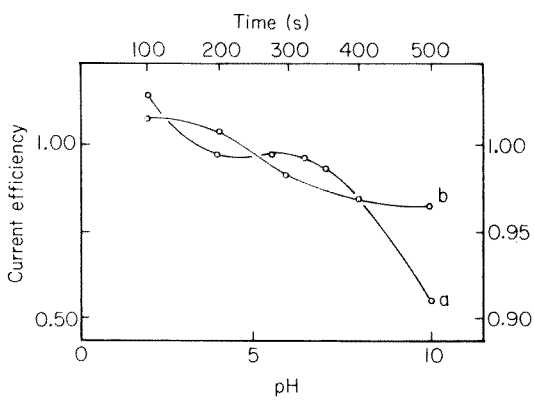

Fig. 3. Current efficiency for the generation of manganese(II) in 1.0 M NaCl as a function of pH (a), and generation time (b).

TABLE 1

Amperometric titration of EDTA with electrogenerated[a] manganese(II)[b]

EDTA taken (mol)	2.220×10^{-5}	1.122×10^{-5}	4.440×10^{-6}
End-point time (s)	221.4 ± 2.4	111.9 ± 2.2	44.6 ± 2.9
EDTA found (mol)	2.214×10^{-5}	1.119×10^{-5}	4.46×10^{-6}
Standard deviation (mol)	0.023×10^{-5}	0.022×10^{-5}	0.029×10^{-5}
No. trials	4	4	6
Relative error (%)	−0.27	−0.27	+0.45

[a]Generation current of 19.30 mA. [b] The EDTA concentration of the analyte solutions was approximately 5×10^{-4} M. The solution volumes ranged from 10 to 40 ml.

Khadeev and Shuartskop [23], who titrated manganese(II) with EDTA in acetate media of pH 5.5.

Aqueous medium. Preliminary titrations of known amounts of EDTA with electrogenerated manganese(II) showed that the titration efficiency was not time-dependent over the range 50—250 s using a current of 19.30 mA. Table 1 shows titration results for EDTA solutions. The average titration efficiency for all determinations of EDTA was found to be 1.00. Therefore, the calculations in Table 1 were made by using Faraday's Law directly without correcting for titration efficiency. Where conditions such as electrode purity, electrolyte concentration, current density, generation time, etc. differ from those stated herein, the titration efficiency may not be 1.00, and corrections should be made when applying the above method.

Other media. It was found that manganese(II) was reproducibly generated with close to 100% current efficiency in aqueous 1.0 M potassium thiocyanate and 0.1 M sodium perchlorate, ethanolic 1.0 M lithium chloride, methanolic 0.1 M lithium perchlorate, and 0.1 M lithium chloride in dimethylsulfoxide.

The current efficiencies for generations into 1.0 M sodium perchlorate were irreproducible and well over 100%. This phenomenon is currently under further investigation. It is likely that many other solvent-supporting electrolyte systems would be useful for the generation of manganese(II).

Generation of permanganate

The anodic formation of permanganate from manganese in aqueous hydroxide media is indicated by the Pourbaix diagram for manganese [20] and the previous work cited. However, in the present studies, the current efficiency for the generation of permanganate in sodium hydroxide solutions was found to be low (<20%) and irreproducible over the current density range 1—40 mA cm^{-2}.

It is interesting to note that the anodic limit of manganese in methanolic sodium methoxide occurred at approximately the same potential (+0.6 V vs. s.c.e.) as it did in aqueous 1.0 M sodium hydroxide. However, no visible activity was observed at the manganese anode in the methanolic case.

Measurements of the weight loss of a metal during generation showed that no manganese dissolved anodically at current densities up to 48.0 mA cm^{-2}. The oxidation in the methanolic methoxide system evidently proceeds with high current efficiency.

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KINETIC FLUORIMETRIC DETERMINATION OF IRON AND THALLIUM BASED ON OXIDATION TRANSFORMATION OF 1,4-DIAMINO-2,3-DIHYDROANTHRAQUINONE

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SUMMARY

Kinetic fluorimetric methods for the determination of iron(III) (0.05—0.6 ppm) and thallium(III) (0.05—0.4 ppm) are described. The fluorescent species obtained results from oxidative transformation of the reagent. The reactions are described and the experimental variables and interferences in each determination are reported.

No method for the determination of iron(III) based on the appearance of fluorescence is to be found in the literature. This is probably due to the slight tendency shown by this ion to form fluorescent products because of its intense paramagnetism. Some fluorimetric methods have been based on quenching phenomena [1—4], but none of these employs kinetic techniques. One method is available for the determination of thallium(III) based on the appearance of fluorescence using rhodamine B as the reagent [5, 6]. Two fluorimetric methods based on quenching caused by this cation on the fluorescence of uranyl sulfate and cochineal red have been described [1, 7]. As with iron(III), neither of these methods is kinetic in nature.

In the present paper, methods are described for the kinetic fluorimetric determination of iron(III) and thallium(III) based on the intense green fluorescence that appears when these cations react with 1,4-diamino-2,3-dihydroanthraquinone at an appropriate pH. The fluorescence is due to the oxidative transformation of the reagent in the presence of these cations.

Generally, the principal advantages of kinetic methods (to be exact, the initial rate method) over non-kinetic methods are the greater speed and selectivity of the former, because there is less time for the effects of the various interferences to become significant. Considering the sensitivity and selectivity of fluorimetric methods, the interest shown in the development of kinetic fluorimetric methods is understandable.

EXPERIMENTAL

Reagents and solutions

A 1,4-diamino-2,3-dihydroanthraquinone (KEK) solution (4×10^{-4} M, 0.1 g l^{-1}) was prepared in ethanol. A standard solution of iron(III), 1.7×10^{-2}

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M, was prepared from $Fe(NO_3)_3 \cdot 9H_2O$ (Merck) and standardized compleximetrically [8]. A thallium(III) chloride solution containing 0.981 g Tl l^{-1} was also standardized complexiметrically [8]. A pH 3.4 buffer solution (HCl—potassium hydrogenphthalate) was prepared by the method of Clark and Lubs [9].

Apparatus and measurement conditions

All spectrofluorimetric measurements were done with a Fika fluorescence spectrophotometer, model 55, MK II. The fluorescence intensity—time curves were obtained by fixing the excitation and emission wavelengths (see below) and using a constant movement of the chart paper (60 s cm^{-1}). A Crison digital pH meter with combined electrode, and a Perkin-Elmer 577 i.r. spectrometer were also used.

Procedure for the determination of iron(III) or thallium(III)

In a 50-ml beaker are placed 0.2 ml of 4×10^{-4} M reagent, 2 ml of pH 3.4 buffer and the volume of cation solution necessary for the final concentration of iron(III) to be between 0.05 and 0.6 ppm and for thallium(III) to be between 0.05 and 0.4 ppm. The final volume is adjusted to 4 ml, if necessary, by addition of deionized water before the addition of the cation (variation in the order of addition does not alter the result). All solutions are thermostatted previously at 20 ± 1°C. When 30 s have elapsed after the addition of the iron(III) or thallium(III), recording of the fluorescence intensity—time curve is started (λ_{ex} = 400 nm, λ_{em} = 470 nm). The duration of each measurement is ca. 10 min.

Isolation of the hydrolysis product

1,4-Diamino-2,3-dihydroanthraquinone (1 g) was dissolved in 200 ml of ethanol, and 1 ml of concentrated hydrochloric acid was added. The mixture was refluxed for 10 min. On cooling to room temperature, crystals appeared in the form of fine dark red needles. The product was recrystallized from ethanol.

RESULTS AND DISCUSSION

Kinetic studies

The reaction substrate produces a yellow fluorescence (λ_{ex} = 475 nm, λ_{em} = 550 nm) in neutral and slightly acidic media while the product obtained in the presence of iron(III) and thallium(III) has a green fluorescence (λ_{ex} = 400 nm, λ_{em} = 470 nm) in both media. Studies carried out to determine the stability of solutions of the reagent at different pH values showed that the reagent is very quickly transformed below pH 1.6, to a green fluorescent product. For pH values between 1.6 and 3.1, this transformation is slower although the speed of the process is noticeably influenced by temperature, thereby making this a pH range where measurements have poor reproduci-

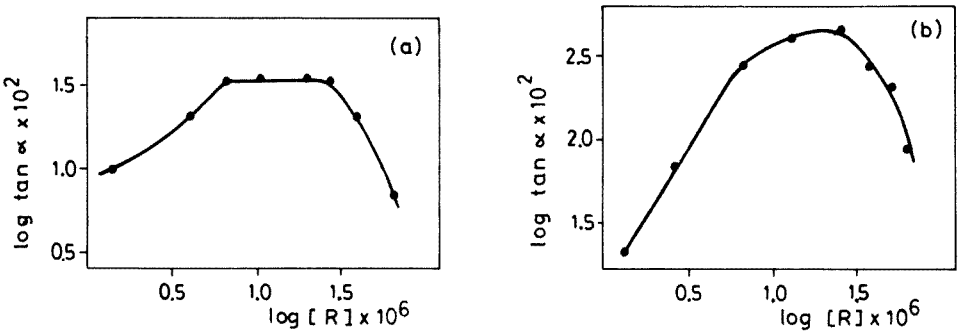


Fig. 1. Influence of the concentration of the anthraquinone on the initial rate: (a) 0.2 ppm Fe(III); (b) 0.5 ppm Tl(III): pH 3.4.

bility. This is less noticeable at higher pH values and for this reason a pH of 3.4 was chosen for further study. At pH 3.4, transformation of the substrate is negligible for at least 2 h.

The fluorescence produced by the reactions depends on the ethanol concentration in the mixture. Maximum fluorescence is produced at an ethanol concentration of 5%, and this concentration was chosen in further work.

In an attempt to optimize the concentration of 1,4-diamino-2,3-dihydroanthraquinone, for both cations, the concentration of the reagent was varied while the other variables were kept constant (Fig. 1). From the analytical point of view, the existence of a plateau in the reaction rate vs. concentration plot is very convenient because small variations in reagent concentrations in the plateau region do not influence the results. For this reason, a final reagent concentration of 2×10^{-5} M was chosen as optimum for the determinations of iron(III) and thallium(III).

Under these optimum conditions, a linear relationship exists between the initial reaction rate and the cation concentration in the range 0.05—0.6 ppm for iron(III) and 0.05—0.4 ppm for thallium(III) (Fig. 2). The kinetic data, resulting from the corresponding logarithmic plots (Figs. 1 and 2), are summarized in Table 1.

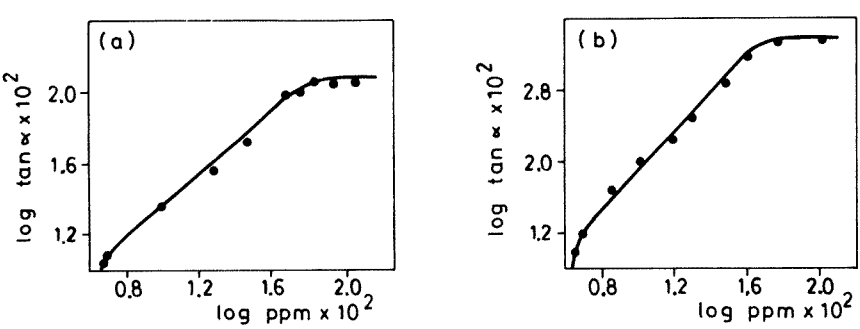


Fig. 2. Influence of the concentration of cation on the initial rate: (a) Fe(III); (b) Tl(III), with 2×10^{-5} M reagent, at pH 3.4.

TABLE 1

Summary of kinetic data

Iron(III)—1,4-diamino-2,3-dihydroanthraquinone (R) system		
$v_0 \propto [R]^1$	for	2.5×10^{-6} M < [R] < 6.7×10^{-6} M
$v_0 \propto [R]^0$	for	6.7×10^{-6} M < [R] < 26.9×10^{-6} M
$v_0 \propto [Fe^{3+}]^1$	for	0.05 ppm < $[Fe^{3+}]$ < 0.6 ppm
Thallium(III)—1,4-diamino-2,3-dihydroanthraquinone (R) system		
$v_0 \propto [R]^{3/2}$	for	1.3×10^{-6} M < [R] < 6.7×10^{-6} M
$v_0 \propto [R]^0$	for	13.5×10^{-6} M < [R] < 31.6×10^{-6} M
$v_0 \propto [Tl^{3+}]^2$	for	0.05 ppm < $[Tl^{3+}]$ < 0.4 ppm

Characteristics of the analytical methods

The fluorescence—time curves were recorded at the maximum excitation and emission wavelengths (λ_{ex} = 400 nm, λ_{em} = 470 nm) for different amounts of iron(III) and thallium(III) (Fig. 3), using optimum pH values and concentrations of reagent. These curves were later treated by various established kinetic methods (Figs. 4 and 5). The data obtained are shown in Table 2, from which it may be deduced that the highest sensitivity and precision are given by the initial rate method.

The selectivity of the methods was tested by obtaining the intensity—time curves in the presence of several foreign ions under the recommended conditions. It was found that the lowest level of interferences was given by the initial rate method. As can be seen in Tables 3 and 4, for the iron(III) method, Pt(IV), Ce(IV), Tl(III), Au(III) and V(V) interfere in concentrations similar to iron, while for the thallium(III) method, only Au(III), Fe(III) and Ce(IV) interfere.

Of the three methods obtaining kinetic data, the one presenting the best analytical characteristics, i.e. the smallest amount determinable, greatest precision and fewest interferences, is the initial rate method. For this reason

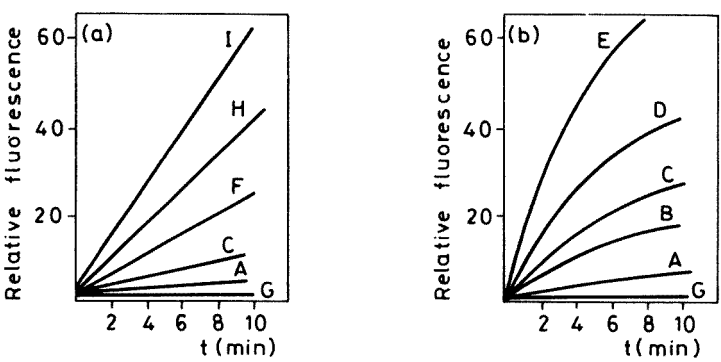


Fig. 3. Fluorescence intensity—time curves for various cation concentrations (ppm); (A) 0.05; (B) 0.07; (C) 0.1; (D) 0.15; (E) 0.2; (F) 0.3; (H) 0.5; (I) 0.7; (G) reagent blank. Otherwise as Fig. 2.

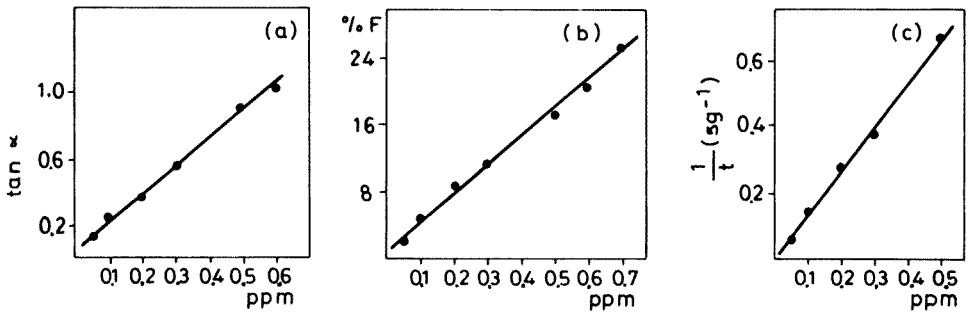


Fig. 4. Calibration graphs for Fe(III): (a) tangent method; (b) fixed-time method; (c) fixed intensity change method, using the recommended procedure.

this method is recommended. The greater sensitivity of this tangent method may be attributed to the shape of the intensity—time response which, for small concentrations, yields precise measurements of the tangent but not of the time and intensity for the intervals chosen. The greater selectivity of the tangent method against fixed-time and fixed intensity change methods, is a consequence of the fact that measurements are made soon after the reaction starts, and so there is probably not enough time for the various interfering effects to become significant.

Nature of the reaction and the product obtained

Further study of the reaction of iron(III) and thallium(III) with 1,4-diamino-2,3-dihydroanthraquinone was undertaken in order to clarify the nature of the reaction and the product formed. It was found that the reagent alone is transformed to a product giving an intense green fluorescence in acidic media. This transformation is very rapid for pH values below 1.6, is relatively rapid between pH 1.6 and 3.1 and is very slow for pH values above 3.1. It was also shown, by operating in an inert atmosphere and eliminating

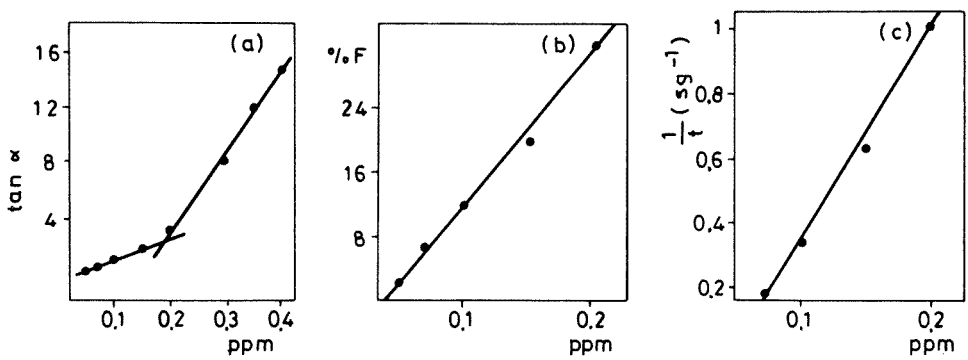


Fig. 5. Calibration graphs for Tl(III): (a) tangent method; (b) fixed-time method; (c) fixed intensity change method, using the recommended procedure.

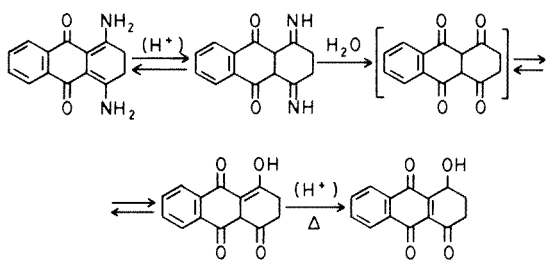
TABLE 2

Characteristics of the kinetic methods

Method	Range of applicability (ppm)	S.d.[a] (M)	C.v.[a] (%)	R.e.[a] (%)
Determination of iron(III)				
Initial rate	0.05—0.6	11.3×10^{-3}	3.8	2.7
Fixed time (4 min)	0.05—0.7	15.0×10^{-3}	5.2	3.7
Fixed intensity change (relative fluorescence = 8)	0.05—0.5	13.5×10^{-3}	4.7	3.4
Determination of thallium(III)				
Initial rate	0.05—0.2	4.3×10^{-3}	4.3	3.1
	0.2—0.4	6.1×10^{-3}	3.1	2.2
Fixed time (3 min)	0.05—0.2	6.9×10^{-3}	3.4	2.4
Fixed intensity change (relative fluorescence = 12)	0.07—0.2	7.4×10^{-3}	5.0	3.6

[a]Standard deviation, coefficient of variation, and relative error (100 t (s.d.)/$\bar{x}$ $n^{1/2}$, where t is Student's t test for 95% confidence) for n = 10 measurements in all instances.

dissolved oxygen from the solution, that the reaction takes place to the same extent as in aerobic conditions. This indicates that the process corresponds to non-oxidative acid hydrolysis of the reagent. The product was isolated and characterized by i.r. and mass spectroscopy and elemental analysis. It was deduced that the product is 1-keto-4-hydroxy-2,3,4-trihydroanthraquinone, resulting from the following reaction



Elemental analysis gave 70.5% C and 4.2% H (calculated for $C_{14}H_{10}O_4$, 69.4% C, 4.1% H). The i.r. spectrum showed bands corresponding to O—H stretching vibrations (3400 cm^{-1}) while bands assigned to N—H amine vibrations disappeared. The initial product showed a single band corresponding to a C=O stretch (1610 cm^{-1}) while the isolated product showed two bands attributable to two different carbonyl groups (1640 and 1620 cm^{-1}). The mass spectrum gave a molecular ion at m/z = 242, compared with the one at m/z = 240 for 1,4-diamino-2,3-dihydroanthraquinone itself. The base peak coincided with the molecular peak for the isolated product, which indicated high stability for the product, while for the initial compound the

TABLE 3

Concentrations of foreign ions tolerated (error ≤2.7%) for 0.3 ppm of iron(III), by the tangent method

Ion	Amount tolerated (ppm)	Ion	Amount tolerated (ppm)
Ca, Sr, Ba, Mg, Zn, Cd, Ni, Co(II), Hg(II), Pb(II), Mn(II), U(VI), Cr(III), Cl^-, SO_4^{2-}, NO_3^-	20	Pd(II)	1[b]
		Pt(IV)	0.5[a]
Ag	10[a]	Ce(IV)	0.1[a]
Al	2 (max)[a]	V(V)	0.01 (max)[a]
F^-	2[b]	Tl(III),	0.01[a]
Be, Cu(II)	1[a]	Au(III)	

[a]Higher concentrations increase fluorescence. [b]Higher concentrations cause quenching.

base peak (m/z = 238) corresponded to a species resulting from the loss of a molecule of hydrogen, producing the corresponding aromatic compound. It can be shown from these data that the 1,4-dihydroxy compound is not obtained, although its formation might be deduced from the above-mentioned process.

The study of the reaction products of thallium(III) and iron(III) with 1,4-diamino-2,3-dihydroanthraquinone showed that the fluorescent compounds formed have identical excitation and emission spectra. These spectra are also the same as those obtained for the product of the transformation of the reagent alone at pH values below 3 and also when reactions occur between the reagent and other cations of similar oxidation strength such as gold(III) and vanadium(V). Oxidizing agents such as hydrogen peroxide accelerate the formation of the fluorescent product.

Once the fluorescent product has formed, it can easily be transformed to non-fluorescent substances by the addition of an excess of the cation

TABLE 4

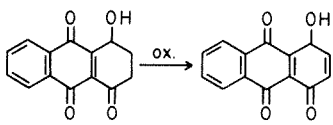
Concentrations of foreign ions tolerated (error ≤2.2%) for 0.2 ppm thallium(III), using the tangent method

Ion	Amount tolerated (ppm)	Ion	Amount tolerated (ppm)
Ca, Sr, Ba, Mg, Zn, Cd, Co(II), Ni, Pb(II), Mn(II), U(VI), Cr(III), SO_4^{2-}, $C_2O_4^{2-}$, F^-, Cl^-, NO_3^-	20	Cu(II)	1[a]
		Pd(II)	1 (max)[b]
Ag, Be, Al, Pt(IV)	20 (max)[a]	Au(III),	0.1 (max)[a]
Hg(II)	20 (max)[b]	Ce(IV)	
V(V)	5 (max)[a]	Fe(III)	0.01 (max)[a]

[a]Higher concentrations increase fluorescence. [b]Higher concentrations cause quenching.

(Tl(III), Fe(III), Au(III), V(V)) or oxidizing agent. This produces solutions of different colours to such an extent, that if the excess of the oxidizing agent is appreciable, clear solutions are eventually obtained. This also occurs when a hydrolysis product is treated with an excess of the cations mentioned above or with an oxidizing agent.

It can be deduced, firstly, that the hydrolysis product is not the same as the reaction product with thallium(III) and iron(III) since the latter seems to imply an oxidation although the two substances must be closely related and have similar structures since they have identical fluorescent spectra. If one considers the molecular structure of the hydrolysis product, it can be seen that it is easily oxidized by an aromatization process requiring only the loss of two hydrogen atoms



(I) (II)

Inasmuch as this oxidation implies a minimal structural change, it can be expected to show fluorescent characteristics similar to those of the substrate (I). This product (II) would be the fluorescent substance resulting from the reaction with the cations in question and the oxidizing agents. It is evident that the formation of this compound favours the hydrolysis reaction. This oxidation product, within a medium of considerable oxidizing strength, would be destroyed, giving way to other, more highly oxidized non-fluorescent substances.

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SPECTROPHOTOMETRIC DETERMINATION OF VANADIUM IN NATURAL WATERS AND ROCKS AFTER SELECTIVE ADSORPTION ON SEPHADEX GEL

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SUMMARY

Vanadium(V) ions are strongly adsorbed on Sephadex G-25 gel at pH 4.2, and are desorbed reversibly into 0.1 M acids. Vanadium present in rocks and in natural waters at $\mu g\ l^{-1}$ levels can be separated and concentrated by means of a gel column at pH 6 (in order to avoid the interference of molybdenum(VI)) and determined spectrophotometrically with 4-(2-pyridylazo)resorcinol. Interference by iron(III) can be suppressed by adding copper(II)—CyDTA. Large amounts of sodium chloride and potassium nitrate have little effect on the adsorption.

Sephadex gels, which consist of dextran cross-linked by epichlorohydrin, show high affinity for some oxo-anions [1—5], probably because of complex formation with the hydroxyl groups of the dextran matrix. Borate ions are adsorbed on Sephadex G-25 gel from alkaline media and desorbed reversibly into acidic media. This property has been utilized to concentrate boron selectively from natural waters and rocks [1]. The behavior of vanadium(V) at high concentrations on Sephadex G-10 gel has been investigated by column chromatography [4]. It has been reported that isopolyvanadates can be separated by a molecular sieve effect, and a tendency for chelation with the gel is observed to increase with decreasing pH values. However, detailed investigations relating to the behavior of vanadium(V) at low concentrations, when isopolymers of vanadium do not exist, have not been described.

In this study, a separation and concentration method for traces of vanadium(V) has been developed by using a Sephadex G-25 gel, which adsorbs vanadium(V) from solutions of pH 3—7 and desorbs it reversibly into acidic solutions. The vanadium(V) can be determined spectrophotometrically with 4-(2-pyridylazo)resorcinol (PAR) [6], which forms soluble complexes of high molar absorptivity with many metals and has appreciable selectivity for vanadium(V) in the presence of trans-1,2-cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (CyDTA).

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EXPERIMENTAL

Chemicals

All the chemicals used were of analytical grade and the water used was deionized.

Buffer—masking solution (pH 9.3). Dilute 38.5 g of ammonium acetate, 2.3 g of CyDTA (Dojin Pharmaceutical Laboratories) and 10 ml of ammonia (28%) to 250 ml with water.

Buffer—masking solution (pH 5.5). Dilute 19.3 g of ammonium acetate, 0.5 g of CyDTA and 2 ml of acetic acid (100%) to 250 ml with water.

PAR reagent (0.001 M). Dissolve 0.054 g of PAR (Dojin Pharmaceutical Laboratories) in 1 ml of 1 M sodium hydroxide and dilute to 250 ml with water. A 2×10^{-4} M solution is prepared by dilution with water as required.

Copper(II)—CyDTA solution (0.1 M). Dissolve 9 g of CyDTA, 4 g of sodium hydroxide and 4.5 g of $CuCl_2 \cdot 2H_2O$ in 250 ml of water.

Sample preparation

For water samples, filter the sample at the sampling point through a glass-fiber filter paper (Whatman GF/D) if necessary. Add 3 ml of 1 M acetic acid and 5 ml of the 0.1 M copper(II)—CyDTA solution to a 150-ml sample and adjust to pH 6 with ammonia solution.

For rock samples, fuse the sample (0.1—0.5 g) for 1 h with 2.5 g of sodium carbonate in a platinum crucible. After cooling, transfer the material to a 300-ml beaker, form a suspension in 150 ml of water by mechanical stirring, and filter. Wash the residue on the filter paper several times with 0.1 M sodium carbonate solution and collect the filtrate in a 200-ml volumetric flask. Add 1 ml of 1 M acetic acid and 5 ml of 0.1 M copper(II)—CyDTA solution to an appropriate aliquot of the solution, adjust to pH 2 with hydrochloric acid, shake to remove carbon dioxide and adjust to pH 6 with ammonia solution.

Separation and concentration of vanadium(V) on a Sephadex G-25 column

Use an acrylic resin column (16 mm i.d., 200 mm long) containing 32 ml of Sephadex G-25 gel. Condition the column with 0.02 M acetate buffer (pH 6) and then pass the sample solution through at a rate of 5 ml min^{-1}. Wash the column with 25 ml of 0.02 M acetate buffer (pH 6) and then desorb the vanadium(V) with 0.12 M hydrochloric acid. Reject the first 15 ml of effluent and collect the next 25 ml. If further concentration is necessary, evaporate to dryness on a water bath after adding 4 ml of 1 M sodium acetate.

Determination of vanadium(V)

To 20 ml of sample containing 0.02—0.75 μmol of vanadium(V), add 2 ml of buffer—masking solution (pH 9.3) and 3 ml of PAR reagent (0.001 M), and stir. The order of addition of the reagents is important. After standing

for 1 h at room temperature, measure the absorbance at 550 nm (Hitachi Model 101 spectrophotometer) against a reagent blank that has also been allowed to stand for 1 h.

Alternatively, to an evaporated sample containing 0.004–0.25 μmol of vanadium(V), add 2 ml of buffer—masking solution (pH 5.5) and 3 ml of 2×10^{-4} M PAR reagent, and stir. Measure the absorbance at 550 nm as above.

In each case, use a calibration graph obtained by taking standards throughout the gel adsorption procedure.

Distribution measurements

To investigate the adsorption of vanadium(V) on Sephadex G-25, the distribution coefficient K_d was measured by the batch technique, using 1 g of Sephadex G-25 and 50 ml of solution. The mixture was stirred overnight and the vanadium(V) in the equilibrated solution was determined by the PAR method. The detailed experimental procedure was described previously [1].

RESULTS AND DISCUSSION

Effects on the adsorption of vanadium(V)

Figure 1 shows the effect of the pH of the equilibrated solution on K_d, i.e., the extent of vanadium(V) adsorption on Sephadex G-25. At pH 3—7, the K_d value exceeds 10; this is less than that for borate [1], but greater than the values reported for almost all other solutes, indicating that selective adsorption of vanadium(V) from solutions at pH 3—7 can easily be accomplished.

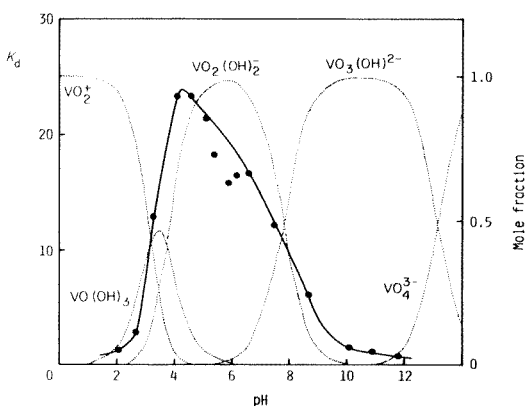


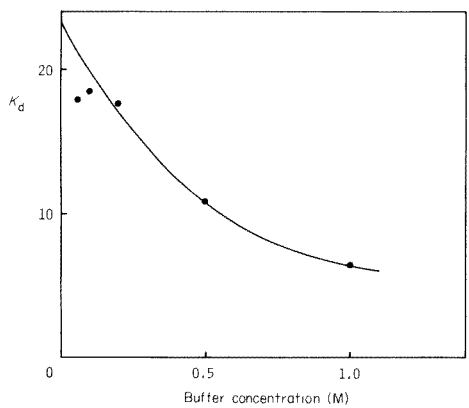
Fig. 1. pH-dependence of the adsorption of vanadium(V) on 1 g of Sephadex G-25 for a 50-ml sample of 5×10^{-5} M NH_4VO_3 in 0.1 M NaCl (HCl—NaOH). Dotted lines indicate the mole fraction curves.

The features of the pH-dependence of the K_d value almost agree with the distributions of $VO(OH)_3$ and $VO_2(OH)_2^-$ as a function of pH (Fig. 1). The dissociation constants of VO_2^+ used for calculation of the distribution diagram were $pK_1 = 3.2$, $pK_2 = 3.8$, $pK_3 = 7.8$ and $pK_4 = 13.2$ [7, 8]. As has already been suggested by Ortner and Dalmonego [4], chelation with the gel may play a part in the adsorption mechanism by analogy with borate adsorption [1].

The K_d value increased with decreasing concentration of vanadium(V), suggesting that vanadium at high concentrations in the gel may be forming bulky isopolymers which are excluded from the gel. This phenomenon, however, did not interfere with the separation and concentration of traces of vanadium(V).

The pH of the sample solutions was controlled by adding acetate buffer. The value of K_d decreased with increasing concentration of acetate (Fig. 2) and the presence of other buffers such as phosphate and tartrate affected adsorption more strongly; therefore the acetate buffer was used in low concentrations.

When vanadium is determined in geochemical samples, it is frequently necessary to separate the vanadium from large amounts of neutral salts. As shown in Fig. 3, the presence of sodium chloride or potassium nitrate hardly affects the adsorption of vanadium(V) on the gel; however, the presence of other sodium perchlorate or calcium chloride results in decreased adsorption. Perchlorate is known to be adsorbed on the gel and thus may exclude vanadate. The effect of calcium may be due to complex formation between calcium and vanadate.



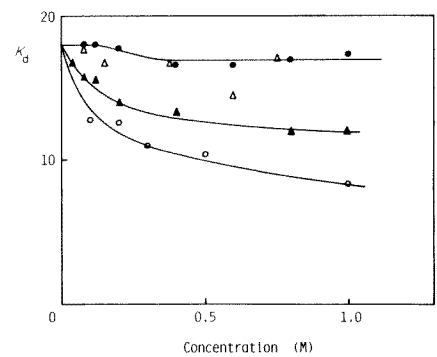


Fig. 2. Variation of K_d with concentration of pH 4.2 acetate buffer on 1 g of Sephadex G-25 for a 50-ml sample of 5×10^{-5} M NH_4VO_3 in 0.1 M NaCl.

Fig. 3. Effect of neutral electrolytes on the adsorption of vanadium(V) on 1 g of Sephadex G-25 from 50-ml samples of 5×10^{-5} M NH_4VO_3 in 0.1 M acetate buffer (pH 4.2): (•) NaCl; (△) KNO_3; (▲) $NaClO_4$; (○) $CaCl_2$.

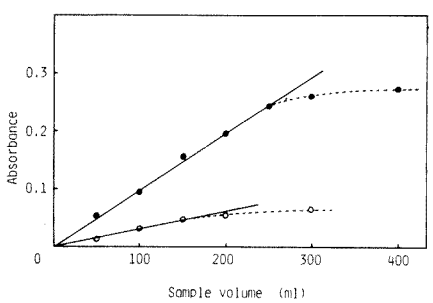
Selective concentration of vanadium(V) on a Sephadex gel column

The characteristic adsorption of vanadium(V) on Sephadex gel was applied to the selective concentration of vanadium. The use of sodium hydroxide as eluent causes tailing of the elution of vanadium(V). When 0.12 M hydrochloric acid was used as eluent, all the vanadium(V) was always found in the 22—34-ml effluent fraction. Therefore, the first 15 ml of effluent was rejected and the next 25 ml was collected. The vanadium(V) in a 20-ml aliquot was determined by the PAR method, the pH of the solution being adjusted to 5.5 by the addition of 2 ml of buffer—masking solution (pH 9.3).

In Fig. 4, the absorbances measured are plotted against the volume of sample solution taken. With sample solutions up to 250 ml at pH 4.2 ± 0.2 or with sample solutions up to 150 ml at pH 6.0 ± 0.2, vanadium(V) could be completely concentrated into a 25-ml solution. Further concentration may be accomplished by evaporation of the effluent to dryness. It is necessary to add sodium acetate before evaporation, otherwise some of the vanadium-(V) is reduced to vanadium(IV) in the presence of hydrochloric acid.

The effects of foreign ions on the recovery of vanadium(V) are listed in Table 1. Iron(III) interferes because of complex formation with vanadium-(V). Strong interaction between hydrated iron(III) oxide and vanadium(V) has been used for the concentration of vanadium(V) [9]. Iron(III) could be masked by adding copper(II)—CyDTA solution (Fig. 5). Copper in the complex is replaced by iron(III) because the copper complex is less stable than the iron complex. In addition, free copper(II) did not interfere with the adsorption of vanadium(V) on the gel column.

Molybdenum(VI) and tungsten(VI) interfere probably by complexing with vanadium(V). The lower the pH of the solution, the larger their interference becomes. Therefore, it is recommended that vanadium(V) should be adsorbed



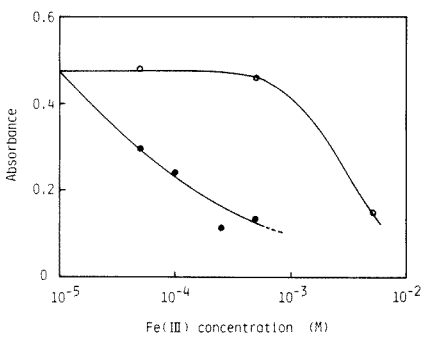


Fig. 4. Effect of sample volume on the recovery of vanadium(V): (•) 0.02 M acetate buffer (pH 4.2) containing 1×10^{-6} M NH_4VO_3; (○) sea water from Shingu, Fukuoka, containing 0.02 M acetate buffer (pH 4.2). For sea water samples, measurements were carried out on effluents evaporated to dryness.

Fig. 5. Masking of iron(III): 5×10^{-6} M of NH_4VO_3 and $FeCl_3$ in 100 ml of 0.02 M acetate buffer (pH 4.2); (○) 5 ml of 0.1 M Cu(II)—CyDTA solution added; (•) no Cu(II)—CyDTA solution added. The dashed line indicates precipitate formation.

TABLE 1

Effects of foreign ions on the recovery of vanadium(V)[a]

	Mole ratio to V(V)	V(V) found ($\times 10^{-6}$ M)		Mole ratio to V(V)	V(V) found ($\times 10^{-6}$ M)
Al	100	5.0	Mo(VI)	1	5.0
	1000	5.2		10	1.8
Ca	1000	5.0		10[c]	5.0
	10000	5.1	W(VI)	0.1	5.0
Fe(III)[b]	10	5.1		1	1.8
	100	4.8		1[c]	4.6
Mn(II)	100	4.9	SiO_2	(0.005 M)[c]	5.1
	1000	4.9		(0.02 M)[c]	0.9
Mn(VII)	100	5.1		(0.05 M)[c]	0.0
Cr(VI)	100	5.2			
	1000	5.0			

[a]Vanadium(V) in a 100-ml sample solution was separated and concentrated to 25 ml on the Sephadex column. Sample solution: 100 ml of 5.0×10^{-6} M NH_4VO_3 (pH 4.2). Absorbance was 0.478 in absence of interferences. [b]0.1 M copper(II)—CyDTA solution was added to the sample solution. [c]The pH of the sample solution was adjusted to 6.

at pH 6. The natural abundance of tungsten is much lower than that of vanadium, and in practice the effect by tungsten may be neglected.

Determination of vanadium in natural waters and rocks

The proposed method was applied to the determination of vanadium in coastal sea waters near Fukuoka. The concentrations of vanadium(V) and molybdenum(VI) have been reported to be at the $\mu g\ l^{-1}$ level [10]. To avoid the interference of molybdenum(VI), adsorption of vanadium(V) on the column was carried out at pH 6. The effluents were evaporated to dryness before vanadium determination. The amount of hydrochloric acid in the

TABLE 2

Recovery of vanadium(V)

Coastal sea-water from Kamiwajiro, Fukuoka[a]; 150 ml					
V added (μg)	0	0.25	0.50	0.75	
Absorbance	0.038	0.065	0.095	0.122	
V found (μg)	0.33	0.57	0.83	1.06	
Rock sample (JB-1[b]); 0.0252 g					
V added (μg)	0	5.00	10.0	15.0	20.0
Absorbance	0.092	0.192	0.291	0.390	0.489
V found (μg)	4.53	9.67	14.7	19.6	24.6

[a]Vanadium contents of coastal sea waters near Fukuoka (in $\mu g\ l^{-1}$) were found to be: Tsuyazaki, 2.1; Shingu, 1.5; Kamiwajiro, 2.1; Kashii, 1.5; Hakozaki, 0.8. [b]Geological Survey of Japan reference sample of basalt.

TABLE 3

Reported vanadium content of JG-1 (granodiorite) and JB-1 (basalt)

Vanadium content (ppm)		Method[a]	Reference[b]
JG-1	JB-1		
25	—	O.e.s.	Champ (1968)
21	390	O.e.s.	Ikeda (1970)
—	205	O.e.s.	Schmidt (1972)
95	170	O.e.s.	Thompson (1972)
28	240	O.e.s.	Champ and Bender (1973)
22	220	O.e.s.	Brenner (1973)
21	217	X.r.f.	Gagnon (1974)
<25	223	A.a.s.	Rubeska (1972)
25	227	A.a.s.	Schafer (1973)
21	215	A.a.s.	Terashima (1973)
25	228	A.a.s.	Gagnon (1974)
25	223	A.s.	Terashima (1970)
23.3	178	A.s.	Akaiwa et al. (1974)
25.2[c]	181[d]	A.s.	This work

[a]Optical emission spectrometry, x-ray fluorescence spectrometry, atomic absorption spectrometry and absorption spectrophotometry. [b]Data cited in [12]. [c]Effluents evaporated to dryness from 0.05 g of rock. [d]0.025 g of rock was used.

effluent cannot be estimated correctly, and so the slope of the calibration curve prepared by the column method is somewhat different from that obtained by direct spectrophotometry. Recovery of added vanadium(V) was almost complete (Table 2). The concentrations found are also given in Table 2.

A convenient separation of vanadium from iron(III) in rock samples is achieved by fusing the sample with sodium carbonate and leaching it with water [11]. However, when the proposed method was applied to such solutions, the amount of vanadium found was linearly related to the mass of sample taken only when that mass did not exceed 75 mg. The higher the concentration of silicic acid, the lower the recovery of vanadium (Table 1). As shown in Table 2, however, recovery of the added vanadium(V) was almost complete when solutions obtained from a small rock sample were loaded onto the column. Table 3 compares previously determined vanadium concentrations in reference samples JG-1 and JB-1 (Geological Survey of Japan), with values obtained by the proposed method. The JG-1 sample was measured after the effluents had been evaporated to dryness. The results obtained by the standard addition method agreed with these other values [12].

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RAPID AND SENSITIVE DETERMINATION OF TOTAL VANADIUM IN AIRBORNE PARTICULATES BY AN EXTRACTION—SPECTROMETRIC METHOD WITH *N*-BENZOYL-*N*-PHENYLHYDROXYLAMINE

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SUMMARY

A method is described for the rapid and sensitive determination of $\leqslant 0.06$ μg of total vanadium in airborne particulates collected in the filter bag of an air-cleaner or on a membrane filter attached to an air sampler. The method is based on decomposition of organic matter in the sample and membrane filter by successive use of nitric, sulfuric and perchloric acids, and decomposition of the suspended silicate residue in a sulfuric—hydrofluoric acid mixture, followed by the extraction of a vanadium—*N*-benzoyl-*N*-phenylhydroxylamine complex into chloroform for spectrophotometric measurement. A comparison with the troublesome fusion method for the residue is included.

Vanadium in airborne particulates has been determined by various methods such as emission spectrography, atomic absorption spectrometry, neutron activation, x-ray fluorescence, spectrophotometric methods, etc. However, significant inter-method or inter-laboratory deviation of the results has often been obtained. Some of these methods are non-destructive and give the total vanadium content of the sample, though sometimes there may be a matrix effect and/or other undesirable effects which are difficult to correct. Others are destructive, and involve acid decomposition of the sample in most cases. The solution from the acid decomposition is used for the determination and the residue is usually discarded in order to simplify the procedure. However, it has been found here that the residue often contains significant amounts of vanadium. The emission spectrographic method has poor precision, the activation method is not universal, and the flame atomic absorption method has too low a sensitivity to determine traces of vanadium in small particulates. The x-ray fluorescence method is not well established. A sensitive kinetic spectrophotometric method has been developed [1] for the determination of acid-extracted vanadium in airborne particulates but it is difficult to apply to the determination of total vanadium.

Extraction—spectrophotometric methods with *N*-benzoyl-*N*-phenylhydroxylamine (BPHA) have been studied for the determination of traces of vanadium in sulfuric [2], phosphoric—sulfuric—hydrofluoric [3], sulfuric—

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hydrofluoric [4] and hydrochloric [4] acid media. The methods seem to be the most sensitive of the spectrophotometric methods for the determination of vanadium. This paper describes the application of the BPHA extraction—spectrophotometric method with a H_2SO_4—HF medium for the simple and sensitive determination of the total vanadium in the particulates collected in the filter bag of an air-cleaner or on a membrane filter attached to an air sampler. The sample of particulates containing organic materials and the membrane filter used for sample collection are first decomposed by successive use of nitric, sulfuric, and perchloric acids, and heating to fumes. After addition of hydrofluoric acid to decompose the suspended residue, which may often contain significant amounts of vanadium as silicates and other forms insoluble in nitric, hydrochloric, sulfuric, perchloric acids and their mixtures, a small amount (3 or 5 ml) of the BPHA—chloroform solution is used for extraction and absorbance measurements are carried out in 50-mm microcells. This method permits up to about 0.06 μg of total vanadium to be determined without the use of any troublesome fusion techniques, and also will be applicable in the analysis of precipitated- or evaporated-metals-membrane-type reference standards for the x-ray fluorescence spectrometry of airborne particulates. The time required is about 85 min.

EXPERIMENTAL

Reagents and apparatus

All chemicals used were of analytical grade. A standard vanadium stock solution (500 μg V ml^{-1}) was prepared by dissolving ammonium metavanadate in water. Working solutions were prepared from this by appropriate dilution with water. Ammonium iron(II) sulfate solution (1%) was freshly prepared before use by dissolving 1 g of ammonium iron(II) sulfate in 100 ml of water containing 0.5 ml of sulfuric acid. The BPHA solution (0.2%) in chloroform was stored in a brown bottle. This solution was usable for several months.

The filter paper was No. 5 (Toyo Filter Paper Co.). The membrane filter was Yumicron MF-90 (47-mm diameter, 0.9-μm pore size; Yuasa Battery Co.), which contains polyvinyl chloride, or FR-100 (47-mm diameter, 1.0-μm pore size; Fuji Photo Film Co. Ltd.) made of regenerated cellulose.

A Sartorius semimicrobalance was used for weighing the samples. The absorbance measurements were done with a Hitachi Model 101 spectrophotometer and 50-mm glass microcells containing 1—5 ml of solution. An Iwaki KM Small Type shaker was employed.

Membrane filters and airborne particulates samples

A glass fiber filter, attached to a high-volume air sampler, has commonly been used for the collection of airborne particulates for routine analysis. However, various kinds of membrane filters, attached to a low- or high-volume air sampler, are also increasingly used because of their lower metal content and low background under x-ray fluorescence spectrometry. Yumi-

cron MF and FR-100 membrane filters seem to be the best from the viewpoint of their strength, durability during sampling and x-ray exposure, and low metal content.

Three homogenized samples (denoted by AS-1, AS-2 and AS-5) were used for this study. AS-1 and AS-2 were prepared by Hashimoto and Ohe [5] and AS-5 by the authors, using airborne particulates collected in filter bags attached to the inlets of air cleaners on the roofs of tall buildings in the office district of Tokyo (AS-1), and in the industrial districts of Chiba (AS-2) and Yokohama (AS-5). Particulates collected on 47-mm diameter membrane filters by a low-volume air sampler were also analyzed to verify the recommended procedure.

Recommended procedure

Airborne particulates contain silicates and other materials which are insoluble in hydrochloric, nitric, sulfuric and perchloric acids and their mixtures. When the sample is treated with these acids, the organic substances in the sample and membrane filter are decomposed but suspended insoluble materials remain in the sample solution, and affect absorbance measurements, as well as being a source of loss of vanadium. The following steps are therefore necessary.

Step 1. Transfer a suitable weight (5—20 mg) of the particulate sample taken from a filter bag, or the sample together with the membrane filter (when the sample was collected on a membrane filter), into a 100-ml tall beaker (a 5—20 mg sample may be collected on a 47-mm diameter membrane filter by a low-volume air sampler in 1—3 weeks depending on the atmosphere). Moisten the sample with 0.5 ml of nitric acid (61%, w/v), and add 7 ml of sulfuric acid (97%, w/v) followed by heating just to fumes, so that all organic substances will be carbonized. After cooling, add 1 ml of perchloric acid (70%, w/v), and heat gently to white fumes. Cover the beaker with a watch glass and continue to fume until the solution becomes clear yellow or colorless. After cooling, add 1 ml of the nitric acid. Cover the beaker with the watch glass, and wash down any carbonized and insoluble matter creeping up the beaker wall by gently boiling the solution. Remove the watch glass and continue to heat to evolve sulfur trioxide fumes. Cool, and dilute with 15 ml of water.

Step 2. Transfer the solution and residue to a 100-ml polypropylene separating funnel with 25 ml of water. Add 4 ml of 40% hydrofluoric acid and allow to stand for about 15 min in order to dissolve the residue of silicates, etc., which may contain vanadium. This standing time is not necessary for a membrane filter without particulates used for the blank.

Step 3. Add exactly 2 ml of 1% (w/v) ammonium iron(II) sulfate solution and mix well to reduce any chromate ions. Add 0.4 g of ammonium peroxodisulfate and mix well. Add exactly 3 or 5 ml of the BPHA—chloroform solution by use of a pipet (10 ml of the BPHA solution can be used for large amounts of vanadium) and shake well. After phase separation, drain the

organic phase through a filter paper for dehydration into a 50-mm microcell. Measure the absorbance at 475 nm against chloroform as reference. Find the amount of vanadium present from a calibration graph. A blank is carried through the whole procedure.

Calibration. The calibration graph is obtained as in step 3 by use of standard vanadium solution and sulfuric and hydrofluoric acids to give similar acid concentrations to the sample solutions.

Methods with H_2SO_4—HF treatment and pyrosulfate fusion

The following procedures which include an H_2SO_4—HF treatment only or the H_2SO_4—HF treatment followed by pyrosulfate (disulfate) fusion of insoluble matter, were also applied for comparison with the above procedure.

H_2SO_4—HF treatment. Step 1 in the recommended procedure was applied as described above. The sample solution containing suspended residue was filtered through filter paper into a polypropylene separating funnel, followed by Steps 2 and 3 for the determination of the acid-extracted vanadium. The residue and the filter paper were ignited in a platinum crucible. After addition of 0.5 ml of (1 + 1) H_2SO_4 and 1 ml of 40% hydrofluoric acid, the residue was gently heated to fumes, dissolved in 10 ml of 2.5 M H_2SO_4, and transferred to another polypropylene separating funnel, using 30 ml of 2.5 M H_2SO_4. Then 4 ml of 40% hydrofluoric acid were added, and the vanadium in the residue was determined as in Step 3.

H_2SO_4—HF treatment and pyrosulfate fusion. The acid-extraction of vanadium and H_2SO_4—HF treatment of the ashed residue were carried out as described above. The residue, which was heated to near dryness in the platinum crucible, was fused with 0.5 g of potassium pyrosulfate. The melt was dissolved with 10 ml of 2.5 M sulfuric acid and transferred to another polypropylene separating funnel using 30 ml of 2.5 M H_2SO_4. Then 4 ml of 40% hydrofluoric acid were added, and the vanadium in the residue was determined as in Step 3.

RESULTS AND DISCUSSION

Decomposition of membrane filter

Though various procedures have been reported for the wet decomposition of organic samples for inorganic analysis, they are generally time-consuming and it is sometimes difficult completely to decompose membrane filters which are used for the collection of airborne particulates. Thus the simple decomposition of membrane filters is an important problem for the wet analysis of airborne particulates collected thus. Some wet procedures were studied, therefore, for their ease of decomposition of two kinds of membrane filters suitable for sample collection.

The procedures tried (Table 1) were as follows. A 100-ml tall beaker covered with a watch glass was used for all decomposition procedures. In procedures 1 and 2, the sample was boiled with nitric acid and hydrogen peroxide. In procedure 3 [6], the sample was heated with nitric acid to near

dryness for preliminary decomposition, and, after addition of sulfuric acid, heated to fumes for carbonization. Finally, dropwise addition of hydrogen peroxide and gentle heating were repeated several times for complete decomposition of the carbonized matter. In procedure 4, the sample was heated with a mixture of nitric, sulfuric and perchloric acids to sulfur trioxide fumes and sometimes followed by further addition of perchloric acid. In procedure 5, the sample was first carbonized by heating with sulfuric acid to fumes followed by addition of perchloric acid, and heated to sulfur trioxide fumes. The decomposition was completed by further addition of nitric acid and heating to fumes as described in the recommended procedure.

Table 1 shows that procedure 5 is best. Thus a modified procedure 5 was employed in the recommended procedure for ease of decomposition of the particulates and membrane filter, and for combination with BPHA extraction—spectrophotometry.

Determination of vanadium by BPHA extraction and spectrophotometry

In Donaldson's procedure [4], which describes the determination of vanadium by extraction with BPHA from a 2 M H_2SO_4—4 M HF medium, the acidic sample solution was extracted three more times with the BPHA solution and all extracts were combined to 25 ml, followed by absorbance measurements in 20-mm cells at the absorption maximum of 475 nm. The present procedure employs a single extraction from a 2.5 M H_2SO_4—2 M HF medium with smaller volumes (3 or 5 ml) of the BPHA solution and 50-mm microcells, which simplifies the procedure and improves the sensitivity. Some of the conditions of the determination and other parameters were studied in some detail.

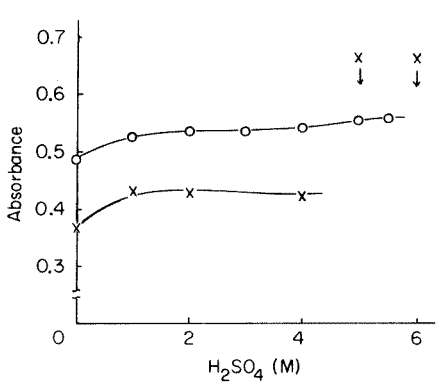
Experiments showed that nearly constant absorbance values were obtained from 1—4 M sulfuric and hydrofluoric acids (Figs. 1 and 2). In greater than 4 M solutions of both acids, however, significant variation of absorbance or unstable absorbance was often obtained, especially when 5 ml of the BPHA solution was used, differing from Donaldson's study [4]. Therefore, a 2.5 M H_2SO_4—2 M HF medium was employed for the extraction of the vanadium—BPHA complex in the recommended procedure.

TABLE 1

Comparison of various procedures for decomposition of membrane filters

Procedure	Decomposition time (min)	
	Yumicron	FR-100
1 Mixed soln. of 10 ml HNO_3 and 1 ml H_2O_2 (30%)	180[a]	90
2 Mixed soln. of 20 ml HNO_3 and 1 ml H_2O_2 (60%)	180[a]	90
3 1 ml HNO_3 and 2 ml H_2SO_4, H_2O_2 (60%) dropwise	180	40
4 Mixed soln. of 10 ml HNO_3, 1 ml H_2SO_4 and 2 ml $HClO_4$	150	45
5 2 ml H_2SO_4, 1 ml $HClO_4$ and 1 ml HNO_3	60	20

[a]Incomplete.



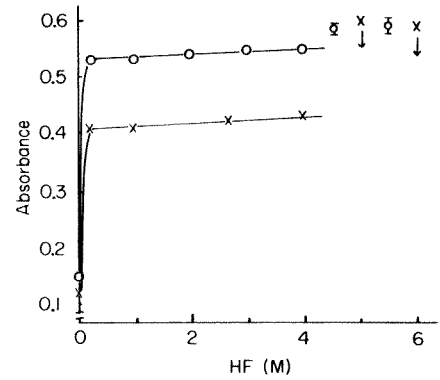


Fig. 1. Effect of sulfuric acid concentration: (-○-) 2 M HF, 3 μg V, 3 ml BPHA—$CHCl_3$; (×) 4 M HF, 4 μg V, 5 ml BPHA—$CHCl_3$; (↓) absorbance decreases.

Fig. 2. Effect of hydrofluoric acid concentration: (○) 2 M H_2SO_4, 3 μg V, 3 ml BPHA—$CHCl_3$; (×) 2 M H_2SO_4, 4 μg V, 5 ml BPHA—$CHCl_3$; (↓) absorbance decreases; (↕) absorbance varies.

The calibration graphs were linear for 0—5 μg of vanadium when 3 ml of the BPHA solution was used, and for 0—10 μg of vanadium when 5 ml of the BPHA solution was used. The sensitivity was 0.06 μg V per 0.01 absorbance when 3 ml of the BPHA solution was used.

In a similar method [3] using an H_3PO_4—H_2SO_4—HF medium in the analysis of stainless steels and nickel—base alloys, the absorbance of the vanadium—BPHA complex was measured at 530 nm, far from the absorption maximum of 475 nm, to eliminate an error caused by extracted manganese. Donaldson [4] measured the absorbance of the complex at the absorption maximum of 475 nm, and showed that <10 mg of many elements, except chromium and cerium, did not interfere in the extraction of vanadium from 2 M H_2SO_4—4 M HF and the subsequent determination. These studies were repeated using a simulated sample solution. Up to 1 mg of manganese(II), 3 mg of iron(III), 200 μg of chromium(III) and 2 ml of concentrated perchloric acid did not interfere. These quantities are more than those contained in 100 mg of particulates.

A series of six determinations of 6.0 μg of vanadium using 5 ml of BPHA solution gave a mean recovery of 6.0 μg and a standard deviation of 0.09 μg (n = 6) for a simulated sample solution also containing 50 mg of SiO_2, 1.5 mg of Fe, 500 μg of Zn, 500 μg of Pb, 200 μg of Cu, 100 μg of Ti, 100 μg of Mn, 50 μg of Cd, 20 μg of Ni and 20 μg of Cr.

Molar absorptivities were calculated from the calibration graphs prepared by the use of 3, 5 and 10 ml of the BPHA—chloroform solution. The results showed that the molar absorptivity was (47 ± 1.5) × 10^2 l mol^{-1} cm^{-1}, and did not depend on the volume of the BPHA—chloroform solution used.

TABLE 2

Analysis of airborne particulates collected in filter bags

Sample	Procedure[a]	Sample taken (mg)	V added (mg)	V found (μg)			Mean V in sample (μg g^{-1})
				Acid-extracted	Residue	Total	
AS-1	1	19.94				4.7	
		20.04				4.6	231
		20.02	2.0			6.5[b]	
		19.93	2.0			6.6[b]	
	2	20.02		4.5	0.3	4.8	233
		19.95		4.4	0.1	4.5	
	3	19.90		4.4	0.3	4.7	
		19.96		4.5	0.1	4.6	228
		20.07		3.5	0.9	4.4	
AS-2	1	5.36				2.3	
		4.77	1.0			3.1	430
		6.36	1.0			3.7	
		20.28				8.8[b]	
		20.19				8.6[b]	430
		20.13				8.2[b]	405[c]
	3	20.66		7.4	1.3	8.7[b]	421
AS-5	1	20.01				4.0	200
		20.12				4.0	
	3	20.29		2.6	1.5	4.1	202

[a](1) Recommended; (2) H_2SO_4 + HF treatment; (3) H_2SO_4 + HF treatment and $K_2S_2O_7$ fusion. [b]5 ml of BPHA—$CHCl_3$ solution. [c]Abnormal value.

Analysis of airborne particulates

Table 2 shows that the values for total vanadium determined by the recommended procedure agree well with the values obtained by the methods with H_2SO_4—HF treatment alone or H_2SO_4—HF treatment followed by pyrosulfate fusion, and emphasize that vanadium should be also determined in any suspended residue. The airborne particulates collected on Yumicron MF

TABLE 3

Analysis of airborne particulates[a] collected on a Yumicron membrane filter, by recommended procedure

Sampling time (h)	Total air volume (m^3)	Collected and analysed particulates (mg)	V determined		In particulates (μg g^{-1})
			(μg)	(ng m^{-3})	
360 (Dec. 1980)	432	19.57	4.5	11	230
264 (July 1979)	317	9.55	1.9	6	200

[a]Collected on a roof at Yamanashi University by use of a low-volume air sampler at 20 l min^{-1}.

membrane filters may also be analyzed by the recommended procedure without any trouble, as is shown in Table 3.

The procedure is simple, and is recommended for the determination of total vanadium in airborne particulates collected in a bag-filter or on a membrane filter. The time required for an analysis is about 85 min.

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MERGING ZONES IN FLOW INJECTION ANALYSIS
Part 6. Determination of Calcium in Natural Waters, Soil and Plant Materials with Glyoxal bis(2-hydroxyanil)

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SUMMARY

A flow injection procedure is proposed for the spectrophotometric determination of calcium in natural waters, soil extracts and plant digests, employing glyoxal bis(2-hydroxyanil) as the colour-forming reagent. The necessary dissociation of this reagent, which is rather slow, proceeds outside the analytical path, and the merging-zones approach is used for reagent addition. Composition of reagents, dissociation time of the colour-forming reagent, ethanol content in the carrier streams and interferences are described. In the analysis of plant and soil materials, zone sampling is required for initial sample dilution. The proposed systems are very stable and permit a sampling rate of 180 determinations per hour. Relative standard deviations are less than 1%. The results compare well with those obtained by inductively-coupled argon-plasma atomic emission spectrometry.

The merging-zones approach in flow injection analysis [1] is a powerful tool in automated chemical analysis, and has been used mainly to reduce the consumption of reagents [2—4]. Previous papers in this series [3] have pointed out other useful aspects of this approach, such as providing initial neutralization of samples, allowing simultaneous determinations of two chemical species in the same analytical path, and avoiding baseline drift [5]. Zone-sampling processes [6] and stopped-flow procedures have already been employed in conjunction with the merging-zones approach [3, 4], resulting in methods with very good analytical characteristics.

The merging-zones approach can also be employed for the addition of unstable reagents. The potential of this approach is considered here in the development of a flow injection procedure for the spectrophotometric determination of calcium in natural waters, soil extracts and plant digests. Glyoxal bis(2-hydroxyanil) (GBHA), which is often recommended as a very sensitive and selective spectrophotometric reagent for calcium [7—9], is used as the colour-forming reagent. Ethanolic solutions of GBHA are slightly coloured and, after addition of sodium hydroxide, become yellowish because of dissociation of the hydroxyl groups of GBHA [7]. This dissociation reaction requires some minutes to be completed. In the presence of calcium, a red complex is formed, in a very fast reaction [9]. If both reactions were

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performed together in the analytical path of a flow injection system, the dissociation step would be a limiting factor for either sampling frequency or sensitivity. The GBHA dissociation must therefore proceed outside the analytical path, more specifically, immediately before its introduction into the analytical path, as the product of this reaction is an unstable species. The merging-zones approach permits the addition of this intermediate to the sample zone in a reproducible manner.

The same basic manifold can be used in analyses of materials with widely differing calcium contents. For samples with high calcium concentrations, the sample is diluted prior to spectrophotometry by using a zone-sampling process [6].

EXPERIMENTAL

Apparatus

The flow systems used are shown schematically in Fig. 1. The peristaltic pump was a Technicon AAII, furnished with Solvaflex pumping tubes, which were replaced weekly. Tubing, connectors, flow-through cuvette, spectrophotometer and recorder were the same as those used in earlier work [3]. The proportional injector, consisting of three 2:3:2 commutation sections [2], was operated electronically. Details of the electronics have been given elsewhere [5, 6].

Reagents, samples and standards

All reagents used were of analytical grade and distilled—deionized water was always employed. Before use, the solutions were heated in a water bath (ca. 60°C) for at least 10 min to eliminate dissolved air, and then cooled to room temperature.

The colour-forming reagent G (Fig. 1) was a 0.2% (w/v) GBHA solution in 96% (v/v) ethanol; this solution is stable for at least two days. The alkaline solution A (Fig. 1) was prepared by dissolving 30 g of sodium hydroxide and 10 g of sodium tetraborate decahydrate in 1 l of 50% (v/v) ethanol. The reagent carrier stream C_R (Fig. 1) was prepared by adding 5 ml of triethanolamine and 1.0 g of potassium cyanide to 200 ml of the alkaline solution A and then diluting to a final volume of 1 l with 50% (v/v) ethanol. The sample carrier stream C_S was 50% (v/v) ethanol.

A 10000 ppm stock solution of calcium was prepared by dissolving calcium carbonate in a slight excess of hydrochloric acid. Working standards were made by diluting the stock solution with nitric acid, potassium chloride or perchloric acid to match the matrix concentrations in natural waters, soil extracts and plant digests, respectively. The following concentrations were employed: waters, 0.014 M HNO_3 (0.0—10.0 ppm Ca); soils (1.0 M KCl, 0—100 ppm Ca); plants, 0.25 M $HClO_4$ (0—500 ppm Ca).

Natural waters were collected in polyethylene bottles and preserved by addition of 1.0 ml of concentrated nitric acid per litre [10]. Soil ex-

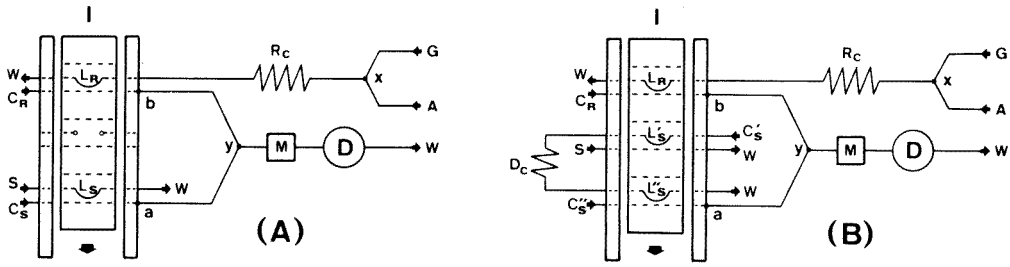


Fig. 1. Flow diagrams of the systems used for calcium determination in waters (A) and in soil extracts or plant digests (B). I, Proportional injector; G, colour-forming reagent (0.7 ml min^{-1}); A, alkaline solution (0.7 ml min^{-1}); R_C, reaction coil (300 cm); L_S and L_R, sample and reagent loops (200 μl and 50 μl); C_S and C_R, sample and reagent carrier streams (7.8 and 3.9 ml min^{-1}); S, sample (aspiration rate of 4 ml min^{-1}); ay and by, synchronization lines (each 20 cm); M, mixing chamber (dead volume of ca. 250 μl); D, detector (555 nm); x and y, confluence points; W, waste. In system B, the first sample carrier stream C'_S (water at 5.0 ml min^{-1}), the first sample loop L'_S (50 μl), the dispersion coil D_C (100 cm), the second sample loop L''_S (200 μl for soil or 50 μl for plant analysis) and the second sample carrier stream C''_S (3.9 ml min^{-1}) are related to the zone-sampling process. For details, see text.

tracts were obtained by shaking 10.0 g of air-dried soil with 100 ml of a 1.0 M potassium chloride solution [11]. Plant digests (75 ml) were prepared by nitric—perchloric digestion of 750 mg of dried and ground, plant material [12].

Flow diagrams

The system in Fig. 1A utilizes only two commutation sections of the proportional injector and was designed for the analysis of natural waters with calcium contents ranging from 0.0—10.0 ppm Ca. The dissociation of GBHA in the alkaline medium starts at the confluence point x, where the colour-forming reagent and the alkaline solution are mixed; this reaction proceeds inside the reaction coil, where a colour gradient can be observed.

The injector rests in the sampling position shown in Fig. 1A, for 10 s. In this situation, both sample and reagent are loading the corresponding loops and going to waste. It should be stressed that the merging-zones configuration permits any change in the continuous preparation of the reagent without disturbing the hydrodynamics in the analytical path of the flow injection system. After switching the injector, the selected volumes of sample and prepared reagent are introduced into the corresponding carrier streams, forming well defined zones which undergo continuous dispersion during transportation by the carrier streams. At point y, the zones merge and in the following chamber M, the red complex is formed. The absorbance is then measured, and the transient signal proportional to the calcium content in the sample is recorded. The injector rests in the injection position for 10 s, which suffices to prevent carryover at the 0.1% level. Next, the injector is switched back to the sampling position, starting another cycle. It must be

emphasized that preliminary experiments indicated that the continuous addition of the reagent via a confluence configuration increased the baseline noise.

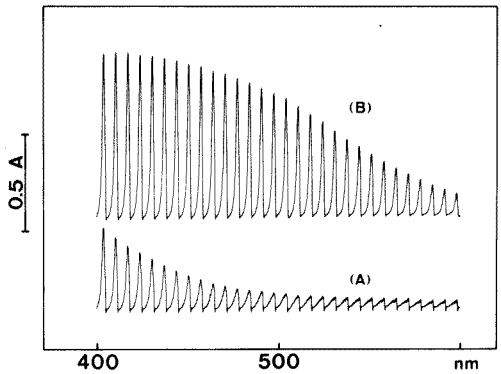
The system in Fig. 1B uses all three commutation sections of the proportional injector and was designed for analyses of soil and plant samples. Since different degrees of sample dispersion are required, a zone-sampling process [6] was employed. In this system, the sample volume selected by the first sample loop is injected into the first sample carrier stream, creating a well defined sample zone. This zone undergoes continuous dispersion inside the dispersion coil D_C while it is transported towards the second sample loop. This loop selects a small fraction of the sample zone to be introduced into the second sample carrier stream. The subsequent processes are as outlined above. The resting time of the injector in the position shown in Fig. 1B is the Δt value [6], which has a marked effect on the total sample dispersion.

RESULTS AND DISCUSSION

Optimization of ethanol concentration and spectrophotometric wavelength

The low solubility of the GBHA in water is well known. Therefore, when the alcohol content in both carrier streams was only 20% (v/v), baseline drift caused by the precipitation process was observed. On the other hand, when the ethanol content was above 70% (v/v), troubles arose from the liberation of air bubbles and the increased blank values caused by the "schlieren" effect [12]. Also, when the ethanol contents in the carrier streams were different, baseline noise increased, as a result of inadequate mixing. In tests to solve these problems, the injected sample volume in system A (Fig. 1) was experimentally defined as 200 μl and a synchronization procedure [3] was applied to define the injected reagent volume and the lengths of the synchronization lines. The effect of ethanol content (20, 50, 70 and 96% v/v) in both carrier streams was investigated, the wavelength of the detector being set at 520 nm. Two standards (0.0 and 15.0 ppm Ca in water) were employed and the reagent was a 0.2% (w/v) GBHA solution in 96% (v/v) ethanol. On the basis of the results obtained, a 50% (v/v) ethanol concentration was chosen for both carrier streams.

To establish the best wavelength for measurement under these conditions, the spectrum was scanned from 600 nm to 400 nm. The speed of scanning and the interval between successive injections (200 μl) were adjusted to permit one measurement every 5 nm. For this experiment, a 15 ppm Ca standard and a blank solution, both in aqueous media were employed. A wavelength of 555 nm was selected for further use, this decision being based on the ratio between the absorbances related to standard and blank solutions and the amplitude of the signal related to the calcium standard (Fig. 2). It may be noted that this flow injection procedure for recording absorption spectra indicates the reproducibility of the measurements and can also be applied to unstable species.



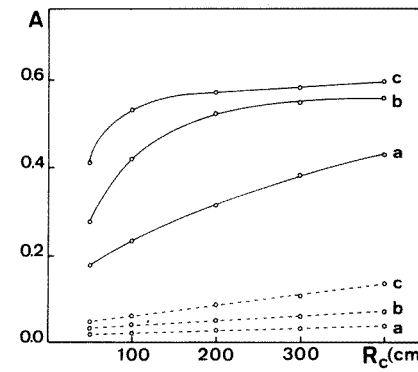


Fig. 2. Absorption spectra related to the blank solution (A) and to the 15 ppm Ca standard (B), with 50% (v/v) ethanol in the carrier streams.

Fig. 3. Effect of reaction coil length and GBHA concentration on the dissociation of the colour-forming reagent. The lengths of the reaction coil, in cm, are indicated by R_C. Curves a, b and c correspond to GBHA concentrations of 0.1, 0.2 and 0.4% (w/v), respectively. (---) Blank solution; (—) 15 ppm Ca standard.

In the flow injection systems (Fig. 1), the pumping rates of the sample and reagent carrier streams were chosen to permit measurements on 180 samples per hour. The total flow rate through the flow cell was higher than that usually employed in flow injection analysis and the mixing conditions inside the mixing coil were found to be critical, since the system involves water—alcohol mixtures. An alternative to a mixing coil was therefore necessary because efficient mixing of water with alcohol requires a long coil which creates pressure problems in the system. A mixing chamber similar to that already described [3] decreased the hydrodynamic pressure and improved mixing.

Dissociation of the glyoxal bis(2-hydroxyanil)

Different reaction coil lengths (50, 100, 200, 300 and 400 cm) and GBHA concentrations (0.1, 0.2 and 0.4% w/v) were tested for investigating the dissociation of the colour-forming reagent inside the reaction coil R_C. Higher reagent concentrations were not employed because the 0.4% (w/v) GBHA solution in 96% (v/v) ethanol was almost saturated. Otherwise, conditions identical to those above were maintained and the wavelength was set at 555 nm.

The effects of reaction coil length and GBHA concentration on the dissociation of the colour-forming reagent are summarized in Fig. 3, where the curves related to the blank solution correspond to the colour gradient established inside the reaction coil. When GBHA concentration was only 0.1% (w/v), the dissociation reaction was not fast enough, causing the ratio between absorbances related to the standard calcium and blank solutions to be too low. From Fig. 3, it can be seen that when a 0.2% (w/v) GBHA concentra-

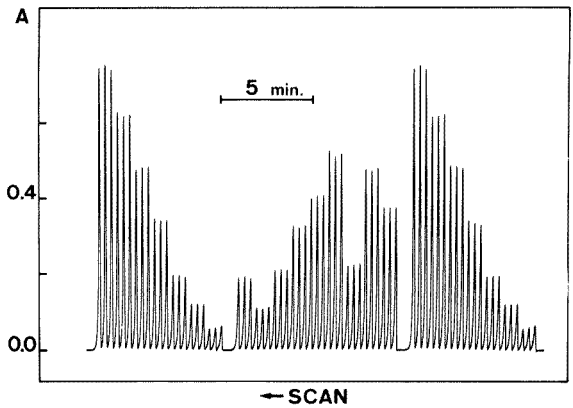


Fig. 4. Routine graph for plant analysis. From left to right, seven standards (0, 50, 100, 200, 300, 400 and 500 ppm Ca), nine samples and the standards again. All measurements in triplicate.

tion and a 300-cm long reaction coil are employed, suitable conditions are attained. In this situation, there is time for the dissociation reaction to be completed, and the calibration graph is almost linear (Fig. 4) with very little reagent decomposition occurring, as observed in test-tube experiments. It must be emphasized that when the reaction coil tends to zero, the absorbance for a calcium standard tends towards the blank value (Fig. 3), i.e., when the dissociation reaction proceeds within the analytical path, the time available for reaction is too short and very little colour is formed. Parallel experiments employing a simple flow injection system, where GBHA and the alkaline solution were added to the sample zone by confluence, confirmed this.

Interference effects

Effects of possible interfering ions were studied with the system of Fig. 1A. Standard solutions containing 1.00 and 10.0 ppm Ca, and also 10 ppm in Fe, Cu and Mn and 20 ppm in Al, were employed. These levels were chosen after considering the probable contents of calcium and other elements in the samples and the degrees of total dispersion in the different flow injection systems. Preliminary tests indicated that magnesium caused no observable interference in the calcium measurement even when its concentration was 200 ppm. Interference effects from the other elements were overcome when triethanolamine (0.5% v/v) and potassium cyanide (0.1% w/v) were added to the reagent carrier stream; the incorporation of these masking agents in the sample zone is efficient because of the high degree of sample dispersion. Without masking, the presence of all the interfering ions mentioned above, together with a 10 ppm Ca standard, caused a signal decrease of 18.9% relative to that for the pure calcium standard. Triethanolamine alone did not eliminate the effects completely, but no interferences were observed when potassium cyanide was also added, either with standard calcium solutions or with the blanks.

TABLE 1

Comparative results for calcium determinations in water, soil and plant materials, obtained by the proposed method (f.i.a.) and by inductively-coupled argon plasma emission spectrometry (i.c.p.e.s.)[a] (Data are given as ppm Ca in the injected sample)

Water		Soil		Plant	
F.i.a.	I.c.p.e.s.	F.i.a.	I.c.p.e.s.	F.i.a.	I.c.p.e.s.
3.67	3.74	36.52	36.60	236	233
6.85	6.98	34.78	34.04	307	300
3.58	3.50	39.13	40.00	123	126
7.23	7.28	22.61	21.28	338	349
3.51	3.49	26.09	24.26	249	241
7.34	7.30	36.52	37.87	195	200
7.40	7.30	34.78	35.32	113	116
3.50	3.48	31.30	31.49	42	47
7.77	8.08	32.17	32.34	98	110

[a]Under the conditions recommended for the Jarrell-Ash Plasma Atom Comp for trace elements in solutions.

Application to natural waters, soils and plants

The system shown in Fig. 1A was applied to the analysis of natural waters. The stability of the system and the reproducibility of measurements were verified by analyzing a typical water sample (3.67 ppm Ca) continuously for two hours. A random selection of 15 measurements from the 300 obtained during this experiment gave a relative standard deviation of 0.57%. The flow system is characterized by a sample dispersion factor of 0.32. It is difficult to improve sensitivity by increasing the dispersion factor because, as the factor increases, the mixing of water and alcohol becomes more critical, causing increased blank value and loss of reproducibility. Studies concerning the replacement of the mixing chamber by a packed reactor, to avoid this limitation, are currently in progress. The accuracy of the method was evaluated by measuring nine samples which had already been measured by inductively-coupled argon plasma atomic emission spectrometry (i.c.p.e.s.). Table 1 indicates that the results obtained from the proposed system are in close agreement with those obtained by i.c.p.e.s.

The system in Fig. 1B was applied to soil and plant analysis. A Δt value of 10 s was employed in both situations, the differences in the required dispersions being attained by changing the second sample loop. Dispersion factors were determined by using a dye solution, as described earlier [6]. The dispersion factors were 0.009 and 0.041 for the soil and plant systems, respectively. As these factors depend on the Δt value, the analytical range of the proposed systems could be changed by employing other Δt values. When these systems were employed, their stability and the precision of the measurements were again satisfactory. Relative standard deviations were less than 1%. Table 1 indicates that for soil and plant analysis, the results agree with those obtained by i.c.p.e.s.

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A NEW METHOD OF DETERMINING THE EXTENT OF BINDING OF AN IONIC DYE TO A POLYELECTROLYTE IN SOLUTION

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SUMMARY

A method of determining the free dye concentration in a dye/polyelectrolyte solution is described. The method utilizes the tendency of cationic dyes to adsorb to a cellulose dialysis membrane, and the nature of the membrane binding is studied by spectrophotometric methods. The binding of the cationic dye toluidine blue to the polyanion sodium carboxymethylcellulose in solution is used as an example. The proposed technique gives reliable estimates of free dye concentration over a wide range of polyanion and dye concentrations, both in the presence and absence of added simple electrolyte. The method possesses inherent advantages over estimates of free dye concentration obtained by absorption spectrophotometry and is more versatile than fluorimetry.

The study of the interaction between cationic dyes and soluble polyanions is extensive and long established [1—4 and refs. therein]. It is well known that this interaction is often accompanied by marked changes in the visible absorption spectrum of the dye, an effect which has been given the name metachromasia. The principal feature in this effect is the appearance of a broad absorption peak at lower wavelengths when a polyanion is added to a solution of a cationic dye (Fig. 1). It is generally thought that this feature is due to aggregation of the dye on the polyanion and that the height of the new peak is related to the amount of dye which is aggregated this way [3]. These changes in the absorption spectrum have often been used as the basis of quantitative attempts to study the binding of dyes to polyelectrolytes [4, 5]. Unfortunately, the different absorption peaks show significant over-

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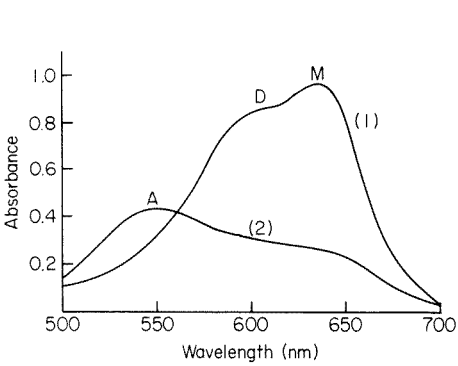

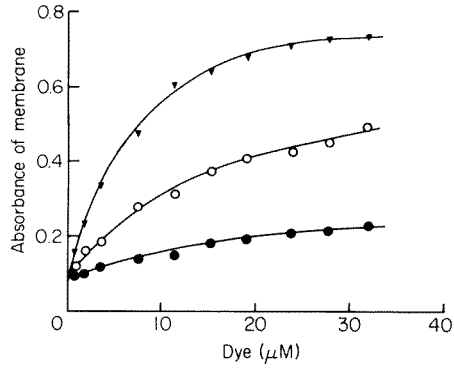

Fig. 1. Spectrophotometer traces for (1) toluidine blue solution (32 μM) showing the monomer absorption band, M, and the dimer absorption band, D; (2) 32 μM toluidine blue—NaCMC solution showing the aggregate band, A. *S/D* ratio = 5.

Fig. 2. The variation of membrane absorbance at 632 nm with concentration of the toluidine blue solution in which the membrane has been immersed. The three curves illustrate the effect of added salt on the binding of dye to the membrane: (•) 25 mM NaCl; (○) 1 mM NaCl; (▾) no added salt.

lap and, in the case of many dyes, the spectra are complicated by the presence of a dye dimer peak in addition to the aggregate peak referred to above [5]. For these reasons it is often difficult to calculate molar absorptivities for the various species present with certainty. In some cases these problems can be overcome by studying the fluorescence spectrum of a dye in the presence of the polyanion [6]. However, this method is restricted to those dyes, such as acridine orange, which fluoresce in the visible region with well resolved emission spectra for the free dye and aggregated dye. Dye binding in solution has also been studied by using equilibrium dialysis [7]. A well known source of uncertainty in this technique has its origins in the Donnan effect which requires the presence of relatively high concentrations of simple electrolyte for its suppression. It often happens, however, that the presence of such electrolyte has a very marked effect on the binding process under study and this severely limits the usefulness of the technique [8]. In addition, the membranes used in dialysis will often bind an appreciable amount of dye, and allowance must be made for this [7]. It was as a result of a study of this latter problem that the technique described here was developed.

The proposed new method of determining the extent of binding of a cationic dye to a soluble polyanion utilizes the adsorption of the dye to a cellulosic membrane. It can be used to obtain unambiguous quantitative data for solutions which contain varying proportions of dye and polyanion and in the presence or absence of simple electrolyte. The method is used here to study the binding of toluidine blue to sodium carboxymethylcellulose (NaCMC) in the presence of different concentrations of simple electrolyte, sodium chloride, using NaCMC with different degrees of substitution. The

results obtained are compared with those obtained by visible absorption spectrometry on similar solutions. The sensitivity of the technique was quite sufficient to permit work with toluidine blue solutions at micromolar concentrations. The sensitivity is determined by the properties of the membrane used, in particular its affinity for dye, and could most probably be improved.

Dialysis membrane is composed of cellulose with protective plasticisers. There is a natural abundance of oxidised glucose units, oxycellulose, on which carboxylic acid groups exist. These can dissociate to produce a net negative charge on the cellulose membrane in water and give it an affinity for a cationic dye [9].

EXPERIMENTAL

Equipment and solutions

The absorbances of the solutions and membranes were measured with a Pye 8/100 spectrophotometer. All measurements were done at 25 ± 1°C.

Toluidine blue (TB) dye was purified by repeated recrystallisation from ethanol. Samples of sodium carboxymethylcellulose were used as supplied, after drying at 70°C in vacuo. The dialysis membrane (Medicell International Ltd., London) was prepared for use as follows: strips of the membrane were heated in a solution of ethanol and water (30:70, v/v) for approximately 3 h to remove plasticiser. During this process the solution was changed several times. The membrane strips were then rinsed thoroughly in twice-distilled water and cut into sections of area 2 cm^2 using a cork borer. The membrane sections were then stored in distilled water.

Stock solutions of the dye and polyelectrolyte were prepared, the dye solution being stored in opaque bottles. The concentration of polyelectrolyte was calculated in terms of the molarity of anionic sites. The degree of substitution of the NaCMC is denoted by symbols such as 4*M*, indicating that the polymer referred to contains, on average, 4 anionic sites per 10 cellulose units. Equal volumes of the dye and polyelectrolyte solutions were mixed so that a solution of the required site to dye ratio (S/D) was obtained. Solutions with different S/D ratios were prepared by keeping the dye concentration fixed and varying the polyelectrolyte concentration. It was assumed that the concentration changes involved did not affect the polyelectrolyte conformation.

Calibration

When a small piece of suitably prepared dialysis membrane is immersed in a solution of cationic dye at constant temperature, the dye is absorbed by the membrane to an extent determined by the dye concentration and, in the initial stage, the duration of immersion. This binding is a reversible process: when a dyed membrane was placed into a large volume of distilled water, it was found that the bound dye is eventually completely removed

from the membrane. The binding of the dye to the membrane can be studied by measuring the optical absorbance of the membrane with a conventional visible absorption spectrometer. For good reproducibility of results a routine was developed to measure membrane absorbance. A membrane was removed from solution using forceps and placed onto a clean microscope slide; a second slide was placed on top of the wet membrane, care being taken to exclude air bubbles. The membrane thus mounted was placed into a spectrophotometer cell holder and the absorbance measured. Dried or rinsed membranes did not give reproducible results. For adsorption measurements, the membrane area chosen was 2 cm^2 and an equilibrium solution volume of 100 ml was used. The solutions containing membranes were continuously shaken at 25°C for 3 h to achieve a constant membrane absorbance before spectrophotometric measurements were taken. Four samples of each solution were prepared and the results obtained represent, in each case, an average of four absorption measurements.

The uniformity of the membrane material was studied by placing six membrane samples into a dye solution and shaking at 25°C for 3 h. The absorbance of each membrane was then measured. The average absorbance was found to be 0.607 with a standard deviation of 0.014.

The effect on the absorbance of the membrane, at the monomer wavelength, of dye concentration and duration of immersion are shown in Figs. 2 and 3 respectively. Figure 2 also illustrates the effect of the presence of simple electrolyte upon this binding process. For the solutions studied, an equilibrium was set up between dye free in solution and dye bound to the membrane within 2 h. The absorbance of a dyed membrane can therefore be used to measure the concentration of the dye solution in which the

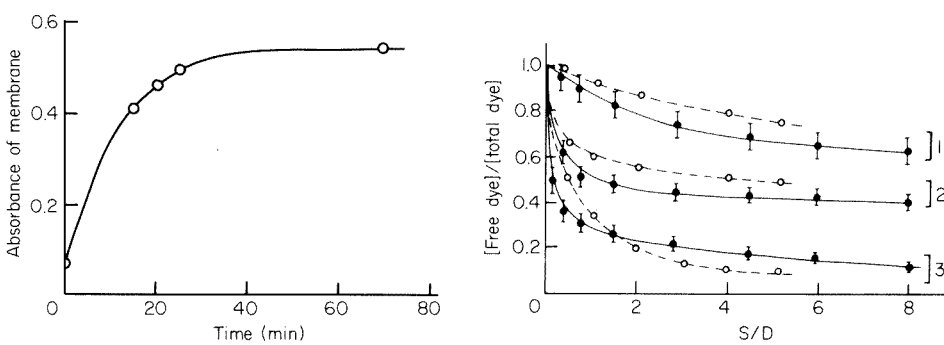

Fig. 3. The variation of membrane absorbance at 632 nm with duration of immersion in 10 μM toluidine blue solution with no added salt.

Fig. 4. The variation of the fraction of free dye with site-to-dye ratio: (•) membrane absorbance measurements; (○) solution absorbance measurements. (1) NaCMC type 4*M* in 25 mM NaCl; (2) NaCMC type 12*M* in 25 mM NaCl; (3) NaCMC type 4*M* in the absence of salt. [TB] = 32 μM in all cases.

membrane has been immersed by use of a calibration curve such as those shown in Fig. 2.

The effect on the dye concentration of placing a membrane into a dye solution was investigated and found to be negligible provided that the ratio of solution volume to membrane area exceeded 25 ml cm^{-2}. For example, with a volume/membrane ratio of 25 ml cm^{-2}, the effect of the presence of the membrane was to reduce the solution absorbance at the aggregate peak wavelength by 2.5% when the composition of the solution was [TB] = 32 μM S/D = 10. In practice, this critical volume/membrane ratio must be determined empirically as different dyes exhibit different affinities for the membrane material. However, if the absorbance of the dye solution is measured before and after inserting the membrane, any change in dye concentration can be allowed for by using an appropriate correction to the calibration curves (Fig. 2). This is simply achieved by calculating the amount of dye bound to the membrane by spectrophotometric determination of the concentration of dye in a calibrating solution before and after insertion of a membrane. Knowing the amount of dye bound to a membrane as a function of membrane absorbance makes it possible to correct for the reduction in dye concentration caused by binding to the membrane.

If the membrane is immersed in a solution of a cationic dye and an anionic polyelectrolyte, the membrane absorbance can, in principle, be used as a measure of the concentration of dye free in solution. Knowing the overall dye concentration then allows one to calculate the proportion of dye bound to the polyelectrolyte. If the dye—polyanion solution contains added simple electrolyte then the calibrating solutions must contain the same concentration of simple electrolyte.

Preliminary tests

Various preliminary experiments were done with a view to checking the validity of the method and also to determining the correct conditions for its use.

The main assumption involved is that the addition of a polyanion to the dye solution affects the amount of membrane-bound dye only by competing with the membrane for dye. The indications are that this is a reasonable assumption as an inspection of the complete absorption spectrum of a membrane which had been immersed in a dye—polyanion solution showed no metachromatic band for the range of dye concentrations used here. One would expect to see a metachromatic band if the polyanion were to be incorporated into or absorbed onto the membrane, in conjunction with stacked dye. Furthermore, one would not expect interaction between the membrane and the polyanion, except possibly at very high ionic strength, as both carry anionic carboxyl groups.

There is the possibility that sodium ions from the polyelectrolyte may influence the binding of dye to the membrane by competing with the dye. However, in these experiments, the concentration of such ions was typically

less than 0.2 mM even if one assumes complete dissociation from the polyelectrolyte. In addition, the low values obtained for the osmotic coefficients [10] and counter-ion activities [11] for polyelectrolytes indicate that a substantial proportion of the counter-ions will be confined within the high electric field region close to the polyion. The effect of these counter-ions on the binding of dye by a membrane is therefore expected to be small especially in the presence of added salt. There are situations, however, in which the effect of counter-ions may not be negligible, e.g., when the polyelectrolyte has a low charge density, is present at relatively high concentration (greater than 0.2 mM in terms of sites), and in the absence of added salt.

RESULTS

The above experiments give confidence that the tendency of a cellulose membrane to absorb dye can be used to monitor the free dye concentration in a dye/polyelectrolyte solution. Typical results for the binding of toluidine blue to the polyelectrolyte sodium carboxymethylcellulose are shown in Fig. 4. For comparison, absorption measurements were taken on the dye/polyelectrolyte solutions themselves and binding data were derived in accordance with the method described by Schwarz et al. [12]. These latter measurements, which are now standard, will be referred to in the following discussion as having been obtained by measurements of solution absorbance, whereas data obtained with membranes will be referred to as having been found from measurement of membrane absorbance.

Measurement errors

Error bars indicating 95% confidence limits are shown on Fig. 4 for the membrane absorbance measurements. These limits were estimated by calculation of the mean and standard deviation of the four absorbance readings taken for each point. The error in free dye concentration is then found from the calibration curve (Fig. 2). The error bars shown represent points within two standard deviations of the mean. The variation in the magnitude of the error is due mainly to the shape of the calibration curves which tend to level off at high dye concentrations. The errors in the solution absorbance measurements are relatively small but there is considerable uncertainty in the interpretation of these data, because the absorption spectra consist of overlapping bands and the shape of separate bands is not known. Attempts to deconvolute such spectra are unlikely to be reliable and this is a major problem in the interpretation of solution absorbance data. Deconvolution has been utilised to study dimerization in dye solutions [13] but the uncertainties become much more severe in the presence of the metachromatic band arising from the addition of a polyelectrolyte to the solution.

DISCUSSION

The results obtained by measurements of membrane absorbance and solution absorbance were found to be qualitatively similar. At low ionic strength and high S/D ratios, a substantial fraction of the dye is bound to the polyanion. It should be noted, however, that measurements of solution absorbance will at best allow discrimination only between forms of dye which have different absorption spectra. It is then assumed that all the dye in one form is bound to the polyelectrolyte and all the dye in the other form is free in solution. There is good evidence, from measurements of solution absorbance at high S/D ratio, that this is not the case and that, under these conditions, some of the bound dye has an absorption spectrum similar to that of free dye [3]. This particular ambiguity does not arise when measurements are made by the membrane technique. It appears that this technique is able to distinguish unequivocally between free and bound dye. In addition, the membrane technique does not require a determination of the dimerization constant for dyes forming dimers in solution. The membrane technique should be particularly useful in the study of dye/polyelectrolyte interactions in which there is no metachromatic effect [2], because only a single absorption band is required. Ordinary spectrophotometric methods would be useless in this case.

The results depicted in Fig. 4 show all the expected behaviour. The binding of dye to the polyelectrolyte is inhibited by the presence of simple electrolyte [8] and is increased as the charge density on the polyelectrolyte is increased. This technique should be a useful addition to existing means of studying the quantitative aspects of dye/polyanion interactions in that it has fewer limitations than the other methods referred to. The sensitivity of the technique is good and it could probably be improved by use of membranes of better optical quality and with a very uniform distribution of anionic sites upon them. Further, it should be possible to extend the method to the study of the interaction between anionic dyes and polycations by employing a membrane bearing cationic surface groups.

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FLUORIMETRIC DETERMINATION OF SECONDARY AMINO ACIDS BY 7-FLUORO-4-NITROBENZO-2-OXA-1,3-DIAZOLE

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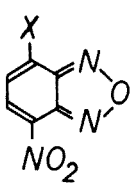
SUMMARY

The fluorigenic reaction of proline with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD—F) is superior, in terms of reactivity and fluorescence yield, to the reactions with the analogous 7-chloro and 7-bromo derivatives. With NBD—F, the reagent blank fluorescence can be suppressed by adjusting the medium to around pH 1 with hydrochloric acid. Many secondary amino acids can be determined by reaction with NBD—F at pH 7.5 at 70°C for 5 min and subsequent acidification to pH 1. The detection limits for proline, hydroxyproline and sarcosine are 0.08, 0.04 and 0.17 nmol ml^{-1}, respectively. Under the same conditions, the primary amino acids, alanine, arginine and aspartic acid, are detected at 1.7, 1.7 and 3.4 nmol ml^{-1}, respectively.

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD—Cl), introduced by Ghosh and Whitehouse [1] as a fluorigenic reagent for amines, reacts easily with secondary amino acids such as proline to yield high fluorescence [2]. The reagent has been used to determine proline and hydroxyproline in blood as a post-column reaction system in liquid chromatography [3]. However, in the course of some studies on the determination of secondary amino acids, the recommended reaction conditions (at 75°C for 20 min) [2] were found to be rather unfavorable, particularly for possible liquid chromatography applications.



X = F : NBD-F
X = Cl : NBD-Cl
X = Br : NBD-Br

In the work described here, the reaction of 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD—F) with proline was investigated in the hope of reducing the reaction time in comparison with NBD—Cl. The 7-fluoro moiety in the *p*-position to a nitro group in an aromatic ring would be more reactive than the 7-chloro moiety [4]. The bromo derivative, 7-bromo-4-nitrobenzo-2-oxa-1,3-diazole (NBD—Br), was also studied. Suitable conditions were found

for proline and were applied to the detection of hydroxyproline, sarcosine and primary amino acids.

EXPERIMENTAL

Reagents and chemicals

NBD—F was synthesized and purified by the method of Nunno et al. [5] from 2,6-difluoroaniline (m.p. 53.5—54.5°C; [5], 52.5—53.5°C). NBD—Br was kindly supplied by the Takeda Yakuhin Kogyo Co., Osaka (m.p. 93.0—94.0°C). NBD—Cl (m.p. 97.0—97.5°C; [6], 96.5—97.0°C) was from Aldrich, Milwaukee. The results obtained for C, H, N and the halogen in these three compounds were in excellent agreement with the theoretical values. Amino acids were from the Kyowa Hakko Kogyo Co., Tokyo. All chemicals were of reagent grade.

Recommended procedure

The aqueous sample solution (0.1 ml) containing up to 10 nmol of amino acids and 2.8 ml of 0.1 M borate buffer solution (pH 7.5) containing ethanol (50%) were mixed well. Then 0.1 ml of a 1 mM NBD—F solution in ethanol was added to the mixture. After heating at 70°C for 5 min on a thermal bath, the reaction mixture was cooled immediately with ice-water and allowed to stand for a few minutes at room temperature; 0.1 ml of 3 M hydrochloric acid was then added (for Figs. 1—3 and 5a, the fluorescence measurements were done at 550 nm with excitation at 470 nm without acid). The fluorescence intensities were measured at 530 nm with excitation at 470 nm using a Hitachi 650-10(S) spectrofluorimeter.

When 0.1 ml of NBD—F solution (1 mM in ethanol), 2.8 ml of 20% ethanolic borate buffer solution (pH 8.5) and 0.1 ml of proline (0.1 mM in water) were mixed and heated at 60°C for 20 min, the net fluorescence intensity (i.e., the observed intensity minus the reagent blank fluorescence) was tentatively regarded as giving a relative fluorescence intensity of 100.

RESULTS

Comparison of the reactions of proline with the halogenated 4-nitrobenzo-2-oxa-1,3-diazoles

The general aim of these experiments was to achieve the best possible compromise between fast reaction times and high net fluorescence intensity.

Effect of pH on the reaction. Time courses of the reaction of proline with each of the three reagents were investigated at various pH values at 60°C in 20% ethanol. (The 20% ethanol solutions were used because precipitation occurred from 50% solutions at the higher pH values.) As the pH increased, the reaction speed became faster to yield higher net fluorescence intensities (the reagent blank fluorescences were also higher; data not shown) whereas the fluorescence decay became faster (Fig. 1) to give lower net intensities.

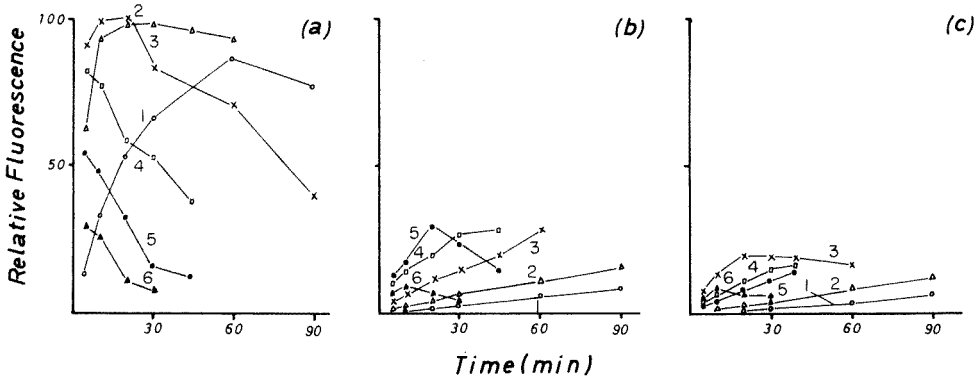


Fig. 1. Effect of pH on the reaction. Mixtures of 0.1 ml of 0.1 mM proline and 0.1 ml of 1 mM NBD—X were heated at 60°C with 2.8 ml of 0.1 M borate buffer solution containing 20% ethanol at different pH values. (a), (b) and (c) relate to NBD—F, NBD—Cl and NBD—Br, respectively. pH values: (1) 7.5; (2) 8.0; (3) 8.5; (4) 9.0; (5) 9.5; (6) 10.0. Net intensities are shown.

NBD—F gave the fastest reactions as well as the highest net fluorescence intensity at any pH investigated.

Effect of ethanol concentration on the reaction. Time courses of the reaction of proline with each reagent were investigated in varying concentrations of ethanol at pH 8.5 at 60°C. As the concentration of ethanol increased, the reaction became faster with higher reagent blank fluorescence and higher net fluorescence intensity, but also faster fluorescence decay (Fig. 2). NBD—F gave the highest fluorescence intensity among the three NBDs with concentrations of ethanol between 3.3 and 50%. Solutions containing more than 50% ethanol were not studied because of precipitate formation in such media. The emission and excitation spectra of the reaction mixtures were constant with varying concentrations of ethanol.

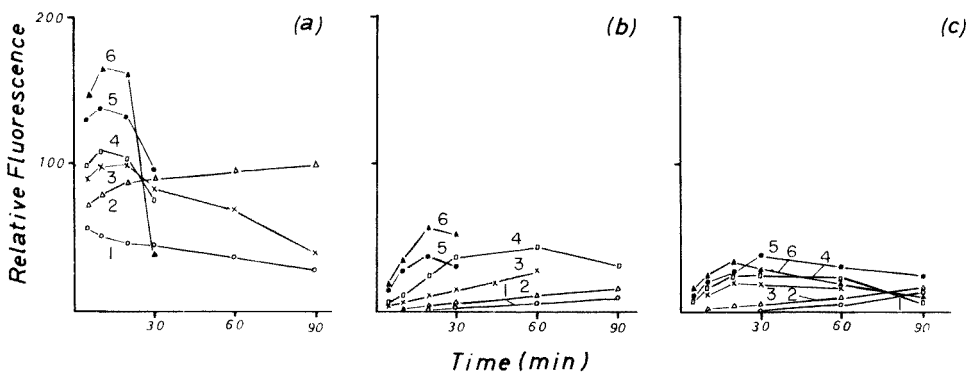


Fig. 2. Effect of ethanol concentration on the reaction at pH 8.5. Conditions as for Fig. 1 except for ethanol concentration. (a), (b) and (c) relate to NBD—F, NBD—Cl and NBD—Br, respectively. Ethanol concentration: (1) 3.3%; (2) 10%; (3) 20%; (4) 30%; (5) 40%; (6) 50%.

Effect of temperature on the reaction. The reactions of proline with each reagent were investigated at 40, 50, 60 and 70°C at pH 8.5 in 20% ethanol; higher temperatures were not studied because of ethanol evaporation. The reaction and the fluorescence decay were accelerated with the increase of temperature, and the higher temperatures gave higher net fluorescence intensity with higher blank fluorescence intensity (Fig. 3).

Reaction with the fluorinated reagent

Effect of solvent on the reaction. Time courses of the reaction of proline with NBD—F were investigated at pH 8.5 at 60°C in 20% organic solvents. Ethanol gave the highest net fluorescence intensity, followed by methanol; *n*-propanol, acetone, dioxane or acetonitrile gave the same level of fluorescence intensities as those observed for the reagent blanks. Similar results were obtained for NBD—Cl and NBD—Br.

To optimize the ethanol concentration, reaction mixtures containing proline with NBD—F at pH 8.5 were diluted with buffer solutions (pH 8.5) having various concentrations of ethanol after heating at 60°C for 20 min. The net fluorescence intensities increased as the ethanol concentrations increased, and doubled when the final ethanol concentration was raised from 20% to 50%.

Stability of the fluorescence and pH effects. When the same reaction mixtures as in the above experiment were diluted with the same volume of buffers (pH 1.0—10.0), and heated again at 60°C, the net fluorescence intensities were constant between pH 1.0 and 6.0 whereas they decayed at basic pH (Fig. 4). When the diluting solution was of pH 2.0—10.0, only straightforward dilution effects were observed for both proline and the reagent blank fluorescence intensities. However, when hydrochloric acid was added to the reaction mixture and the reagent blank solution, the observed fluorescence intensity of the reaction mixture was slightly increased while that of the reagent blank solution was decreased remarkably. Thus the net fluorescence intensity was significantly higher when hydrochloric acid was

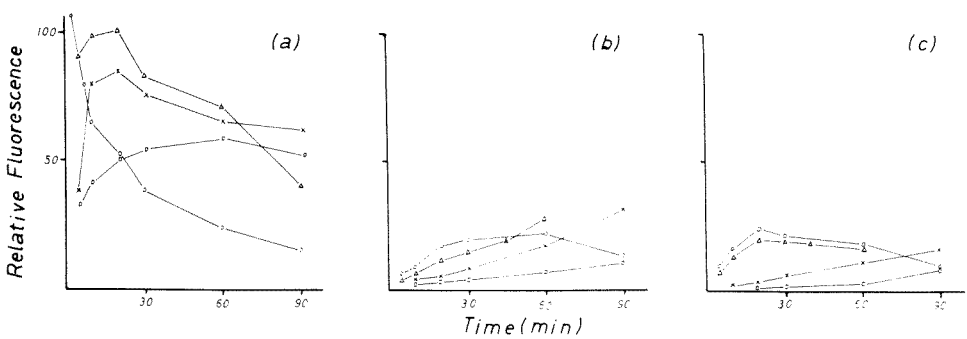


Fig. 3. Effect of temperature on the reaction at pH 8.5. Conditions as for Fig. 1 except for temperature. (a), (b) and (c) relate to NBD—F, NBD—Cl and NBD—Br, respectively. Heating temperature: (○) 70; (△) 60; (×) 50; (□) 40°C.

added to the reaction mixtures. The maximum wavelength of the emission spectrum was also slightly changed at pH 1.0 from 550 nm to 530 nm.

Re-investigation of the reaction of proline with NBD—F. The reactions of proline with NBD—F were then re-investigated at pH 7.5, 8.0 and 8.5 in 50% ethanolic solution at 70°C with and without a final addition of hydrochloric acid. As shown in Fig. 5a, the reagent blank fluorescence at lower pH was smaller and the net fluorescence intensity without the addition of hydrochloric acid was higher than those at higher pH. When the solution was

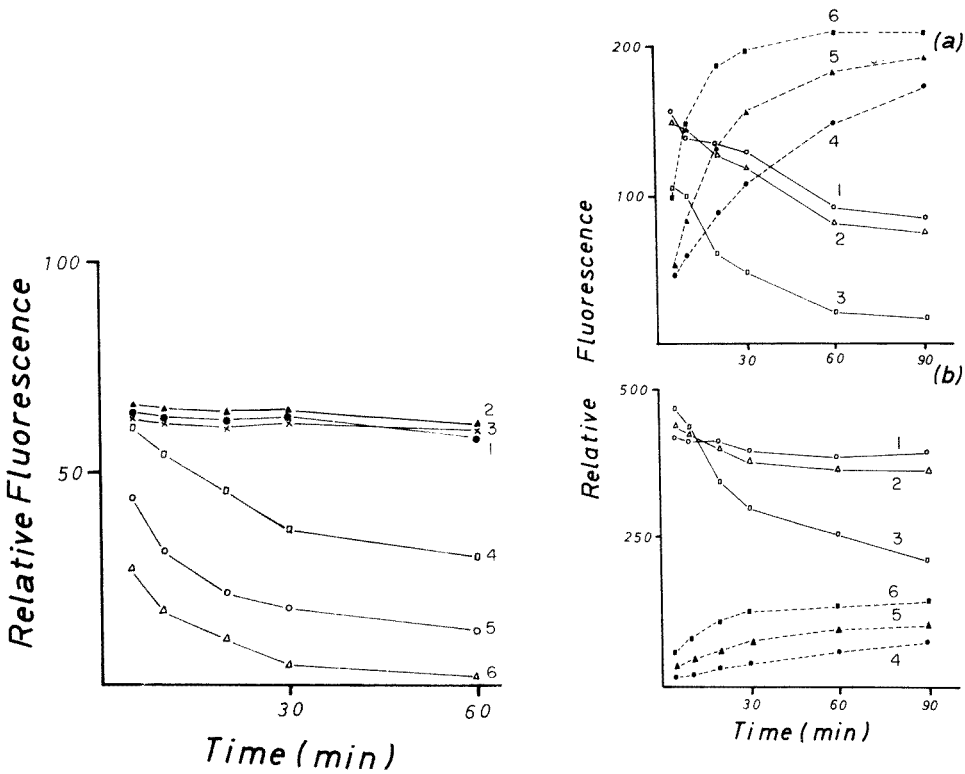


Fig. 4. Stability of fluorescence. Mixtures as for Fig. 1 (pH 8.5) were heated at 60°C for 15 min, and then the same volume of a buffer solution (0.1 M borate, 0.1 M phosphate or 1 M acetate) or hydrochloric acid was added to the reaction mixture, and heated at 60°C again. The pH of the added solutions were: (1) 1.0; (2) 3.0; (3) 5.0; (4) 7.0; (5) 9.0; (6) 10.0.

Fig. 5. (a) Time courses of the net fluorescence intensity and the reagent blank fluorescence at various pH values. Mixtures of 0.1 ml of 0.1 mM proline, 0.1 ml of 1 mM NBD—F and 2.8 ml of 50% ethanolic 0.1 M borate buffer at different pH were heated at 70°C. pH values: (1) 7.5; (2) 8.0, (3) pH 8.5; curves 4—6 are the reagent blanks corresponding to curves 1—3, respectively, magnified 3-fold for better legibility. (b) Effect of hydrochloric acid on the fluorescence intensity; each of samples in Fig. 5 (a) was mixed in the volume ratio 3.0:0.1 with 3 M hydrochloric acid.

acidified with hydrochloric acid, the net fluorescence intensity increased three times compared with the original value because of the great reduction of the reagent blank fluorescence (Fig. 5b).

Detection limits for proline, hydroxyproline, sarcosine and primary amino acids. In these tests, 1.5 ml of amino acid solution in phosphate (pH 7.0 or 7.5) or borate (pH 7.5, 8.0 or 8.5) buffer was mixed with 1.5 ml of 0.06 mM NBD—F in ethanol and heated at 70°C. After the reaction mixture had cooled in an ice-bath, 100 μl of 3 M hydrochloric acid was added to reduce the pH to 1. The highest net fluorescence intensity was obtained with about 5 min of heating at 70°C at pH 8.5. But the buffer of pH 7.5 was preferred for the final method because sodium borate precipitated from the reaction mixture at pH 8.5 at room temperature. By this manual method, the detection limits (the net fluorescence intensity of twice the reagent blank fluorescence intensity) for proline, hydroxyproline, sarcosine, alanine, arginine and aspartic acid were 0.25, 0.13, 0.5, 5.0, 5.0 and 10.0 nmol, respectively.

DISCUSSION

As was expected, the reactivities of NBD derivatives were in the order of NBD—F, NBD—Cl and NBD—Br. NBD—F seemed to be the best of these fluorigenic reagents for proline, giving the highest net fluorescence intensities and the highest reaction rate under any condition tested.

The reaction was accelerated by increased pH and temperature, and seemed to be partially accelerated by addition of ethanol. Ethanol, used as a solvent for NBD—Cl in earlier papers [1—3], was also a better solvent for NBD—F than methanol, etc., and tended to increase the net fluorescence intensity of the reaction mixture. A 50% concentration of ethanol was chosen, as outlined under Results.

Tests involving transference of reaction mixtures of proline with NBD—F in basic medium into solutions at other pH values showed that the intensity was not changed by pH changes from 2 to 9. Thus the intensity of the reaction mixture in basic medium below pH 10 seems to reflect the amount of the fluorophore present in the medium.

The production of the fluorophore was greater and its degradation was faster in basic medium, but the reagent blank was also more significant at basic pH. Therefore, for the determination of amino acids, changing the reaction medium to pH 1 was desirable, particularly as the reagent blank fluorescence was greatly reduced at pH 1. Thus, reaction in pH 7.5 medium at 70°C for 5 min was selected for the present manual method. In the case of h.p.l.c. determinations, a higher temperature might be used to reduce the reaction period because the instantaneous reaction could be handled mechanically.

Under the best conditions with NBD—F, 0.08 nmol ml^{-1} proline, 0.04 nmol ml^{-1} hydroxyproline and 0.17 nmol ml^{-1} sarcosine were measurable. Under the same conditions, alanine, arginine and aspartic acid were detected

at 1.7, 1.7 and 3.4 nmol ml^{-1}, respectively. NBD—F did not give better sensitivity for primary amino acids than fluorescamine, with which as little as 0.5 nmol ml^{-1} alanine can be detected [7]. However, NBD—F permits detection of both primary and secondary amino acids in situ. In the case of *o*-phthaldialdehyde [8] and fluorescamine [9], oxidative decarboxylation of secondary amino acids is a prerequisite for their detection [10, 11].

According to Fager et al. [2], who used NBD—Cl, primary amino acids such as alanine afforded one hundredth of the intensity compared to proline and hydroxyproline. The same was true for NBD—F under most reaction conditions. However, under the best conditions found here, i.e., with acidification after the reaction, the detection limit was one twentieth of the limit for proline. In some preliminary experiments with NBD—Cl and acidification, the same reaction ratio between proline and alanine was obtained. In either case, the reason why alanine does not give a higher intensity is not clear. Quenching of the fluorophore by the medium seems unlikely because the intensity of the reaction medium did not change under various conditions of pH. The kinetics of the reactions of NBD—F with amino acids are being studied in an attempt to clarify this.

The novel fluorigenic reagent, NBD—F, is superior to NBD—Cl which has already proved useful as a post-column derivatization reagent [3], and might be useful in the h.p.l.c. of secondary amino acids in biological fluids. Work is in progress in this laboratory to examine such possibilities.

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Short Communication

A NOVEL APPROACH FOR DETERMINATION OF TIN, LEAD AND COPPER IN BIOLOGICAL SAMPLES AND SEDIMENTS BY ALTERNATING CURRENT ANODIC STRIPPING VOLTAMMETRY

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Summary. Simultaneous determination of Sn, Pb and Cu in biological samples (plants, animal tissues) and sediments is possible in methanolic 1 M HCl by alternating current anodic stripping voltammetry after digestion of the samples with Lumatom. This digesting agent eliminates some common sources of error and increases the intensity of the anodic current. With a 1 min pre-electrolysis time, 1 ppb Sn, Pb or Cu can easily be determined.

Polarographic techniques are relatively sensitive, but are susceptible to numerous interferences. For most samples, it is normally necessary to conduct a digestion in acidic solutions or other media. In some cases, it is necessary to complex the ion to be determined, in order to separate the half-wave potentials. These steps normally increase the errors from contamination, and the analysis becomes complicated and time-consuming.

Numerous studies have been reported for the determination of Sn, Pb, Cu and other elements in relatively simple media (e.g. water, beer, wine) using voltammetric techniques [1—3], and speciation studies may be possible [2]. Applications of voltammetry to environmental samples such as plants, animal tissues and sediments are limited because tedious sample preparations are usually involved [4].

In this work, a quaternary ammonium hydroxide in isopropanol (Lumatom) was used to digest biological samples and sediments. This technique of digestion is very simple and convenient for routine analysis. The voltammetric determinations cannot be done in aqueous supporting electrolyte because Lumatom is not completely miscible with water. Accordingly, 1M HCl in methanol was used as the supporting electrolyte, as it permits a good separation of the tin and lead peaks [5].

Experimental

Reagents. The hydrochloric acid used was of Suprapur grade and the methanol was of analytical grade (both from Merck). Standard Sn(IV), Pb(II) and Cu(II) solutions were prepared by diluting 1.000 g l^{-1} stock solutions (Titrisol Merck) with 1 M HCl in methanol. Lumatom, a digesting agent containing a quaternary ammonium hydroxide in isopropanol (H. Kürner,

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D-6451 Neuberg, FRG), was used. The supporting electrolyte was 1 M HCl in methanol containing 2.5% (w/v) Lumatom. All the polarographic determinations were carried out in this media.

Apparatus and polarographic conditions. A Metrohm E506 Polarecord instrument was used with Metrohm accessories. Polarographic conditions were as follows: ACT_1 (Tast alternating current) mode, 10 mV amplitude, 75 Hz frequency, 5 mV s^{-1} sweep rate, 0° phase angle. The mercury drop surface area was 2.2 mm^2 and the reference electrode was Ag/AgCl/KCl (sat.)//0.1 M KNO_3. The pre-electrolysis potential of −0.8 V was applied for 1 min with stirring and 15 s without stirring. The solutions were deoxygenated with nitrogen for 5 min before measurement and for 1 min after each standard addition. All measurements were made at 20°C. All quoted potentials are referred to the Ag/AgCl electrode.

Preparation of samples. Biological samples and sediments (ca. 100 mg) were digested in sealed glass vials with 1 ml of Lumatom at 50°C. Dried samples must be rehydrated with distilled water before the digestion. The digestion was complete in 3 h or more, depending on the amount and nature of the sample. Cellulose and some polysaccharides in plant tissue and some inorganic components of sediments were not dissolved.

Generally, 4 ml of supporting electrolyte and 0.1 ml of the digested sample were introduced into the electrochemical cell. If required, samples containing high concentrations of metal may be diluted with 1 M HCl in methanol and then 0.1 ml taken for analysis. The standard addition method was used for all determinations.

Results and discussion

The increased sensitivity in the determination of Sn, Pb and Cu caused by

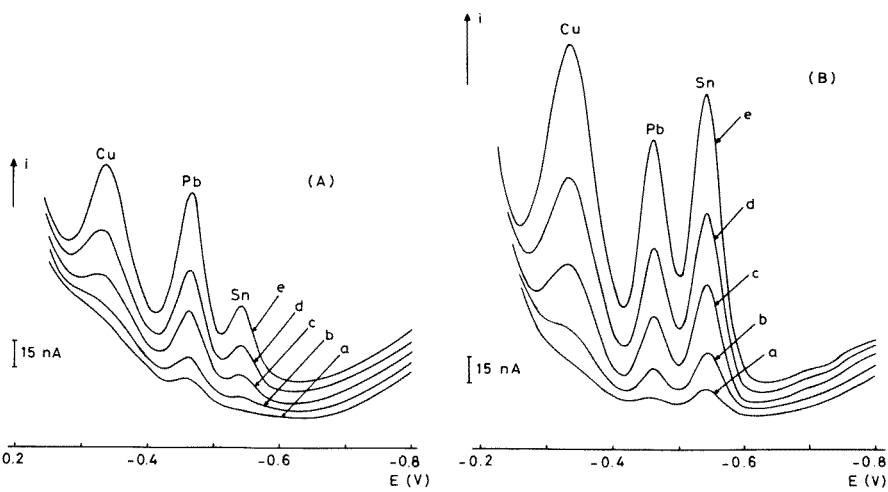

Fig. 1. Typical polarograms of Sn, Pb and Cu by a.c. anodic stripping voltammetry. Pre-electrolysis time, 1 min. Concentration of Sn, Pb and Cu: (a) blank, (b) 2 ppb, (c) 6 ppb, (d) 10 ppb, (e) 20 ppb. (A) In the absence of Lumatom; (B) in the presence of 2.5% (w/v) Lumatom.

Lumatom has been reported [6]. The anodic current increases with the percentage of Lumatom in the supporting electrolyte, the increase being particularly significant for tin and copper. The addition of 2.5% (w/v) Lumatom was found to increase the sensitivity for tin and copper by factors of about 3 and 2, respectively. Figure 1 shows typical anodic stripping voltammograms in the presence and absence of Lumaton for different concentrations of Sn, Pb and Cu; the peak potentials of Sn, Pb and Cu are not affected, being −0.56 V, −0.47 V and −0.34 V, respectively, in both cases. Lumatom improved the base line (Fig. 1B, curve a) and the blank contained about 1 ppb tin.

The effect of the pre-electrolysis time on the peak heights is shown in Fig. 2. Without Lumatom (Fig. 2A), the current for Pb and Cu increases with the time of pre-electrolysis, whereas for tin a plateau is reached after 3 min. Glodowski and Kublik [3] reported the same phenomenon for tin(IV) in perchloric acid supporting electrolyte, and the formation of an intermetallic compound may be responsible. In the presence of Lumatom (Fig. 2B), the currents for Sn, Pb and Cu increase rapidly and maxima are obtained after pre-electrolysis times of about 3 min. The later decreases in the anodic currents of tin and lead are probably due to adsorption of active components of Lumatom on the mercury drop. It is well known that cations such as tetraalkylammonium or tribenzylammonium have surface-active properties [7]. In the case of copper, the increased sensitivity after pre-electrolysis times of 10 min cannot be explained fully at the present time. For practical purposes, a pre-electrolysis time of 1 min was selected for all determinations, and 1 ppb can easily be determined; the sensitivity can be almost doubled with pre-electrolysis times of 3 min.

Organotin compounds. The decomposition of some organotin compounds (dimethyltin dichloride, dibutyltin dichloride and triphenyltin chloride) with Lumatom was found to be complete. The voltammetric determination of total tin (without speciation) may therefore be possible in some cases (see below).

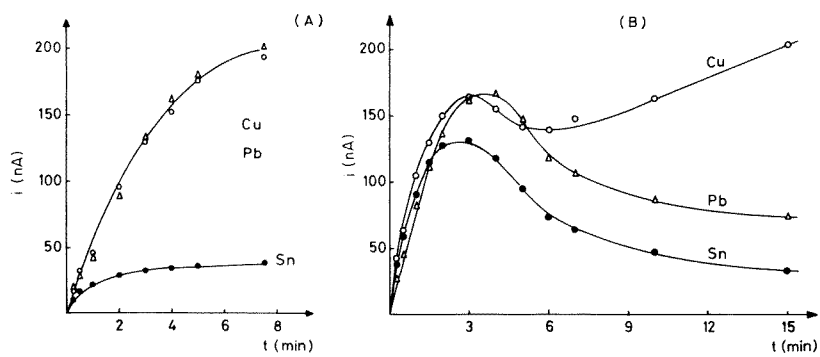

Fig. 2. Effect of pre-electrolysis time on the sensitivity for (•) Sn, (△) Pb and (○) Cu (20 ppb of each element): (A) in the absence of Lumatom; (B) in the presence of 2.5% (w/v) Lumatom.

TABLE 1

Determination of copper and lead in reference materials by the recommended procedure[a]

SRM	Sample weight (mg)	Water volume for rehydration (ml)	Obtained (ppm)		Certified (ppm)	
			Pb	Cu	Pb	Cu
Orchard Leaves (SRM 1571)	89.5	0.06	47 ± 5[b]	ND[c]	45 ± 3	12 ± 1
Bovine Liver (SRM 1577)	50.2	0.06	ND[d]	190 ± 15	0.34 ± 0.08	193 ± 10
River Sediment (SRM 1645)	92.5	0.05	695 ± 45	96 ± 14	714 ± 28	109 ± 19

[a]In these three cases, the digested sample was stirred well before the pipetting, diluted 10 times with 1 M HCl in methanol and then 0.1 ml of this solution was used for voltammetry. [b]Standard deviation for 6 determinations. [c]Copper could not be determined because the copper peak was masked by some interferences. [d]The concentration of copper compared to lead was very high (568 times) so that lead could not be determined.

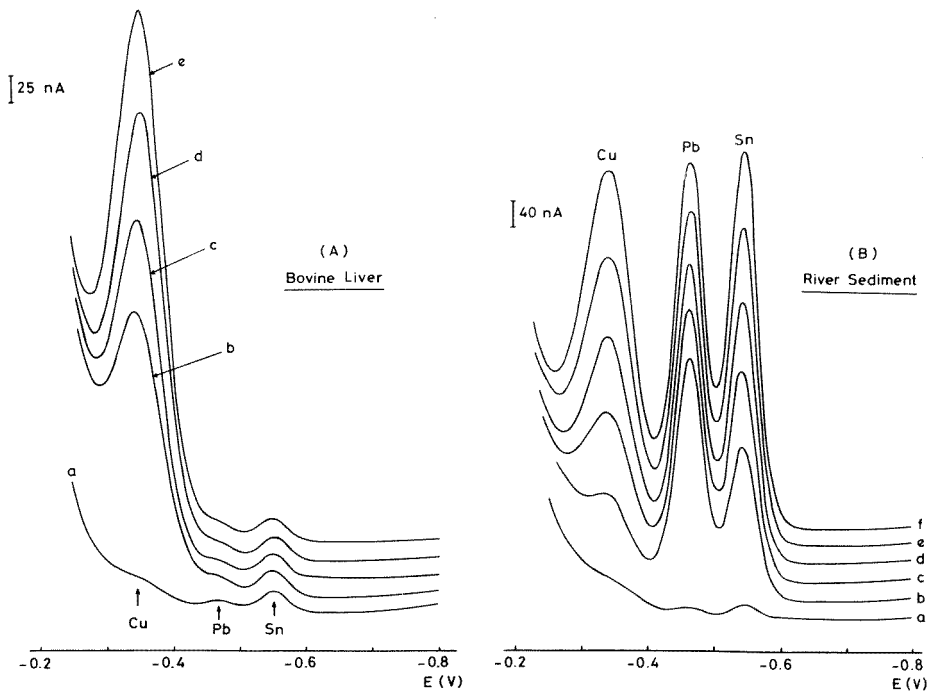

Fig. 3. Determination of Sn, Pb and Cu in NBS standard reference materials with pre-electrolysis times of 1 min. (A) Cu in SRM 1577: (a) blank; (b) bovine liver solution; added Cu: (c) 40 ng, (d) 80 ng, (e) 120 ng. (B) Cu, Pb and Sn in SRM 1645: (a) blank; (b) river sediment solution; added Cu, Pb and Sn; (c) 80 ng, (d) 160 ng, (e) 240 ng, (f) 320 ng.

TABLE 2

Determination of tin in some syrups or liquids from canned fruit

Sample	pH	Tin found (ppm)	
		Proposed method	A.a.s. [8]
Peach halves	3.90	105	99
Peach slices	3.96	28	30
Pear halves	3.63	45	43
Pineapple slices	3.98	83	86
Fruit cocktail	3.74	51	57
Mandarin	3.30	70	68
Peeled tomato	4.56	50	52

Analysis of reference materials. Copper and lead were determined in NBS standard reference materials with the results shown in Table 1. The determinations of copper in bovine liver (SRM 1577) and Cu, Pb and Sn in river sediment (SRM 1645) are shown in Fig. 3. Although the tin content of the river sediment sample is not mentioned by NBS, it was found to be of the order of 220 ppm in the present work. Attempts to verify this result by electrothermal atomic absorption spectrometry produced a much higher value, showing that total tin was not measured by the proposed voltammetric method. For the determination of total tin in sediment samples, a complete digestion with strong acids is probably necessary.

Determination of tin in canned fruit syrups. Tin was determined in the syrups from commercial canned fruits. The acidity of the fruits stored in cans causes dissolution of tin from the can. The results obtained (Table 2) are in reasonable agreement with those obtained by electrothermal atomic absorption spectrometry (a.a.s.) [8].

Analysis of sediments. Copper and lead were determined by the proposed method in sediment samples from Lake Léman (Geneva) and the Mediter-

TABLE 3

Determination of copper and lead in sediment samples

Sample	Element found (ppm)			
	Proposed method		A.a.s. [9]	
	Cu	Pb	Cu	Pb
Marine sediment	196	49	190	44
Lake sediment	70	20	68	ND
	38	34	41	38

ranean (Villefranche Bay, France). The results obtained (Table 3) are in good agreement with results obtained by using acid digestion and atomic absorption spectrometry [9].

Conclusions

Lumatom has been used previously as a solubilizing agent in order to facilitate the determination of trace metals by atomic absorption spectrometry [10]. The use of Lumatom to digest environmental samples permits a direct voltammetric determination of tin, lead and copper after suitable dilution, without the extensive manipulation often required in mineralization steps. The use of Lumatom as a digesting agent has some major advantages: contamination errors normally associated with the use of acids are eliminated and the time necessary for the digestion is considerably reduced. Lumatom also increases the sensitivity in the determinations of copper and tin by anodic stripping voltammetry. It should also be noted that the use of this technique makes it possible to apply a.s.v. measurements in the presence of organic matter (contained in the sample and in Lumatom). Unfortunately, the exact composition of Lumatom is unknown, so that these phenomena cannot be explained fully at the present time.

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Short Communication

ELECTROCHEMICAL DETERMINATION OF NITRITE AND NITRATE BY PNEUMATOAMPEROMETRY

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Summary. Nitrite in aqueous solution is reduced to nitric oxide with hydroquinone in pH 2 phosphate buffer. The nitric oxide is swept with nitrogen to an anodically polarized membrane-covered platinum electrode, where it is oxidized to nitrate. The resulting current is linearly related to nitrite concentration in the original solution from the detection limit of 18 nmol to 5 μmol of nitrite. Nitrate is subsequently determined similarly after reduction to nitric oxide with hydroquinone in 50% sulfuric acid containing ammonium molybdate catalyst. The linear range is from the detection limit of 40 nmol to 10 μmol of nitrate.

Nitrite in aqueous solution is routinely determined colorimetrically through diazotization and coupling to form an azo dye; nitrate ion is usually determined by reduction to nitrite before application of the same colorimetric procedure, although less sensitive direct colorimetric procedures for nitrate are available [1]. Cox [2] has recently determined nitrite and nitrate by selective reduction to nitric oxide and chemiluminescent detection of the purged vapors. This communication reports on electrochemical detection of purged nitric oxide vapors through oxidation at a membrane-covered anodically polarized platinum electrode. A similar detector has been used to detect mercury vapor produced from mercury(II) ion [3], iodine vapor produced from iodide [4], and sulfur dioxide and hydrogen sulfide vapors produced from sulfite and sulfide, respectively [5]. Electrochemical detection at a polarized electrode of gases generated during a preliminary chemical reaction has been termed pneumatoamperometry by Gifford and Bruckenstein [6]. The same workers have developed a theoretical treatment of pneumatoamperometry [7], and have used the method to determine cyanide [8].

Experimental

Reagents. All solutions were prepared from ACS Reagent Grade chemicals and deionized, distilled water. Standard nitrate solutions were prepared by weighing predried potassium nitrate and diluting to volume. Stock nitrite solutions were prepared in 0.1 M NaOH to prevent disproportionation and were standardized periodically by titrimetry [1]. Solutions of reducing agents

were prepared daily. Compressed nitrogen was used as the purge gas.

Apparatus. The purge cell and electrochemical detector were as described previously [5], except that the fritted glass sparge tube in the purge cell was replaced by a capillary tube to minimize sulfuric acid mist formation during nitrate reduction. A teflon-coated magnetic stir bar was also introduced to the purge cell to give efficient mixing of reagents. The gas-permeable membrane was 1-mil polyethylene and the electrolyte was 1 M sulfuric acid. The electrochemical instrument, recorder, and purge gas flow system were also as described previously [5].

Procedure for nitrite. The sample (5 ml) containing nitrite and/or nitrate is placed in the purge cell and made 0.1 M in NaOH to prevent disproportionation of nitrite. The detector electrode is polarized at +1.05 V vs. SCE, and the nitrogen flow rate is adjusted to 150 ml min^{-1}. An aliquot (1 ml) of a 1 M H_3PO_4 solution saturated with benzoquinone is added to the sample to produce a pH 2 buffer and to oxidize any sulfide or sulfite which may be present in the sample. Nitrite is then reduced by the introduction of 0.5 ml of a 0.4 M hydroquinone solution. The detector peak current resulting from nitric oxide evolution is measured and plotted against nitrite ion concentration.

Procedure for nitrate. After all the nitric oxide from nitrite has been evolved and the detector current has returned to baseline, nitrate is reduced to nitric oxide by adding 0.25 ml of a 4% ammonium molybdate solution as catalyst, followed by 5 ml of concentrated sulfuric acid. The detector peak current resulting from nitric oxide evolution is measured and plotted against nitrate ion concentration.

Results and discussion

Electrochemical detection of nitric oxide. It has been shown [9] that nitric oxide is oxidized stepwise in sulfuric acid medium. At potentials between approximately +0.7 and +0.9 V vs. SCE, it is oxidized to HNO_2, and at more positive potentials, it is oxidized to HNO_3. The polarizing potential used in this work (+1.05 V) is on the plateau of the second oxidation wave. Less positive potentials result in a smaller detector response current because of incomplete oxidation of NO, whereas more positive potentials result in a larger background current because of slow oxidation of the electrolyte. No pretreatment or activation of the electrode is required.

Reduction of nitrite and nitrate. In addition to hydroquinone [10], mercury [10], vanadium(II) [11], iron(II) [12], titanium(III) [12], and iodide [13] have been used to reduce nitrite and nitrate to nitric oxide. It was found that, of these, only hydroquinone and iodide react rapidly enough with nitrite and nitrate to be useful, and iodide is unsatisfactory because its reaction product, iodine, is both volatile and electroactive. Therefore, hydroquinone was selected as the reducing agent. It reacts rapidly with nitrite in a pH 2 buffer as is shown in Fig. 1A, and, under the same conditions, as much as 10 mmol of nitrate produces no detectable response.

The reduction of nitrate by hydroquinone under the recommended con-

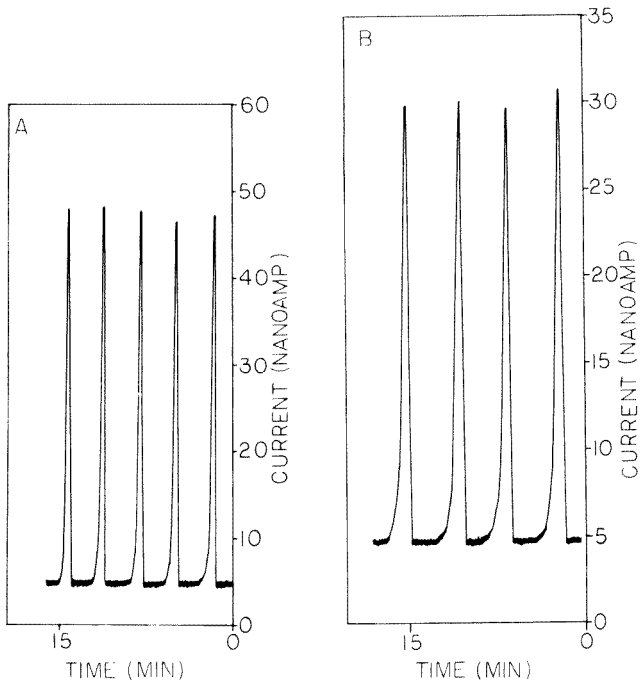

Fig. 1. Detector responses: (A) to five consecutive samples, each containing 2 μmol nitrite ion; (B) to four consecutive samples, each containing 2.5 μmol nitrate ion.

ditions is reasonably rapid, as is shown in Fig. 1B. Without ammonium molybdate present as catalyst, the peak response is smaller by about 15%. The heat generated by rapid mixing of sample and concentrated sulfuric acid is also critical to rapid reduction of nitrate; the reduction of nitrate by hydroquinone in 9 M H_2SO_4 at room temperature proceeds only slowly, whereas the same reaction carried out in 3 M H_2SO_4 at 95°C proceeds almost instantaneously.

Interferences. Oxygen present in the purge gas interferes by oxidizing nitric oxide to nitrogen dioxide; the use of compressed air as the purge gas reduces the detector response shown in Fig. 1 by approximately 30%. Therefore, nitrogen is suggested as the purge gas. Sulfide and sulfite present in the sample interfere with nitrite determination by evolving hydrogen sulfide and sulfur dioxide respectively at pH 2, both of which are oxidized at the detector [5] resulting in a false positive response. Sulfide and sulfite can be eliminated by oxidation with benzoquinone prior to addition of hydroquinone. The addition of benzoquinone to the sample has no adverse effect on nitrite and nitrate determinations. Other anions tested, chloride, bromide, carbonate, sulfate, and cyanide, do not interfere.

Linearity and detection limits. A linear detector response to nitrite is observed over the range 0.05—5.0 μmol of nitrite. The regression equation for 18 samples in this range is $I_p = (21.3 \pm 0.1)n_{NO_2} + (4.7 \pm 0.1)$ with a

standard error of 0.3 nA, where I_p is the peak current (nA) and n_{NO_2} is μmoles of nitrite. The detection limit, defined as three times the peak-to-peak baseline noise, is approximately 18 nmol of nitrite.

A linear detector response to nitrate is observed over the range 0.1—10.0 μmol of nitrate. The regression equation for 13 samples in this range is $I_p = (9.79 \pm 0.03)n_{NO_3} + (4.7 \pm 0.1)$ with a standard error of 0.3 nA, where, again, I_p is peak current (nA) and n_{NO_3} is μmoles of nitrate. The detection limit, defined as above, is approximately 40 nmol of nitrate.

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Short Communication

THE USE OF POTASSIUM HYDROXIDE IN 2-ETHYLHEXANOIC ACID AS IONIZATION SUPPRESSOR IN THE DETERMINATION OF CALCIUM IN LUBRICATING OILS BY ATOMIC ABSORPTION SPECTROMETRY

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Summary. Calcium in lubricating oil cannot always be determined merely by diluting the sample and aspirating into the flame of an atomic absorption spectrometer. The production of free calcium atoms depends on the type of calcium additive, and is influenced by the solvent. The calcium in both the sample solution and the standard solution must be available in the same form. Application of a solution of potassium hydroxide in 2-ethylhexanoic acid appears to meet this requirement.

The determination of calcium in lubricating oils by atomic absorption spectrometry (a.a.s.) is commonly applied in the petroleum industry. The method is claimed to be simple and fast, and in principle consists of diluting the oil with a suitable solvent and aspirating the solution into the flame. The actual concentration is found by comparison with organometallic standards dissolved in the same solvent. When a.a.s. was used initially for this determination, various solvents were applied with moderate success with certain types of additives [1—3]. Chemical interferences by phosphorus compounds had to be eliminated by addition of an excess of an organic lanthanum compound [1, 3]. From the writer's experience certain organic sulfur compounds also interfered with the measurement. At that time the measurements were performed with an air—acetylene flame, but later the nitrous oxide—acetylene flame became more attractive. Holding and Matthews [4], and Guttenberger and Marold [5] investigated various mixtures of solvents either to eliminate errors from measurements in an air—acetylene flame or to apply aqueous solutions of inorganic salts for standardization. Organometallic standards which are stable in organic solvents, inexpensive, and of sufficiently high purity, are currently available, thus making the application of mixed solvents less necessary.

The introduction of the nitrous oxide—acetylene flame and the use of white spirit (a petroleum fraction, b.p. 150—200°C) as diluent improved the measurement considerably. This use of white spirit has been accepted in standard methods [6, 7]. Application of a high temperature, however,

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necessitated the suppression of ionization by adding an excess of an easily ionizable element [1, 2]. Sodium or potassium as naphthenate or sulfonate [1, 7], potassium cyclohexanebutyrate [2, 6] and sodium dioctylsulfosuccinate [6] are commonly applied as ionization suppressors. This communication discusses the advantages of using a solution of potassium hydroxide in 2-ethylhexanoic acid (KEHA) as an ionization suppressor, and as a regulator for the chemical reactions in the flame. KEHA has been applied successfully for more than 10 years. Recently, it was shown to be useful in the determination of calcium in lubricating oils to which calcium was added as its acetate. This is in contrast to the German standard method [6], according to which calcium levels are found too low for this type of calcium compound.

Initial development

In the first studies of the determination of calcium in lubricating oils in this laboratory, tests with the solvents cyclohexane, methyl isobutyl ketone, and *n*-heptane aspirated into an air—acetylene flame gave some unacceptable results. Calcium contents 20—50% lower than the actual contents were recorded for products containing calcium as overbased sulfonates and overbased sulfurized alkyl phenates. (Overbased sulfonates are manufactured by heating a mixture of certain promoters or solvents with a normal sulfonate and a large excess of metal base.) Furthermore phosphorus interfered with the measurement. Replacement of these solvents by white spirit showed some improvement. Replacement of the single-slot burner head by a three-slot burner head further improved the measurement. However, the results found were occasionally 10% lower than the true values and, therefore, not acceptable.

The improvement obtained with the three-slot burner head indicated that the atomization was affected by the condensation of calcium compounds in the relatively cool outer zones of the flame. In a further evaluation, it was noticed that, after lubricating-oil samples containing the overbased calcium compounds had been mixed with 2-ethylhexanoic acid prior to dilution, the results obtained were in excellent agreement with the true values. It was concluded, therefore, that 2-ethylhexanoic acid reacts with the calcium compounds in the oil to form the hexanoate which consequently determines the degree of conversion to free calcium atoms in the flame. Treatment of the standard in a similar way meant that the resulting solution for analysis also contained the calcium as the hexanoate and therefore provided the same rate of conversion.

The introduction of the nitrous oxide—acetylene flame [1—3] helped to eliminate interferences caused by condensation of calcium compounds in cooler flames, and made superfluous the addition of an organic lanthanum salt to eliminate phosphorus interference. The high temperature, however, requires addition of a metal of low ionization potential to suppress the calcium ionization; organic potassium or sodium salts are applied for this

purpose [1, 2, 6, 7]. Preference was given to potassium because it is a stronger electron donor than sodium, and because the intensely yellow sodium flame is tiring to the eye in long series of measurements when the burner has to be inspected for carbon deposition. Further, the use of high sodium concentrations can easily contaminate the glassware and burner and, therefore, may cause problems with the determination of sodium. (Potassium is an element of minor interest in the petroleum industry compared with sodium.)

In early work in this laboratory, attempts were made to prepare a suitable organic potassium compound soluble and stable in white spirit. It was found that potassium hydroxide can be dissolved in hot (80—90°C) 2-ethylhexanoic acid; complete dissolution is achieved in 1 h with continuous stirring. This solution, called KEHA, contains 50 g K l^{-1}. In order to avoid contamination with sodium, potassium hydroxide with a sodium content of less than 0.005% (Merck) is used. For the determination of calcium in lubricating oils by a.a.s., 1 ml of KEHA is added to a weighed sample in a 50-ml volumetric flask, and the mixture is diluted to volume with white spirit. Prior to use, the white spirit is filtered through a 0.45-μm filter. Standard solutions are prepared similarly, using concentrated Conostan calcium standards (0.5%). This procedure will be referred to below, as the KEHA procedure.

TABLE 1

Determination of calcium in lubricating oils and additives containing calcium as acetate by various a.a.s. methods

Sample		Calcium (% m/m)[c]				
DIN no. (year)	Gulf Tech No.	By a.a.s. after ashing	DIN 51 391		"Erding procedure" [5]	KEHA procedure[b]
			White spirit	White spirit + ethanol (8 + 2)		
1 (1978)	8-7470	0.201	0.164	0.181	0.192	0.198
		0.202	0.172	0.180	0.194	0.193
2 (1978)	8-7471	0.301	0.250	0.268	0.299	0.297
		0.302	0.247	0.265	0.297	0.297
Calcium acetate		9.30	8.30			9.31
additive[a] (1978)		9.30	8.37			9.38
		9.37				
5 (1979)	9-7699	0.148	0.129	0.141		0.147
		0.149	0.132	0.141		0.146
		0.148				
6 (1979)	9-7700	0.381	0.338	0.366		0.379
		0.378	0.334	0.364		0.380
		0.381				

[a]Obtained from ARAL-Forschung, Bochum, Germany. [b]A Perkin-Elmer 303 or 400 atomic absorption spectrometer was used under the manufacturer's recommended conditions with a non-luminous nitrous oxide—acetylene flame. [c](Mass/mass).

Recent experience with KEHA

The application of KEHA recently proved its reliability in the analysis of lubricating oils containing calcium acetate as additive (Table 1). In contrast, the German standard method [6] gave calcium contents which were generally about 15% lower than the true values. To investigate this phenomenon, four lubricating-oil samples containing the acetate additive, and a sample of the pure additive, were analyzed by a.a.s. after various preliminary procedures: (a) ashing the sample after addition of sulfuric acid, followed by dissolution in hydrochloric acid, (b) German standard method as described in [6]; (c) German standard method after modification of the solvent, namely replacing white spirit by a mixture (80 + 20) of white spirit and absolute ethanol; (d) the "Erding procedure" as developed by Guttenberger and Marold [5]; (e) the KEHA procedure.

The results in Table 1 show excellent agreement between procedures (a), (d) and (e). However, the results obtained with the German standard method (b) are lower by 10—20%. Application of the mixed solvent (c) showed some improvement, but negative deviations of 4—10% were still found. A similar investigation was carried out with lubricating-oil samples of various FAM correlation programs [8], containing calcium additives other than calcium acetate (Table 2). Comparison of the results found by the above methods does not show any significant deviations. Since it was observed that the deviations did not change with varying dilution ratios it is unlikely that this

TABLE 2

Determination of calcium in lubricating oils containing common calcium additives by various a.a.s. methods

Sample		Calcium (% m/m)				
DIN no. (year)	Gulf Tech No.	By a.a.s. after ashing	DIN 51 391		"Erding procedure" [5]	KEHA procedure
			Mean of FAM corr. program[a]	Gulf results		
111 (1974)	4-7566		0.169	0.168		0.172
				0.175		0.172
112 (1974)	4-7567		0.066	0.061		0.061
				0.062		0.060
3 (1978)	8-7472	0.052	0.051	0.052	0.049	0.049
		0.052		0.052	0.048	0.048
4 (1978)	8-7473	0.198	0.198	0.202	0.197	0.207
		0.196		0.197	0.194	0.203
7 (1979)	9-7703		0.151	0.148		0.147
				0.148		0.147
8 (1979)	9-7678		0.204	0.205		0.202
				0.206		0.204

[a]See ref. 8.

phenomenon was caused by incomplete dissolution of the acetate in the solvent. Obviously the dissociation behavior in the flame was influenced by the type of calcium compound and solvent.

Finally the influence of measuring-height in the flame on the absorption signal was investigated with solutions prepared with 0.5% (m/m) Conostan calcium standard (Continental Oil Company, Ponca City, OK) and lubricating-oil sample no. 5, Table 1, containing calcium acetate (Ca content 0.148% (m/m)). The calcium content of both standard and oil was determined in triplicate by a.a.s. after ashing in the presence of sulfuric acid and dissolution in hydrochloric acid. The standard was a mixture (1 + 1) of two different batches, in which the calcium content was found to be 0.507%. The individual results were 0.502, 0.507 and 0.512%.

The measuring solutions were prepared as in the German standard method, applying white spirit as well as a mixture (80 + 20) of white spirit and absolute ethanol, and as in the KEHA procedure. These solutions, each containing 10.0 μg Ca ml^{-1}, were subsequently aspirated into a nitrous oxide—acetylene flame, all at the same rate (4.6 ml min^{-1}). The absorbances were measured at 5 different heights in the flame, varying from 4 mm to 16 mm. Comparison of the absorption signals recorded for the Conostan calcium standard with the signals recorded for the lubricating oil containing the calcium acetate in the respective solutions showed several features. Maximum absorbance was recorded at a height of 13 mm in the flame, independent of the type of solvent and calcium compound applied (Fig. 1). The relative difference in absorbance recorded for the Conostan calcium standard solution and the calcium acetate solution prepared as in the German standard method did not change with measuring-height. The calcium acetate solution showed absorbances which were ca. 14% lower than the corresponding Conostan standard solution (Fig. 1a). A similar, although smaller, effect was observed with the mixture of white spirit and ethanol (Fig. 1b). Excellent agreement of the absorption signals was obtained at selected measuring-heights with the solutions of both calcium compounds when prepared by the KEHA procedure (Fig. 1c).

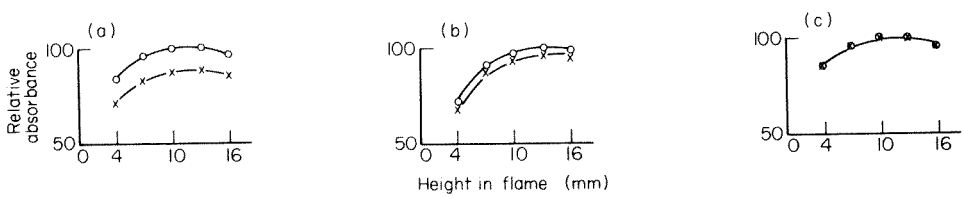

Fig. 1. Influence of measuring-height in flame, type of calcium compound and solvent on the absorption signal. Solvent: (a) white spirit containing 10 g l^{-1} of dioctyl sodium sulfosuccinate (52 mg Na l^{-1}) DIN 51 391; (b) mixture (80 + 20) of white spirit and ethanol containing 10 g l^{-1} of dioctyl sodium sulfosuccinate (52 mg Na l^{-1}) DIN 51 391, modified; (c) white spirit containing 20 ml l^{-1} of KEHA (1000 mg K l^{-1}); (○) Conostan calcium standard; (×) calcium acetate additive.

Discussion and conclusion

These experiments indicated that the actual calcium content in lubricating oils cannot always be determined merely by diluting the sample in a solvent and aspirating the solution into the flame of an atomic absorption spectrometer. The production of free calcium atoms in the flame depends on the type of calcium compound, and is influenced by the solvent used. Application of KEHA made it possible to obtain the calcium in the same form in both sample and standard solutions and so to establish the same degree of conversion.

Similar reliable results were obtained with the "Erding procedure", in which the sample is treated with dimethylsulfoxide and subsequently dissolved in a mixture of toluene and acetic acid. A disadvantage of that procedure might be the corrosiveness of the dimethylsulfoxide, while the solvents applied are more expensive than white spirit.

The German standard method clearly gives erroneous results when the lubricating oils contain the calcium acetate additive, though the errors are less significant after modification of the solvent. Other methods similar to the German standard method, e.g. the tentative method of the Institute of Petroleum [7], will very probably suffer from the same defect.

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Short Communication

CHEMICAL SPECIATION OF CHROMIUM IN SEA WATER
Part 2. Effects of Manganese Oxides and Reducible Organic Materials on the Redox Processes of Chromium

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Summary. Experiments designed to clarify the role of manganese oxides in the oxidation process of Cr(III) to Cr(VI) in sea water are described. The theoretical redox equilibrium controlled only by dissolved oxygen is shown not to be always attained for the Cr(III)/Cr(VI) system in practice. Some naturally occurring materials can reduce Cr(VI) under the conditions of natural sea water.

It is probable that chromium is present in sea water in two oxidation states, trivalent and hexavalent, considering the analytical results of past research as outlined in Part 1 of this series [1]. According to thermodynamic calculations, however, only the hexavalent species should theoretically be present in natural oxygenated waters. For instance, if the pH of sea water is taken as 8.1 and the theoretical redox condition for sea water, $pE = 12.5$ is used, the ratio of Cr(VI) to Cr(III) comes to 10^{21} [2]. Even if the redox condition, $pE = 8.5$, measured for sea water [3] is employed, the ratio is reduced only to 10^9 [4]. The reason why Cr(III) is present in sea water has been assumed to be that the redox equilibrium is only slowly attained once Cr(VI) has been reduced to Cr(III). As is clarified in Part 1, this discrepancy may partly be due to the fact that the existence of stable organic species has not been fully taken into account in past research. In the present communication, the effect of manganese oxides on the oxidation process of Cr(III) to Cr(VI) in sea water is discussed because of the geochemical evidence [5] that the chromium content is extremely low in manganese nodules compared with that in marine sediments composed of clay minerals. In addition, the effect of several naturally occurring reducible organic materials on the reduction process of Cr(VI) is described.

It is confirmed that Cr(III) is easily oxidized to Cr(VI) in the presence of manganese oxides under the conditions prevalent in natural sea water and it is shown that some organic materials can reduce Cr(VI) at the normal pH of sea water.

Experimental

Equipment and chemicals. These were essentially the same as described earlier [1]. In addition, a Shimadzu UV 200S spectrophotometer was used. Two kinds of manganese oxides were employed for oxidation experiments: γ-MnO(OH) was synthesized [6] and a natural deep-sea manganese nodule was washed with 4 M hydrochloric acid after being finely powdered in an agate mortar.

Procedures. The oxidation rate of Cr(III) to Cr(VI) was examined as follows: a 1-l sample of sea water spiked with 10^{-5} M Cr(III) at pH 8.1, either alone or with the further addition of manganese oxides or organic materials, was thermostated at 25°C and then bubbled with air or nitrogen. After the stated period, Cr(VI) was determined spectrophotometrically with diphenylcarbazide in a 5-ml aliquot of the solution. Reduction of Cr(VI) with organic materials was examined as follows: a 50-ml sample of sea water containing 10^{-8} M Cr(VI) labeled with ^{51}Cr tracer and 5×10^{-6} M organic materials was thermostated at 25°C for 72 h. A freshly prepared hydrated iron(III) oxide precipitate (equivalent to 5 mg Fe) [1] was added to the solution after the pH of the latter had been adjusted to 9 to separate Cr(III) from residual Cr(VI) [7]. After 3 h, the precipitate was filtered through a Millipore filter and dissolved in 4 M hydrochloric acid. The solution was diluted to 50 ml and an aliquot was used for γ-activity measurement together with an aliquot of the supernatant liquid.

Results and discussion

The effect of manganese oxides on the oxidation process of Cr(III) to Cr(VI). Figure 1 shows the relationship between the oxidation rate of Cr(III) and the storage time under different conditions. As can be seen, no significant Cr(VI) could be detected when the solution was bubbled with only air for more than 300 h (curve 4). When 30 mg of γ-MnO(OH) was added to the solution, 10% of Cr(III) was oxidized within 100 h (curve 1). Even when almost all the dissolved oxygen was removed by bubbling with nitrogen, a fair amount of Cr(III) was oxidized within 75 h. A similar effect was observed (curve 2) in the presence of 50 mg of powdered nodules (manganese content equivalent to 30 mg of γ-MnO(OH)). These results suggest that the catalytic action of manganese oxide is important in the oxidation process in sea water, because manganese oxides are present in naturally oxygenated sea water; the results also indicate that direct oxidation by dissolved oxygen alone does not occur in the kinetic sense. However, in the presence of 10^{-3} M citric acid, Cr(III) was not oxidized even when an excess (500 mg) of γ-MnO(OH) was present. This indicates that when Cr(III) is combined in a stable complex, as happens with citric acid, the catalytic action of manganese oxide becomes ineffective; the resulting Cr(III) complex does not coprecipitate appreciably with metal hydroxides [1].

Reduction of Cr(VI). It has been generally supposed that the reduction of Cr(VI) occurs in some biological process after Cr(VI) has been taken up

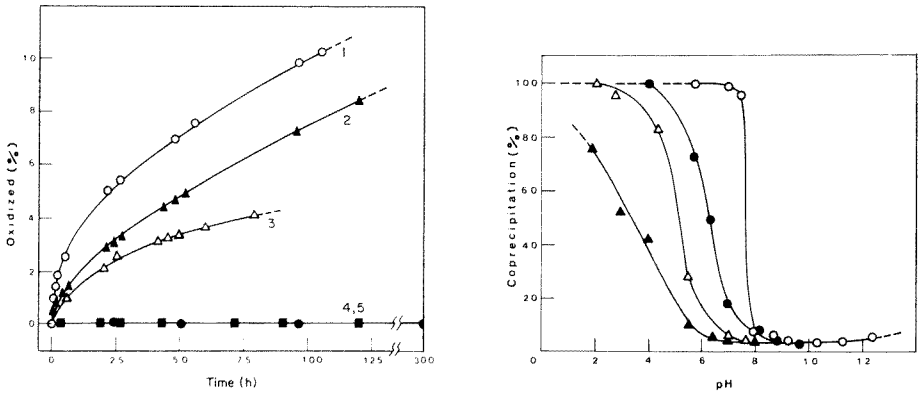

Fig. 1. Oxidation rate of Cr(III) (10^{-5} M spiked) to Cr(VI) in sea water under different conditions (pH 8.1 with 0.02 M borate buffer, 25°C): (1) air bubbling in the presence of 30 mg of γ-MnO(OH); (2) air bubbling in the presence of 50 mg of manganese nodules; (3) nitrogen bubbling in the presence of 30 mg of γ-MnO(OH); (4, •) air bubbling only; (5, ▪) air bubbling in the presence of 500 mg of γ-MnO(OH) and 10^{-3} M citric acid.

Fig. 2. Relationship between the percentage coprecipitation of chromium and the pH at which sea water spiked with 10^{-8} M Cr(VI) and 5×10^{-6} M reducible material was stored for 72 h at 25°C: (○) ascorbic acid; (●) hydroxylamine; (△) humic acid; (▲) formaldehyde.

by organisms. In the present study, only the direct effect of reducible organic materials was examined in order to estimate the extent to which they may contribute to the reduction of Cr(VI) in sea water. Shown in Fig. 2 are the relationships between the percentage coprecipitation of chromium and the pH at which the Cr(VI) solution was treated with different reducing agents. Chromium(VI) coprecipitates only slightly (<5%) from sea water at pH 9 so that coprecipitating chromium can be regarded as Cr(III) reduced from Cr(VI). Accordingly, the order of reducing ability is ascorbic acid > hydroxylamine > humic acid > formaldehyde. As is evident from Fig. 2, only ascorbic acid and hydroxylamine reduce Cr(VI) at the natural pH of sea water, i.e., 7.5—8.2.

Model for the circulation of chromium in sea water. On the basis of this study, a model for the circulation of chromium in sea water was developed (Fig. 3). This model explains the behaviour of chromium in sea water as follows. Inorganic Cr(III) can be oxidized to Cr(VI) by the catalytic action of manganese oxide either by adsorption during sedimentation or in the marine sediments. Direct oxidation of Cr(III) with only dissolved oxygen, i.e., the theoretical redox equilibrium, cannot always be attained in practice. Meanwhile Cr(VI) can be reduced in marine organisms or by some reducible materials. Organic complexes of Cr(III) are not oxidized and the trivalent state is stabilized. Thus chromium can be present in sea water as any form of inorganic Cr(III), Cr(VI) and as organic species.

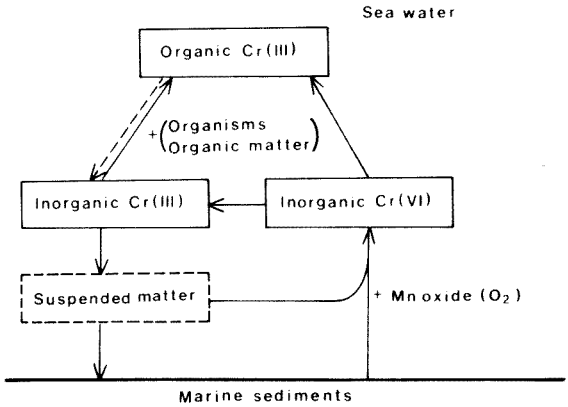

Fig. 3. A model for the circulation of chromium in sea water.

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Short Communication

EXTRACTION DU PARACETAMOL PAR RELARGAGE DE SOLVANT ET APPLICATION AU DOSAGE PAR CHROMATOGRAPHIE EN PHASE GAZEUSE DANS LE PLASMA

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(Reçu le 19 mai 1981)

Summary. (A rapid extraction technique for the determination of paracetamol in plasma by gas chromatography.) Plasma proteins are precipitated by a sulfotungstic acid reagent, and the supernatant liquid is mixed with pyridine containing the internal standard. Paracetamol is extracted by salting-out with sodium sulfate and determined by gas chromatography of its acetylated derivative on OV17. No interference was found with 26 different drugs. The method is suitable for 10^{-4} M levels of paracetamol in plasma.

Résumé. Les protéines du plasma sont d'abord précipitées avec le réactif sulfotungstique. Le surnageant est mélangé avec de la pyridine contenant l'étalon interne. Le paracétamol est extrait par relargage de la pyridine par le sulfate de sodium puis il est dosé par c.p.g. sur OV17 après acétylation. Aucune interférence n'a été constatée avec les médicaments testés.

Dans une publication récente [1] sur le dosage du paracétamol dans les milieux biologiques par polarographie sinusoïdale, nous avions rappelé l'intérêt de ce dosage dans les cas d'intoxication ainsi que les différentes méthodes qui ont été proposées pour l'effectuer. La polarographie permet une détermination rapide et sensible du taux de paracétamol, mais l'appareillage nécessaire ne se trouve pas dans tous les laboratoires. Aussi avons-nous recherché une technique employant des réactifs et un matériel courant. Les méthodes colorimétriques n'étant pas assez sensibles pour évaluer des taux thérapeutiques, nous avons utilisé la chromatographie en phase gazeuse (c.p.g.). Le paracétamol étant très polaire, il est nécessaire de former un dérivé par silylation [2—6], acétylation [7, 8] ou trifluoroacétylation [9]. Pour que la technique soit utilisable par le plus grand nombre de laboratoires, nous avons choisi d'effectuer une acétylation par le mélange anhydride acétique—pyridine et une séparation sur phase OV17.

Avant l'acétylation, il est nécessaire d'extraire le paracétamol par un solvant organique. Les solvants les plus couramment employés sont l'éther éthylique et l'acétate d'éthyle. L'éther éthylique donne des rendements d'extraction très faibles, même si on ajoute du chlorure de sodium. Avec l'acétate d'éthyle le rendement est plus élevé mais de nombreux composés susceptibles d'interférer au cours du dosage sont extraits. C'est pourquoi

nous avons mis au point une nouvelle technique d'extraction basée sur le relargage de la pyridine qui servira ensuite à la réaction d'acétylation.

Partie expérimentale

Les produits chimiques utilisés sont des produits purs pour analyse.

Les dosages par c.p.g. sont réalisés sur un appareil Packard modèle 427 équipé d'un détecteur thermoionique et d'une colonne de verre (2 m) contenant OV17 3% sur Chromosorb WAW-DMCS (80—100 mesh). Les températures sont: injecteur 260°C, détecteur 300°C, four 190°C. Les temps de rétention sont de 7,2 min pour le paracétamol et de 12 min pour l'étalon interne (*N*-butyryl-*p*-aminophénol).

Le dosage colorimétrique du paracétamol est effectué sur le dérivé nitré [10].

Précipitation des protéines. Les différents réactifs ajoutés à 1 ml de sérum pour précipiter les protéines sont: 1 ml d'hydroxyde de sodium 0,5 M et 1 ml de sulfate de zinc 0,6 M; 2 ml d'acide perchlorique 0,65 M; 2 ml d'acide trichloracétique 0,6 M; 4 ml d'une solution contenant du tungstate de sodium 0,18 M et de l'acide phosphorique 1,7 M; ou 3 ml d'acide sulfurique 0,17 M et 1,5 ml de tungstate de sodium 0,15 M.

Extraction par relargage. On ajoute à la solution aqueuse 1 ml de pyridine et on agite pour homogénéiser. On ajoute ensuite 3—4 g de sulfate de sodium anhydre; on agite 15 s et on centrifuge 5 min. La couche supérieure formée par relargage de la pyridine contient le paracétamol.

Dosage dans le plasma. A 1 ml de plasma, on ajoute 3 ml d'acide sulfurique 0,17 M et 1,5 ml de tungstate de sodium 0,15 M. On agite et on centrifuge 15 min. On verse le surnageant dans un tube où on introduit ensuite 1 ml de pyridine contenant l'étalon interne (*N*-butyryl-*p*-aminophénol) à la concentration 3×10^{-4} M. L'extraction par relargage est effectuée comme indiqué ci-dessus. La couche supérieure est reprise dans un tube où on ajoute 1 ml d'anhydride acétique. On ferme hermétiquement le tube et on le porte à 70°C pendant 30 min. On évapore ensuite son contenu sous azote à 50°C. On reprend le résidu dans 0,2 ml d'acétate d'éthyle et on injecte 4 μl.

Résultats et discussion

Pour réaliser l'extraction, nous avons d'abord introduit la pyridine directement dans le sérum pur ou dans le sérum dilué dans de l'eau. Après relargage du solvant et centrifugation, les protéines qui sont insolubilisées se trouvent dans la couche organique, ce qui rend impossible la reprise de celle-ci. C'est pourquoi nous avons ajouté une étape de défécation. Pour choisir le réactif permettant cette défécation, il faut vérifier qu'il ne précipite pas le paracétamol et qu'il n'interfère pas dans son extraction.

Des essais ont été effectués en ajoutant à 1 ml de plasma, 0,1 ml d'une solution aqueuse de paracétamol $6,6 \times 10^{-3}$ M et différents réactifs précipitant les protéines. Le paracétamol est dosé dans le surnageant par colorimétrie. Les résultats sont donnés dans le Tableau 1. Les rendements les plus

TABLEAU 1

Pourcentage de paracétamol en solution aqueuse après défécation du plasma (a), et en solution dans la pyridine après extraction (b)

	NaOH—Zn	$HClO_4$	CCl_3COOH	Na_2WO_4—H_3PO_4	Na_2WO_4—H_2SO_4
Défécation (a)	48	90	92	59	80
Extraction (b)	32	—	65	55	75

faibles sont obtenus avec le réactif soude—zinc et avec le réactif phosphotungstique.

Les rendements d'extraction ont été également déterminés en extrayant le surnageant, après précipitation des protéines, par relargage de 1 ml de pyridine. La pyridine est évaporée et le paracétamol contenu dans le résidu est dosé par colorimétrie. L'acide perchlorique est extrait par la pyridine et forme un sel non volatil en quantité importante, rendant impossible le dosage du paracétamol par colorimétrie et par c.p.g. après acétylation. L'acide trichloracétique forme également un sel de pyridine auquel correspond en c.p.g. un pic qui interfère avec le pic de paracétamol. Le réactif sulfotungstique donnant le meilleur rendement est choisi comme réactif de défécation.

La Fig. 1 représente les chromatogrammes obtenus en c.p.g. La courbe d'étalonnage, qui a été tracée en portant en ordonnée le rapport entre les hauteurs des pics du paracétamol et de l'étalon interne, est linéaire de 0,2 $\times 10^{-4}$ M à 7 $\times 10^{-4}$ M pour le paracétamol dans le plasma.

Afin de rechercher d'éventuelles interférences en c.p.g., plusieurs médicaments ont été ajoutés dans du plasma à des concentrations correspondant à des doses toxiques. Ces essais ont été effectués avec acide salicylique,

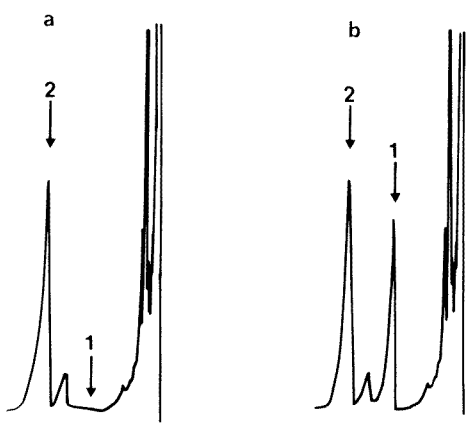

Fig. 1. Exemples de chromatogramme: (a) blanc plasma; (b) plasma contenant 0,13 mM l^{-1} de paracétamol. (1) Paracétamol; (2) étalon interne.

acide acétylsalicylique, salicylamide, méprobamate, phénobarbital, pental, butalbital, méthaqualone, imipramine, désipramine, trimépramine, clomipramine, amitriptyline, oxazépam, diazépam, desmethyldiazépam, prazépam, clonazépam, médazépam, chlordiazepoxide, clobazam, bromazépam, lorazépam, flunitrazépam et nitrazépam. Seule la salicylamide donne un pic sur le chromatogramme mais son temps de rétention étant de 3 min (paracétamol 7,2 min), il n'y a aucune interférence.

Conclusion

Cette méthode de dosage du paracétamol est particulièrement simple à mettre en œuvre. L'extraction par relargage de solvant évite l'utilisation d'extractions répétées avec des solvants difficiles à manipuler comme l'éther. La précipitation préalable des protéines empêche la formation d'émulsions. Aucun des médicaments testés n'a donné d'interférences, ce qui montre la sélectivité du procédé d'extraction.

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Short Communication

SPECTROPHOTOMETRIC FLOW INJECTION DETERMINATION OF CHLORIDE IN ETHANOL

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Summary. A flow injection procedure is described for the spectrophotometric determination of chloride in ethanol, based on the mercury(II) thiocyanate—iron(III) reaction. Effects of reagent composition and ethanol content of the sample are investigated in detail. The proposed system can analyse 120 samples of ethanol (94—100% v/v) per hour, with a relative standard deviation lower than 1%, when the chloride content ranges from 0.1—6.0 ppm. Recoveries of ca. 96% are found.

The determination of chloride in ethanol is of importance because chloride is often involved in corrosion processes and in catalytic systems [1]. With the increasing demand for analysis of ethanol, a sensitive, accurate and fast method for chloride determination is needed to replace the common manual colorimetric [2] and turbidimetric [1] procedures. In the last five years, flow injection analysis [3] has proved to be an excellent tool in laboratory routines. In this communication, a flow injection method is proposed for the spectrophotometric determination of chloride in ethanol, based on the mercury(II) thiocyanate—iron(III) reaction [4].

Experimental

Apparatus. The peristaltic pump was an MP13 Ismatec, furnished with Solvaflex pump tubes, which were replaced weekly. A Varian model 634S spectrophotometer, equipped with a Hellma 178OS flow cell (light path 10 mm, inner volume 80 μl), was connected to a Radiometer REC61 recorder with a REA112 high-sensitivity unit. The proportional injector and all components of the manifold have already been described [5].

Reagents, standards and samples. All reagents, including the ethanol, were of analytical grade and distilled—deionized water was used throughout. The 96% (v/v) ethanol was redistilled before use.

The working standards were prepared daily, in 96% (v/v) ethanol, to cover the range 0.00—6.00 ppm Cl^- (as NaCl). The mercury(II) thiocyanate reagent was prepared by dissolving 0.06 g of $Hg(SCN)_2$ in 100 ml of 96% (v/v) ethanol. The iron(III) reagent was prepared by dissolving 1.0 g of $Fe(NO_3)_3 \cdot 9H_2O$ in about 50 ml of 96% (v/v) ethanol, adding 7 ml of concentrated nitric acid and diluting to 100 ml with 96% (v/v) ethanol. Both reagents were stable for at least one week.

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The samples, commercially available ethanol, were produced at 75 sugar-cane distilleries of S. Paulo State, Brasil. They were collected in 1-l polyethylene bottles provided with good stoppers. Prior to the chloride determinations, the alcohol content of the samples was determined by densitometry.

Procedure. The flow diagram of the proposed system is presented in Fig. 1. The operation of the system is similar to that already described [5] and the chemical reactions involved have already been discussed [4, 6, 7]. A single reagent solution could not be employed here because, in ethanolic medium, severe incompatibility between the two reagents was verified in preliminary experiments.

The pumping rate of the sample carrier stream was chosen to permit a sampling rate of about 120 determinations per hour and the other flow rates were defined to provide a minimum sample dilution at the confluence point x (Fig. 1), associated with suitable mixing conditions. The alcohol content, around 95% (v/v) in all streams, was similar to that of the samples in order to avoid the refractive index effect [8] and the formation of air bubbles. The length of the mixing coil R_1 is not relevant in the system design, as this coil lies outside the analytical path; a 200-cm long coil was chosen to provide good mixing between the two reagents. The length of the reaction coil R_2 was defined after experiments with a 6.00 ppm chloride standard in an infinite volume configuration [9] and the volume of sample injected was chosen in order to provide a low degree of sample dispersion. Several reaction coils (25—200 cm) and sample loops (25—100 cm) were tested.

The precision of the proposed method was evaluated in terms of relative standard deviation. The stability of the system was checked by analysing a typical sample (1.08 ppm Cl^-) repeatedly for 2 h, and standard additions were tested by adding 50 and 100 μg Cl^- (5.00 ml) to 50-ml samples. Comparisons with other procedures for the determination of chloride in ethanol were not made because the available colorimetric or turbidimetric procedures are not sensitive enough [1, 2].

Results and discussion

In the proposed method, the reagent is coloured so that, when mixing inside the reaction coil R_2 (Fig. 1) is insufficient, baseline noise can be a limiting factor in sensitivity. Increasing the length of this coil improves mixing and attenuates the baseline noise but it also enhances the degree of sample dispersion, thus diminishing the sampling frequency and the analytical sensitivity. As a compromise between sampling rate, sample dispersion and signal:noise ratio, a 50-cm long reaction coil and a 500-μl injected sample volume were chosen. In this situation, the baseline noise is small (Fig. 2) and the peak height related to an injected sample corresponding to about 80% of the signal obtained in the steady-state condition when the sample is placed in the infinite volume configuration (dispersion factor ≈ 0.8).

The iron(III) thiocyanate absorption spectra in water and ethanolic media were compared. These spectra were measured for 6 ppm of chloride in

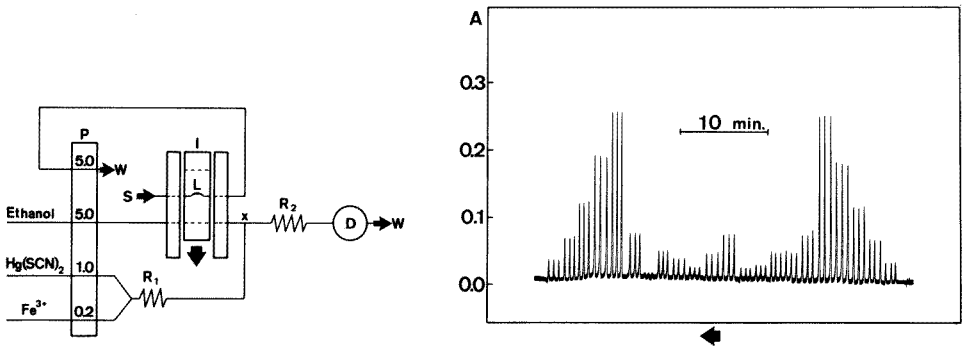

Fig. 1. Flow diagram of the proposed system. P represents the peristaltic pump with indication of flow rates in ml min^{-1}; ethanol (95% v/v) is the sample carrier stream; the reagent streams are as specified in the text; S indicates the sample under aspiration, filling the sample loop L (500 μl) and going to waste W. R_1 is the mixing coil (200 cm), R_2 is the reaction coil (50 cm), and D is the spectrophotometer set at 500 nm. For details, see text.

Fig. 2. Recording of a routine determination of chloride in 94.5—95.5% (v/v) ethanol samples. From left to right, a series of standards (0.25, 0.50, 1.00, 1.50 and 2.00 ppm Cl^-), the samples and then the standards again. All measurements in triplicate.

ethanol or 20 ppm of chloride in water, with the system in the infinite volume configuration [9]; for aqueous media, the single reagent solution [10] was used. The results showed that ethanol displaces the absorption maximum from 460 to 500 nm in a typical bathochromic shift [11]. Also, the measured absorbance is higher in an ethanol than in a water medium (about three times) as a consequence of displacement of chemical equilibria and change in the molar absorptivity of the complex.

Variations in the concentration of iron(III) nitrate have very little effect on the measured peak height in the concentration range 1—3% (w/v) in 1 M nitric acid, for chloride standards in the range 0—6 ppm. When the iron(III) nitrate concentration lies below 0.5% (w/v), loss of linear range in the calibration graph is observed. This reagent has, however, a marked influence on the baseline absorbance. Thus, when the iron(III) nitrate concentration changed from 0.1% to 3.0% (w/v), an increase in the baseline absorbance of 0.32 was measured and, in consequence, the signal:noise ratio deteriorated. A 1% (w/v) iron(III) nitrate concentration was therefore chosen.

Nitric acid is required in the iron(III) nitrate solution as a preservative, because the ethanol enhances the instability of the solution. Thus, when the nitric acid concentration was below 0.1 M for 1% (w/v) iron(III) nitrate solution, a brown precipitate was formed in a few hours; with 0.2 M nitric acid, the reagent was too strongly coloured to be employed in the flow injection system. When the concentration of nitric acid changed from 0.3 M to 1.0 M, the colour of the reagent varied from yellow—brown to yellow, decreasing the absorbance of the baseline and consequently the baseline noise. Increase in this acid concentration from 0.5 M to 1.0 M caused a very

TABLE 1

Standard addition in ethanol samples

Sample	μg Cl^-			Recovery (%)	Sample	μg Cl^-			Recovery (%)
	Originally measured	Added	Found			Originally measured	Added	Found	
1	18	50	65.5	96	20	41	50	88.5	97
1	18	100	114.0	97	20	41	100	136.0	96
2	35	50	83.6	98	21	22.5	50	72.5	100
2	35	100	128.7	95	21	22.5	100	119.5	98
7	13.5	50	62.2	98	29	10.5	50	58.5	97
7	13.5	100	104.5	92	29	10.5	100	104.5	100
13	61	50	103.0	93	30	27	50	72.6	94
13	61	100	148.0	92	30	27	100	121.6	96

slight variation in peak height. A 1 M nitric acid concentration was, therefore, chosen.

When the sample carrier stream was 96% (v/v) ethanol, as recommended above, increase in the ethanol content of the samples in the range 80—96% (v/v) leads to a slight increase in the measured peak height (about 3% per ethanolic degree). The ethanol content of the samples must, therefore, be known prior to the chloride determinations, to avoid inaccurate results. Although 96% (v/v) ethanol covers the main production in Brasil for motor fuel, there is a significant production of 92% and 99% (v/v) ethanol. In both cases, preparation of standards and the sample carrier stream with an alcohol content similar to that of the samples is recommended.

The proposed system is very stable. After two hours of continuous analysis of a typical sample, slight changes (less than 5%) in peak height were observed. A typical run for routine chloride determinations in commercial ethanol (94.5—95.5% v/v) is shown in Fig. 2. The relative standard deviations were usually lower than 1%, indicating the good precision of the measurements. The recoveries presented in Table 1 indicate the accuracy of the proposed method.

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Short Communication

ELECTROGENERATED CHEMILUMINESCENCE OF LUCIGENIN IN AQUEOUS ALKALINE SOLUTIONS AT A PLATINUM ELECTRODE

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Summary. Lucigenin is shown to emit light during electrolysis in aqueous alkaline solutions at a platinum electrode. In the mechanism proposed, lucigenin is initially reduced at −0.30 V vs. Ag/AgCl and subsequently the reaction product reacts either with oxygen or a reduction product of oxygen. Previous evidence on this phenomenon is contradictory.

The chemiluminescence of lucigenin (*N,N'*-dimethyl-9-9'-biacridinium dication) has been known since 1935 [1]. Perhaps the best known chemiluminescent reaction of lucigenin in aqueous alkaline solution is that with hydrogen peroxide. Several investigations concerning its mechanism have been reported [2, 3], and it has been applied in trace metal determinations [4—6]. The electrogenerated chemiluminescence of lucigenin has been studied in non-aqueous [7, 8] and aqueous [8, 9] solutions. Tamamushi and Akiyama [9] observed light emission during electrolysis in aqueous alkaline solutions at a platinum cathode. Legg and Hercules [8] investigated this electrolysis under the same conditions and observed no light emission; by substituting a mercury pool cathode for a platinum cathode and by using an unbuffered solution at pH 7, however, they observed light emission. In their opinion, light emission at the platinum electrode is not possible, because oxygen is directly reduced to hydroxide ions under these conditions, whereas in neutral solution at a mercury cathode, oxygen is reduced to hydrogen peroxide.

The electrogenerated chemiluminescence of lucigenin is of interest because of its possible applications in trace metal determinations. In this communication, the electrogenerated chemiluminescence of lucigenin in aqueous alkaline solution at a platinum electrode is confirmed, in agreement with the earlier work of Tamamushi and Akiyama [9].

Experimental

Reagents. Special attention was paid to the purity of the reagents. Lucigenin (Pfaltz & Bauer) was recrystallized twice from a 1:1 mixture of methanol and ethanol. Sodium chloride (Merck, Suprapur) and sodium hydroxide (Merck, p.a.) were used without further purification. All water used was distilled once from a conventional stainless steel still, and then from a quartz, double-distillation unit.

Apparatus. The chemiluminescence apparatus is described in detail elsewhere [10]. The surface area of the platinum working electrode (Pine Instruments, ring-disk electrode with the ring disconnected) was 0.461 cm^2. The reference electrode was silver/silver chloride; the auxiliary electrode was a short platinum wire mounted so that any light emitted at this electrode could not reach the photomultiplier. The electronic circuits were conventional and constructed by using MP-System 1000 (Danville, CA). The output signal from the photomultiplier (Hamamatsu R 375) was passed through a current—voltage converter to an x-y recorder (Model 29350, Bryans Southern Instruments, England). The voltammetric circuit was conventional in all respects.

Procedure. Before each measurement, the platinum working electrode was washed with ethanol, rinsed with water, electrolyzed for 1 min both anodically and cathodically at 2 V in 0.5 M perchloric acid and finally rinsed carefully with water. A 15-ml portion of lucigenin solution was pipetted into the cell and saturated with oxygen for 15 min. The cell voltage was turned on and the output from the photomultiplier measured.

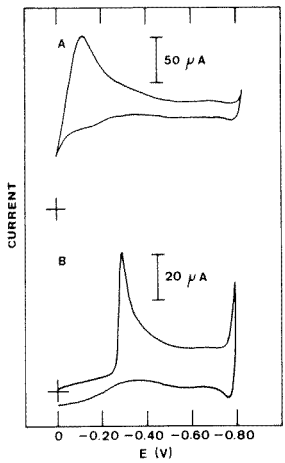
Results and discussion

The cyclic voltammogram of oxygen under the stated conditions is presented in Fig. 1A. The peak potential of the oxygen reduction wave is —0.12 V vs. Ag/AgCl. The literature survey shows that the role of hydrogen peroxide in oxygen reduction at the platinum electrode in aqueous alkaline solutions is ambiguous. It has been postulated that oxygen is reduced directly to hydroxide ions without hydrogen peroxide as an intermediate [11], or that hydrogen peroxide is an intermediate in a reaction path parallel to the path involving direct reduction of oxygen to hydroxide ions [12]. Further, depending on the reduction potential, oxygen is said to be reduced to hydrogen peroxide or to hydroxide ions via hydrogen peroxide [13]. Under some conditions, hydrogen peroxide is the only product of oxygen reduction [14]. Thus, hydrogen peroxide must be taken into consideration in investigations concerning the mechanism of the electrogenerated chemiluminescence of lucigenin at a platinum electrode.

Figure 1B presents the cyclic voltammogram of lucigenin under the stated conditions. The peak potential of the lucigenin reduction wave is —0.30 V vs. Ag/AgCl. The reduction product of lucigenin was insoluble and plated out on the platinum electrode.

In Fig. 2, the height of line A shows the intensity of the chemiluminescence formed in aqueous alkaline solutions of lucigenin in the presence of oxygen. This emission disappeared when the solution was deaerated with nitrogen. Maskiewicz et al. [2] have discussed the mechanism of this chemiluminescent reaction of lucigenin.

Curves B—E in Fig. 2 show the chemiluminescence intensities observed during the electrolysis of lucigenin at the platinum electrode as a function of potential, under the stated conditions. These four curves are consecutive measurements with no intervening conditioning of the platinum electrode.



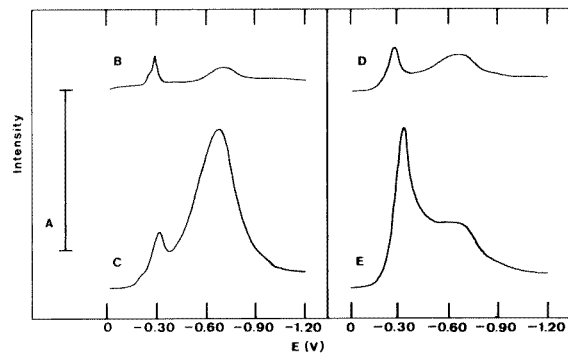


Fig. 1. Cyclic voltammograms for the reductions of (A) oxygen and (B) lucigenin (5×10^{-4} M) in aqueous 0.01 M NaOH solution at a platinum electrode. (A) Solution saturated with oxygen; (B) solution deaerated with nitrogen. Sweep rate 15 mV s^{-1}; NaCl added to give an ionic strength of 0.1.

Fig. 2. Intensity vs. potential curves for the electrogenerated chemiluminescence of lucigenin (2×10^{-4} M) in aqueous 0.01 M NaOH solution at a platinum electrode: (A) without electrolysis; (B)—(E) first, second, third and fourth measurements without electrode purification. Solution saturated with oxygen; other conditions as in Fig. 1.

These curves have two intensity maxima with peak potentials at −0.30 V and −0.67 V vs. Ag/AgCl. The intensities at these potentials vary considerably in consecutive measurements; the intensity at −0.30 V increases with successive measurements while the intensity at −0.67 V is greatest during the second measurement.

Based on the results obtained by cyclic voltammetry and the intensity vs. potential measurements, the following general reaction scheme is proposed for the electrogenerated chemiluminescence of lucigenin (Luc) at the peak potential of −0.30 V:

$$\text{Luc} + ne^- \rightarrow \text{Luc (red)}$$

$$O_2 + ne^- \rightarrow \text{O (red)}$$

$$\left.\begin{array}{lll} \text{Luc (red)} + O_2 & \rightarrow & \text{product 1} \\ \text{Luc (red)} + \text{O (red)} & \rightarrow & \text{product 2} \end{array}\right\rightarrow \text{intermediate} \rightarrow \text{emitter}$$

The formation of a reduction product of lucigenin, Luc (red), which is most probably a radical, is necessary for the lucigenin to produce electrogenerated chemiluminescence. Luc (red) reacts either with oxygen or with a reduction product of oxygen, O (red), to produce a light-emitting species. The possible reduction product of oxygen which may be involved is either the one-electron reduction product, the superoxide radical, or the two-electron reduction

product, hydrogen peroxide. On the available evidence, a more detailed reaction scheme cannot be formulated. However, it seems evident that the reaction proceeds in principle as described previously [2]: i.e., products 1 and 2, most probably peroxides, decompose via an intermediate to the emitter, *N*-methylacridone. The light emission at −0.67 V may be caused by the precipitation of the lucigenin reduction product onto the platinum electrode and subsequent surface processes on this electrode.

Metal ions in general, have either a catalytic or an inhibitive effect on the electrogenerated chemiluminescence of lucigenin in aqueous alkaline solutions. Thus, there is no doubt that lucigenin can be applied to trace metal determinations in a similar way to luminol [10, 15]. There appears to be no sensible reason why Legg and Hercules [8] observed no light emission under essentially similar conditions.

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Short Communication

DETERMINATION OF DIAMINE OXIDASE BY A KINETIC METHOD WITH 2,2′-AZINO-DI(3-ETHYLBENZTHIAZOLINE-6-SULFONATE)

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(Received 8th May 1981)

Summary. Hydrogen peroxide, the product of diamine oxidase-catalyzed putrescine or cadaverine oxidation, formed in proportion to the enzyme activity, is measured spectrophotometrically by using the above sulfonate (ABTS) and peroxidase. Only one reagent solution containing 3 mmol of putrescine or 10 mmol of cadaverine, 4 mmol of ABTS and 3000 U of peroxidase per litre of 0.2 mol l^{-1} Tris—0.1 mol l^{-1} HCl buffer pH 7.5 is needed. Absorbance changes are measured at 410 nm over the first 3 min of the reaction. This initial oxidation rate of the chromogen enables diamine oxidase activity up to 230 U l^{-1} to be determined.

Diamine oxidase [DAO, histaminase, amine:oxygen oxidoreductase (deaminating), EC 1.4.3.6] catalyses diamine oxidation to the aminoaldehyde, ammonia and hydrogen peroxide

$$H_2N(CH_2)_nNH_2 + O_2 + H_2O \rightleftharpoons H_2N(CH_2)_{n-1}CHO + H_2O_2 + NH_3$$

Hence the diamine oxidase activity may be determined by oxygen consumption [1], disappearance of substrate [2—4], aldehyde production [5] or indirectly by measuring the peroxide formed [6]. The subject has been reviewed by Zeller [7] and Bardsley et al. [8, 9] where the commonest methods are compared.

The kinetic method presented here for DAO assay is based on 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate) (ABTS) as chromogen in the presence of peroxidase and 1,5-diaminopentane (cadaverine) or 1,4-diaminobutane (putrescine) as substrate. The rate of oxidation of ABTS by hydrogen peroxide, measured at 410 nm, is proportional to DAO activity.

Experimental

Apparatus. All kinetic measurements were carried out with a Unicam SP 8000A spectrophotometer, equipped with a Haake FS thermostat and a Weyfringe ADCP-2 printer with digital display.

Reagents. Cadaverine and putrescine dihydrochlorides and diamine oxidase (from porcine kidney, activity 0.06 U mg^{-1}) were obtained from Sigma Chemical Company. Diammonium ABTS and horseradish peroxidase (activity 250 U mg^{-1}) were from Boehringer, Mannheim.

Tris—HCl buffers of different pH values were prepared from 0.2 mol l^{-1} Tris and 0.1 mol l^{-1} HCl. The reagent solution for DAO assay was composed of 4 mmol of ABTS, 3000 U of peroxidase, 10 mmol of cadaverine dihydrochloride or 3 mmol of putrescine dihydrochloride in 1 l of Tris—HCl buffer pH 7.50. The diamine oxidase was dissolved in 1 ml of Tris—HCl buffer, pH 7.50.

Procedure. To 2.5 ml of reagent solution at 30°C, add 0.1 ml of enzyme solution, mix well and measure the increase in absorbance at 410 nm in a 1-cm cell, at 1, 2 and 3 min after the start of the reaction against air as the reference. Calculate the absorbance change per min $(\Delta A_{410}\ \text{min})^{-1}$. Calculate the diamine oxidase activity, expressed as U l^{-1}, from $\Delta A_{410}\ \text{min}^{-1} \times 1019.6$, on the basis of the molar absorptivity of $ABTS_{ox}$ of 25.5×10^3 l mol^{-1} cm^{-1} [10, 11].

Results

To determine the optimal conditions for the diamine oxidase (DAO) assay approximate optimal substrate concentrations were used. A series of solutions (0—50 mmol l^{-1} for cadaverine, 0—25 mmol l^{-1} for putrescine) was prepared in Tris—HCl buffer, pH 7.20. Measurements were made at 410 nm with 2.5 ml of reagent solution, containing 2 mmol of ABTS, 2500 U of peroxidase and various amounts of cadaverine or putrescine in 1 l of the Tris—HCl buffer, pH 7.20, and 0.1 ml of DAO solution (230 U l^{-1}) at 30°C. Under these conditions the maximum reaction rate was obtained with 20—30 mmol l^{-1} cadaverine and 5—10 mmol l^{-1} putrescine; 25 mmol l^{-1} cadaverine and 10 mmol l^{-1} putrescine were used in further studies.

Because both substrates were used as their dihydrochlorides, in concentrations at or above 10 mmol l^{-1} they decreased the pH of phosphate buffers from 7.20 to 6.60. Similar changes in pH were observed when triethanolamine and borate buffers were used. A Tris—HCl buffer was more resistant to pH changes and was selected for further study. The effect of pH on the reaction rate was monitored by dissolving the substrate in 0.2 mol l^{-1} Tris and adjusting the pH with 0.1 mol l^{-1} hydrochloric acid to values from 7.0 to 8.50. Figure 1 shows that maximum DAO activity was obtained at pH 7.4 for cadaverine and pH 7.6 for putrescine. For all further experiments pH 7.5 was used for both substrates.

In the previous experiments 2500 U l^{-1} peroxidase solutions were used, as in previous methods for aerobic dehydrogenases [10, 11] or their substrates [12, 13]. In order to determine the optimal activity of peroxidase for DAO assay, peroxidase activity was varied in the range 0—10000 U l^{-1} (in the reagent solution) for both substrates. The results are shown in Fig. 2. The Lineweaver—Burk plot [14] gave values of K_m for peroxidase of 142 U l^{-1} with cadaverine and 63 U l^{-1} for putrescine. However, maximum reaction rate with both substrates was achieved with 3000 U l^{-1}, and that concentration of peroxidase was used in all further experiments.

The effect of varying the ABTS concentration in the reagent solution

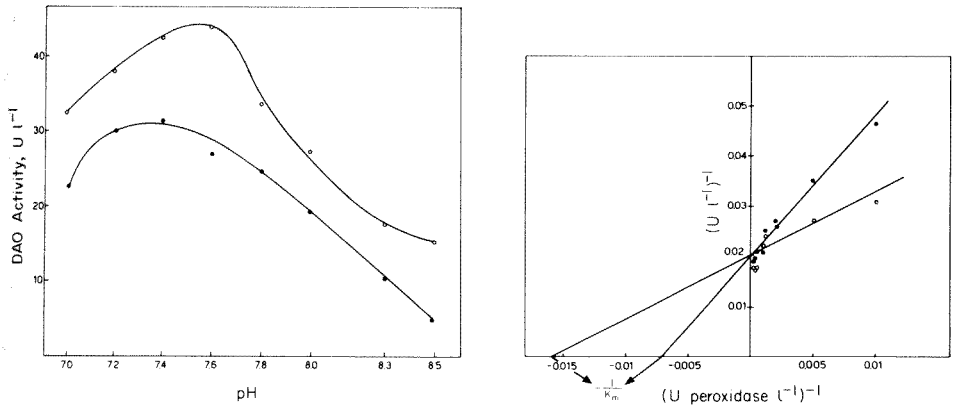


Fig. 1. Optimal pH for DAO assay with (•) cadaverine and (○) putrescine as substrates. Conditions: 2.5 ml of reagent solution (2 mmol ABTS, 2500 U peroxidase and 25 mmol cadaverine or 10 mmol putrescine in 1 l of Tris—HCl buffer) and 0.1 ml of enzyme solution (230 U DAO l^{-1} in Tris—HCl buffer) recorded every min at 410 nm.

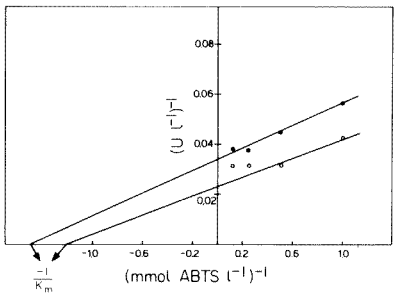
Fig. 2. Effect of peroxidase concentration using (•) cadaverine ($Y_x = 0.0197 + 2.8056X$, $r = 0.990$) and (○) putrescine ($Y_x = 0.0200 + 1.2573X$, $r = 0.893$) as substrates. Conditions otherwise as in Fig. 1, with buffer pH 7.5.

from 0 to 8 mmol l^{-1} is shown in Fig. 3. The Michaelis—Menten constant for ABTS with putrescine as substrate was 0.78 mmol l^{-1} ($10K_m \approx 8$ mmol l^{-1}) and for cadaverine was 0.67 mmol l^{-1} ($10K_m \approx 7$ mmol l^{-1}). Since the maximum velocity for both substrates was obtained with just 4 mmol ABTS l^{-1}, this concentration was used in further tests.

When the optimal values for pH, ABTS and peroxidase concentrations had been determined, it was necessary to estimate the accurate optimal concentrations of cadaverine and putrescine by varying their concentrations over the ranges 0—40 mmol l^{-1} and 0—15 mmol l^{-1}, respectively. The resulting Lineweaver—Burk plot gave $K_m = 0.22$ mol l^{-1} for putrescine and 0.85 mmol l^{-1} for cadaverine (Fig. 4). Thus, for estimation of DAO activity, 10 mmol l^{-1} cadaverine or 3 mmol l^{-1} putrescine in the reaction mixture should be used, as these concentrations are close to $10K_m$, and thus give maximum reaction rate.

The temperature dependence of DAO activity was determined under the established optimal conditions, for 230 U enzyme l^{-1}, between 20 and 70°C. For putrescine, maximum activity was obtained at 60°C, while for cadaverine the optimal temperature was 45°C. However, all further assays of DAO activity using either substrate were done at 30°C, as this was most convenient.

Since the DAO activity is determined by measuring the initial rate, it was necessary to establish the greatest DAO activity that gives zero-order kinetics. For that purpose, DAO activities ranging from 28.8 to 345 U l^{-1} were used, and the enzyme activities were determined with 0.1 ml of these solutions under the recommended conditions. Regression analysis showed that ab-



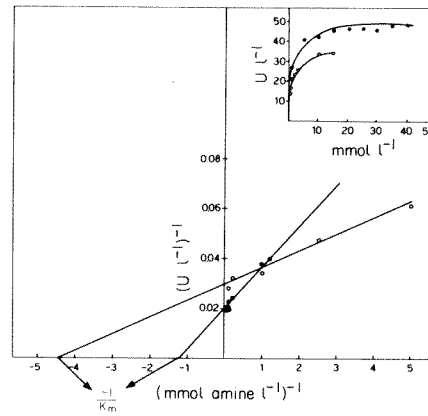


Fig. 3. Lineweaver—Burk plot for ABTS concentrations used for DAO assay with (•) cadaverine (Y_x = 0.0345 + 0.0292X, r = 0.993, K_m = 0.67 mmol l^{-1}); and (○) putrescine (Y_x = 0.0234 + 0.0182X, r = 1.000, K_m = 0.78 mmol l^{-1}).

Fig. 4. Lineweaver—Burk plot for (○) putrescine (Y_x = 0.030011 + 0.00672X, r = 0.998, K_m = 0.22 mmol l^{-1}) and (•) cadaverine (Y_x = 0.0204 + 0.172X, r = 0.978, K_m = 0.85 mmol l^{-1}). The Michaelis plot is inset.

sorbance changes registered every minute at 410 nm, are constant for up to 230 U DAO l^{-1} for both substrates. The regression lines were Y_x = 0.00235 + 0.00008X, r = 0.988, $S_{y,x}$ = 0.00113 with cadaverine and Y_x = 0.00189 + 0.00012X, r = 0.996, $S_{y,x}$ = 0.00132 with putrescine.

Discussion

As mentioned above, there are many different methods for diamine oxidase assay. The titrimetric, monometric, fluorimetric and biological procedures [7, 8] are time-consuming and too insensitive for precise measurements of the low enzyme activities occurring in biological materials. With regard to its sensitivity and selectivity, the radioactive method [5] appears best, but it is time-consuming, expensive and not readily applicable in routine work. Spectrophotometric determination of the colour formed with *o*-dianisidine [6] is lengthy. The proposed kinetic assay of DAO activity is relatively fast, taking advantage of the high aqueous solubility and molar absorptivity of ABTS.

Assay of DAO under the recommended conditions gave K_m = 0.22 mmol l^{-1} for putrescine (pH 7.60) and 0.85 mmol l^{-1} (pH 7.40) for cadaverine. Estimating the activity of the same enzyme at pH 7.5, Hill and Bardsley [15] obtained values of 1.28 mmol l^{-1} and 0.71 mmol l^{-1} for putrescine and cadaverine, respectively.

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ANALYTICA CHIMICA ACTA, Vol. 130/2 (1981)

AUTHOR INDEX

Anderson, C. W., see Cushman, M. R. 323

Bakker, F. P., see Slanina, J. 1
Bennett, B. G., see Cushman, M. R. 323
Bergamin F°, H., see Jacintho, A. O. 243
Berkeš, I., see Majkić-Singh, N. 419
Berzas Nevado, J. J., see Román Ceba, M. 183
Boer, H. S. de, see de Boer, H. S. 1
Bratin, K.
—, Kissinger, P. T. and Briner, R. C. Determination of nitro aromatic, nitramine, and nitrate ester explosive compounds in explosive mixtures and gunshot residue by liquid chromatography and reductive electrochemical detection 295
Briner, R. C., see Bratin, K. 295
Brown, C., see Janshekar, H. 81
Bruhn F., C.
—, Navarrete A., G. Matrix modification for the direct determination of cadmium in urine by electrothermal atomic absorption spectrometry 209
Bruns, R. E., see Jacintho, A. O. 243
Bye, R., see Holen, B. 257

Carlson, R., see Cox, J. A. 313
Carnahan, J. W.
—, Mulligan, K. J. and Caruso, J. A. Element-selective detection for chromatography by plasma emission spectrometry 227
Caruso, J. A., see Carnahan, J. W. 227
Cedergren, A., see Korečková, J. 267
Cejas, M. A.
—, Gomez-Hens, A. and Valcarcel, M. Fluorimetric determination of magnesium by ternary complex formation with pyridoxal nicotinylhydrazone and amines 73
Covington, A. K.
—, Robinson, R. A.† and Sarbar, M. Determination of carbonate in the presence of hydroxide. Part 2. Evidence for the existence of a novel species from first-derivative potentiometric titration curves 93
Covington, A. K.
—, Goldberg, R. N. and Sarbar, M. Computer simulation of titration curves with application to aqueous carbonate solutions 103
Cox, J. A.
— and Carlson, R. Quantitative enrichment of trace levels of ions by electrodialysis 313
Cushman, M. R.
—, Bennett, B. G. and Anderson, C. W. Electrochemistry at carbon fibers. Part 1. Characteristics of the mercury film carbon fiber electrode in differential pulse anodic stripping voltammetry 323

Davies, I. M., see Shepard, R. J. 55
Dawson, A., see Gormally, J. 369
de Boer, H. S.
—, van Oort, W. J. and Zuman, P. Polarographic analysis of corticosteroids. Part 6. Mechanism of polarographic electroreduction of some Δ^4-3-ketosteroids and $\Delta^{1,4}$-3-ketosteroids 111
Deguchi, T., see Sanemasa, I. 149
Dits, J. S.
— The use of potassium hydroxide in 2-ethylhexanoic acid as ionization suppressor in the determination of calcium in lubricating oils by atomic absorption spectrometry 395
Dočekal, B., see Slovák, Z. 203
Dogan, S.
—, Nembrini, G. and Haerdi, W. A novel approach for determination of tin, lead and copper in biological samples and sediments by alternating current anodic stripping voltammetry 385
Dyck, P. van, see Vos, L. 167

Fiechter, A., see Janshekar, H. 81
Fischer, C., see Uhlemann, E. 177
Frech, W., see Korečková, J. 267
Fujinaga, T., see Nakayama, E. 289

Fujinaga, T., see Nakayama, E. 401
Fukasawa, T.
—, Miyata, S. and Matsunaga, S.
Rapid and sensitive determination of total vanadium in airborne particulates by an extraction—spectrometric method with *N*-benzoyl-*N*-phenylhydroxylamine 353

Genestar, C., see Salinas, F. 337
Głodowski, S.
—, and Kublik, Z.
Polarographic investigation of the stability of tin(II) solutions in the presence of some stabilizing agents 133
Goldberg, R. N., see Covington, A. K. 103
Gomez-Hens, A., see Cejas, M. A. 73
Gormally, J.
—, Panak, J., Wyn-Jones, E., Dawson, A., Wedlock, D. and Phillips, G. O.
A new method of determining the extent of binding of an ionic dye to a polyelectrolyte in solution 369
Gorton, L.
— and Ögren, L.
Flow injection analysis for glucose and urea with enzyme reactors and on-line dialysis 45
Grases, F., see Salinas, F. 337
Gricken, R. van, see Vos, L. 167
Gu, H., see Riley, J. P. 199
Guilbault, G. G., see Ho, M. H. 141

Haapakka, K. E.
— and Kankare, J. J.
Electrogenerated chemiluminescence of lucigenin in aqueous alkaline solutions at a platinum electrode 415
Haerdi, W., see Dogan, S. 385
Ho, M. H.
—, Guilbault, G. G. and Scheide, E. P.
Determination of nanogram quantities of mercury in water with a gold-plated piezoelectric crystal detector 141
Holen, B.
—, Bye, R. and Lund, W.
Atomic absorption spectrometry of selenium in an argon—hydrogen flame after electrochemical preconcentration 257
Holthuis, J. J. M.
—, Pinedo, H. M. and van Oort, W. J.
A sensitive high-performance liquid chromatographic method for determination of the anti-neoplastic agents VP 16-213 and VM 26 in biological fluids 23

Imai, K.
— and Watanabe, Y.
Fluorimetric determination of secondary amino acids by 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole 377
Irr, L. G.
— and Safford, H. W.
The coulometric generation of manganese(II) from a manganese electrode: the titration of EDTA 329

Jacintho, A. O.
—, Zagatto, E. A. G., Bergamin F°, H., Krug, F. J., Reis, B. F., Bruns, R. E. and Kowalski, B. R.
Flow injection systems with inductively-coupled argon plasma atomic emission spectrometry. Part 1. Fundamental considerations 243
Jacintho, A. O.
—, Zagatto, E. A. G., Reis, B. F., Pessenda, L. C. R. and Krug, F. J.
Merging zones in flow injection analysis. Part 6. Determination of calcium in natural waters, soil and plant materials with glyoxal bis(2-hydroxyanil) 361
Jacintho, A. O., see Krug, F. J. 409
Janshekar, H.
—, Brown, C. and Fiechter, A.
Determination of biodegraded lignin by ultraviolet spectrophotometry 81
Jongejan, P. A. C., see Slanina, J. 1

Kaji, H., see Yoshimura, K. 345
Kankare, J. J., see Haapakka, K. E. 415
Kissinger, P. T., see Bratin, K. 295
Konjović, M., see Majkić-Singh, N. 419
Korečková, J.
—, Frech, W., Lundberg, E., Persson, J.-Å. and Cedergren, A.
Investigations of reactions involved in electrothermal atomic absorption procedures. Part 10. Factors influencing the determination of arsenic 267
Kowalski, B. R., see Jacintho, A. O. 243
Krug, F. J., see Jacintho, A. O. 243
Krug, F. J., see Jacintho, A. O. 361
Krug, F. J.,
—, Pessenda, L. C. R., Zagatto, E. A. G., Jacintho, A. O. and Reis, B. F.
Spectophotometric flow injection deter-

mination of chloride in ethanol 409
Kublik, Z., see Głodowski, S. 133
Kuwamoto, T., see Nakayama, E. 289
Kuwamoto, T., see Nakayama, E. 401

Lamoen, J. van, see Slanina, J. 1
Lochmüller, C. H.
—, Marshall, D. B. and Wilder, D. R.
An examination of chemically-modified silica surfaces using fluorescence spectroscopy 31
Lund, W., see Holen, B. 257
Lundberg, E., see Korečková, J. 267

Majkić-Singh, N.
—, Konjović, M., Stojanov, M., Spasić, S. and Berkeš, I.
Determination of diamine oxidase by a kinetic method with 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonate) 419
Marshall, D. B., see Lochmüller, C. H. 31
Matsunaga, S., see Fukasawa, T. 353
Mickler, W., see Uhlemann, E. 177
Miyata, S., see Fukasawa, T. 353
Moesta, B., see Weisz, H. 193
Möls, J. J., see Slanina, J. 1
Moselhy, M. M.
— and Vijan, P. N.
Simultaneous determination of trace metals in sewage and sewage effluents by inductively coupled argon plasma atomic emission spectrometry 157
Mulligan, K. J., see Carnahan, J. W. 227
Muñoz Leyva, J. A., see Román Ceba, M. 183

Nagai, H., see Sanemasa, I. 149
Nakayama, E.
—, Kuwamoto, T., Tsurubo, S., Tokoro, H. and Fujinaga, T.
Chemical speciation of chromium in sea water. Part 1. Effect of naturally occurring organic materials on the complex formation of chromium(III) 289
Nakayama, E.
—, Kuwamoto, T., Tsurubo, S. and Fujinaga, T.
Chemical speciation of chromium in sea water. Part 2. Effects of manganese oxides and reducible organic materials on the redox processes of chromium 401
Navarrete A., G., see Bruhn F., C. 209
Nembrini, G., see Dogan, S. 385
Nygaard, D. D.
— Electrochemical determination of nitrite and nitrate by pneumatoamperometry 391

Ögren, L., see Gorton, L. 45
Oort, W. J. van, see de Boer, H. S. 111
Oort, W. J. van, see Holthuis, J. J. M. 23

Panak, J., see Gormally, J. 369
Parczewski, A.
— The use of empirical mathematical models in the examination of homogeneity of solids 221
Pegon, Y.
— et Vallon, J. J.
Extraction du paracétamol par relargage de solvant et application au dosage par chromatographie en phase gazeuse dans le plasma 405
Persson, J.-Å., see Korečková, J. 267
Pessenda, L. C. R., see Jacintho, A. O. 361
Pessenda, L. C. R., see Krug, F. J. 409
Phillips, G. O., see Gormally, J. 369
Pinedo, H. M., see Holthuis, J. J. M. 23
Poppe, H., see van Rooijen, H. W. 9

Reis, B. F., see Jacintho, A. O. 243
Reis, B. F., see Jacintho, A. O. 361
Reis, B. F., see Krug, F. J. 409
Riley, J. P.
— and Gu, H.
A physically-coated mercury film electrode for anodic stripping voltammetry 199
Robberecht, H., see Vos, L. 167
Robinson, R. A†, see Covington, A. K. 93
Román Ceba, M.
—, Muñoz Leyva, J. A. and Berzas Nevado, J. J.
New spectrophotometric method for determining the stoichiometry and equilibrium constants of some redox reactions 183
Rooijen, H. W. van, see van Rooijen, H. W. 9

Saeed, K.
— and Thomassen, Y.
Spectral interferences from phosphate matrices in the determination of arsenic, antimony, selenium and tellurium by electrothermal atomic absorption spectrometry 281

Safford, H. W., see Irr, L. G. 329
Salinas, F.
—, Genestar, C. and Grases, F.
Kinetic fluorimetric determination of iron and thallium based on oxidation transformation of 1,4-diamino-2,3-dihydroanthraquinone 337
Sanemasa, I.
—, Takagi, E., Deguchi, T. and Nagai, H.
Preconcentration of inorganic mercury with an anion-exchange resin and direct reduction—aeration measurements by cold-vapour atomic absorption spectrometry 149
Sarbar, M., see Covington, A. K. 93
Sarbar, M., see Covington, A. K. 103
Scheide, E. P., see Ho, M. H. 141
Shepherd, R. J.
— and Davies, I. M.
A semi-automatic alkaline peroxodisulphate method for the routine determination of total dissolved nitrogen in sea water 55
Slanina, J.
—, Bakker, F. P., Jongejan, P. A. C., van Lamoen, L. and Möls, J. J.
Fast determination of anions by computerized ion chromatography coupled with selective detectors 1
Slovák, Z.
— and Dočekal, B.
Some aspects of electrothermal atomization of elements from large amounts of involatile matrices 203
Smith, M. E.
— Determination of carbon in stainless steel and plutonium using infrared detection of carbon dioxide 215
Spasić S., see Majkić-Singh, N. 419
Stojanov, M., see Majkić-Singh, N. 419

Takagi, E., see Sanemasa, I. 149
Tarutani, T., see Yoshimura, K. 345
Thomassen, Y., see Saeed, K. 281
Tokoro, H., see Nakayama, E. 289
Tsurubo, S., see Nakayama, E. 289
Tsurubo, S., see Nakayama, E. 401

Uhlemann, E.
—, Mickler, W. und Fischer, C.
Zur Flüssig—Flüssig-Extraktion von Gallium mit zweizähnigen Chelatbildnern 177

Valcarcel, M., see Cejas, M. A. 73
Vallon, J. J., see Pegon, Y. 405
van Dyck, P., see Vos, L. 167
van Grieken, R., see Vos, L. 167
van Lamoen, L., see Slanina, J. 1
van Oort, W. J., see de Boer, H. S. 111
van Oort, W. J., see Holthuis, J. J. M. 23
van Rooijen, H. W.
— and Poppe, H.
An electrochemical reactivation method for solid electrodes used in electrochemical detectors for high-performance liquid chromatography and flow injection analysis 9
Vijan, P. N., see Moselhy, M. M. 157
Vos, L.
—, Robberecht, H., van Dyck, P. and van Grieken, R.
Multi-element analysis of urine by energy dispersive x-ray fluorescence spectrometry 167

Watanabe, Y., see Imai, K. 377
Wedlock, D., see Gormally, J. 369
Weisz, H.
— and Moesta, B.
Amplification reactions by multiplication with an ion exchanger in an inversion tube 193
Wilder, D. R., see Lochmüller, C. H. 31
Wyn-Jones, E., see Gormally, J. 369

Yamaguchi, E., see Yoshimura, K. 345
Yamane, T.
— Flow injection determination of traces of cobalt by catalysis of the SPADNS—hydrogen peroxide reaction with spectrophotometric detection 65
Yoshimura, K.
—, Kaji, H., Yamaguchi, E. and Tarutani, T.
Spectrophotometric determination of vanadium in natural waters and rocks after selective adsorption on Sephadex gel 345

Zagatto, E. A. G., see Jacintho, A. O. 243
Zagatto, E. A. G., see Jacintho, A. O. 361
Zagatto, E. A. G., see Krug, F. J. 409
Zuman, P., see de Boer, H. S. 111

AUTHOR INDEX

VOLUMES 121–130

The volume number is placed in parentheses before the page number

Abe, H. (122) 87, (122) 241
Adams, F. (122) 57, (122) 373
Al-Abachi, M.Q. (125) 215
Alder, J.F. (123) 329, (129) 163, (129) 175
Alexander, P.W. (121) 61, (125) 55
Alkayer, M. (124) 113
Allen, T.W. (126) 225
Ancian, B. (124) 415
Anderson, C.W. (130) 323
Anderson, J.L. (124) 321
Anderson, W.C. (129) 49
Andrews, R.W. (124) 107, (124) 333
Aoyama, M. (129) 237
Arbige, V.A. Jr. (124) 409
Archer, A.W. (128) 235
Ariel, M. (128) 147
Arnold, M. (124) 341
Arnold, M.A. (128) 121
Arrebola Ramírez, A. (124) 201
Assenza, S.P. (123) 33
Athanasiou-Malaki, E. (128) 239
Aue, W.A. (125) 175
Azogu, C.I. (125) 171

Bagliano, G. (123) 45
Bailey, K. (123) 75
Baker, A.A. (125) 93
Bakker, F.P. (130) 1
Baltensperger, U. (123) 107
Bardsley, A.H. (128) 251
Barker, B.J. (122) 223
Barlič, B. (122) 103
Barofsky, D.F. (124) 357
Barrett, P. (122) 67
Basson, A.J. (126) 217
Batley, G.E. (124) 121
Bayliss, R. (129) 29
Bem, H. (124) 373
Benischek, F. (123) 45
Bennett, B.G. (130) 323
Berg, E.W. (121) 239, (123) 1
Bergamin, H.F°. (123) 221, (125) 29, (125) 37, (130) 243
Berge, D.G. (123) 19
Berkeš, I. (130) 419
Bernier, W.E. (124) 365
Bertrand, C. (126) 23
Berzas Nevado, J.J. (124) 201, (130) 183
Bhat, S.R. (128) 263
Bichon, C. (124) 113
Bierowska, A. (122) 279
Bilewicz, R. (123) 201
Bocken, M.C.Y.M. (127) 47
Bond, A.M. (121) 1, (127) 121, (127) 135
Bos, M. (122) 81, (122) 193, (122) 387
Bower, J.N. (124) 155
Bradfield, D.Z. (129) 243
Bradshaw, J.D. (124) 155
Bratin, K. (130) 295
Briggs, M.H. (127) 135
Briner, R.C. (130) 295
Britz, D. (122) 331
Broekaert, J.A.C. (124) 421
Broering, W.B. (128) 213
Brown, A. (127) 223
Brown, C. (130) 81
Brown, M.E.B. (126) 125
Brown, P.L. (124) 431
Brown, P.R. (123) 33
Brown, S.D. (123) 187
Bruhn, C.F. (130) 209
Bruins, C.H.P. (125) 85
Bruns, R.E. (130) 243
Brunt, K. (125) 85
Bruntlett, C.S. (130) 295
Bryson, W.G. (123) 339, (124) 391
Buck, R.P. (121) 101, (122) 171
Budini, R. (124) 215
Buldini, P.L. (124) 99, (124) 233, (126) 247
Burggraf, L.W. (129) 19
Burguera, J.L. (127) 199
Burguera, M. (127) 199, (127) 227
Burns, D.T. (128) 257
Burns, R.R. (124) 281
Bye, R. (130) 257
Bykovskaya, L.A. (125) 1

Cardwell, T.J. (121) 175
Carlson, R. (130) 313
Carnahan, J.W. (130) 227
Carpenter, R.C. (125) 209
Carr, P.W. (121) 135, (124) 229, (129) 269
Caruso, J.A. (130) 227
Casebolt, R.G. (129) 247
Catterall, A. (128) 245
Cattrall, R.W. (123) 213
Cauchetier, P. (124) 443, (124) 449
Cedergren, A. (128) 1, (130) 267
Cejas, M.A. (130) 73
Chambers, J.Q. (129) 49

Chan, P.-K. (124) 365
Chao, H.-E. (125) 139
Chao, T.T. (128) 225
Chao-Sheng, H. (124) 177
Chen, N.H. (128) 163
Cheney, M.C. (126) 213
Chesney, D.J. (124) 321
Christensen, J.K. (121) 71
Christian, G.D. (123) 125
Chung-Gin, H. (124) 177
Clardy, P. (124) 155
Coetzee, C.J. (126) 217
Collinge, A. (121) 337
Coomans, D. (122) 347
Copeland, T.R. (122) 67
Corn, R.M. (123) 187
Coulet, P.R. (126) 23
Covington, A.K. (127) 1, (130) 93, (130) 103
Cox, A.G. (128) 67
Cox, J.A. (130) 313
Crisp, P.T. (123) 355
Crowell, M.A. (129) 29
Curran, D.J. (126) 213
Cushman, M.R. (130) 323
Cutié, S.S. (123) 25
Czech, N. (121) 275
Czichon, P. (126) 221

Dajková, M. (127) 165
Danielsson, L.-G. (127) 147
Daud, H. (123) 213
Davidowski, L.J. (122) 67
Davidson, I.E. (121) 51
Davies, I.M. (130) 55
Davis, T.L. (128) 213
Dawson, A. (130) 369
De Boer, H.S. (130) 111
De Goeij, J.J.M. (127) 181
De Jager, E.M. (122) 1, (122) 151
De Roy, G. (122) 373
Deguchi, T. (130) 149
Delayette-Mills, M. (124) 365
Den Hartigh, J. (127) 47
Desimoni, E. (124) 143
Di Paolantonio, C.L. (128) 121
Diamandis, E.P. (123) 143, (123) 341, (127) 173, (128) 239
Dilli, S. (128) 101, (128) 109
Dits, J.S. (130) 395
Dittrich, K. (121) 205
Djingova, R. (123) 303
Dočekal, B. (129) 263, (130) 203
Dogan, S. (130) 385
Donche, H. (127) 251
Doornbos, D.A. (125) 85
Downey, D.M. (121) 239, (123) 1
Downey, S.W. (121) 165
Doxtader, M.M. (124) 409
Dresler, G. (127) 87
Dubois, J.-E. (124) 415
Dullnig, S. (127) 219
Duyckaerts, G. (129) 113

Ebdon, L. (128) 45, (128) 67
Eckert, J.M. (123) 355, (124) 351, (128) 263
Edmonds, T.E. (129) 69
Efstathiou, C.E. (127) 173
Eggli, R. (123) 107
Egsgaard, H. (124) 1
Eisenreich, S.J. (124) 31
Ellis, J. (124) 431
Elton-Bott, R.R. (127) 213
Elvecrog, J.M. (121) 135
Epifanoff, P. (121) 321
Epstein, M.S. (124) 155
Erbe, D.A. (124) 409
Evans, J.F. (124) 229, (129) 269
Evans, O.M. (129) 79
Evstiferov, M.V. (128) 85
Ex, G. (128) 207

Fell, G.S. (124) 281, (128) 57, (129) 205
Ferri, D. (124) 99, (124) 233, (126) 247
Fiechter, A. (130) 81
Fields, B. (124) 39, (124) 65
Filipović, I. (122) 395
Fischer, C. (130) 177
Fletcher, K.S. III (126) 213
Fligier, J. (126) 221
Forcé, R.K. (124) 409
Formánek, Z. (127) 109
Forsman, U. (128) 135
Fortin, R.C. (127) 103
Fowler, W.K. (124) 225
Frazer, J.W. (122) 403
Frazzini, T.L. (129) 125
Frech, W. (128) 1, (130) 267
Freiser, H. (125) 179
Friese, B. (121) 275
Fritz, G. (123) 239
Fritz, J.S. (121) 225
Fujinaga, T. (121) 119, (130) 289, (130) 401
Fujita, M. (124) 267
Fujiwara, I. (122) 87
Fujiwara, S. (128) 185
Fukasawa, T. (130) 353
Fulcher, C. (129) 29
Fuwa, K. (123) 57

Gampp, H. (122) 303
Garbett, K. (126) 135, (126) 147
Garcia-Sanchez, F. (121) 295, (125) 21
Gardiner, P.E. (124) 281, (128) 57
Gattavecchia, E. (124) 215
Gautheron, D.C. (126) 23
Geissler, M. (124) 237
Genestar, C. (130) 337
Georges, J. (121) 29, (127) 233
Gibbons, J.J. (129) 247
Gibson, N.A. (123) 355, (128) 263
Giraudi, G. (128) 169
Girotti, S. (124) 215
Głodowski, S. (130) 133
Going, J.E. (123) 19
Gold, H.S. (122) 171
Goldberg, R.N. (130) 103
Gomez-Hens, A. (130) 73
Goodall, C.M. (124) 391
Goodfellow, G.I. (126) 135, (126) 147
Gormally, J. (130) 369

Gorton, L. (130) 45
Goto, M. (126) 95
Gough, D.S. (124) 259
Grabarić, B.S. (122) 395
Graner, C.A.F. (123) 347
Grases, F. (125) 21, (130) 337
Gregorowicz, Z. (126) 221
Gribov, L.A. (122) 249
Grier, R.A. (124) 333
Grillo, C. (128) 169
Grime, J.K. (121) 125
Gritzapis, P.C. (126) 51
Groves, J.A. (123) 175
Gu, H. (130) 199
Guilbault, G.G. (124) 169, (130) 141
Gulberg, E.L. (123) 125
Guogang, P. (129) 69
Gustavsson, A. (125) 65

Haapakka, K.E. (130) 415
Haartz, J.C. (121) 187
Hadjiioannou, T.P. (123) 143, (123) 341, (126) 43, (126) 51, (127) 173, (128) 239
Hadži, D. (122) 103
Haerdi, W. (130) 385
Hairyan, E.K. (124) 185
Halls, D.J. (128) 57, (129) 205
Hamada, C. (126) 237
Hamada, S. (125) 109
Hamilton, I.C. (123) 213
Hamon, M. (121) 321
Han, H.-B. (128) 9
Hanck, K.W. (129) 79
Hanekamp, H.B. (121) 13
Hansen, E.H. (129) 1
Hara, H. (121) 119
Harada, T. (129) 195
Haraguchi, H. (123) 57
Harner, R.S. (127) 23
Hart, J.P. (128) 245
Hassan, S.S.M. (126) 35
Haugen, G.R. (123) 255
Hawn, G.G. (127) 223
Headridge, J.B. (125) 93
Heineman, W.R. (126) 57
Heller, S.R. (122) 117
Helmer, J.C. (122) 27
Hepel, T. (123) 151, (123) 161
Herget, C.J. (122) 403
Heritage, I.D. (127) 135
Hernandis, V. (129) 213
Hieftje, G.M. (121) 165, (123) 255, (123) 319, (124) 245, (126) 167
Hippe, Z. (122) 279
Hirai, Y. (121) 281
Hirao, Y. (125) 203
Hirata, Y. (127) 55
Hlaváč, R. (127) 109
Ho, M.H. (130) 141
Hobo, T. (129) 237
Holen, B. (130) 257
Holland, K.B. (129) 29
Holland, M.K. (129) 125
Hollod, G.J. (124) 31
Holthuis, J.J.M. (130) 23
Holub, K. (121) 23
Hörmann, P.K. (124) 421
Horváth, Z. (121) 219
Hoste, J. (121) 147
Houpt, P.M. (124) 15
Hsieh, J.Y.-K. (123) 41
Huber, I. (123) 45
Huber, J.F.K. (122) 139
Hudson, H.A. (127) 121
Hull, R.D. (121) 187
Hume, D.N. (123) 335
Hurley, M.F. (129) 49
Hurtubise, R.J. (126) 225
Hutton, A.T. (128) 261

Ighodalo, E.C. (123) 351
Ihn, G.-S. (121) 101
Iino, A. (124) 427
Ikeda, M. (125) 109
Imai, K. (130) 377
Imasaka, T. (124) 381, (125) 161, (129) 195
Imura, H. (126) 199, (129) 221
Ingle, J.D. Jr. (123) 247, (124) 23
Irr, L.G. (130) 329
Irving, H.M.N.H. (128) 261
Isaac, C.A. (129) 163, (129) 175
Isabelle, L.M. (124) 357
Ishibashi, N. (124) 381, (125) 161, (129) 195
Ishida, Y. (122) 95
Ishii, D. (126) 95
Ishizuka, T. (121) 197
Ito, A. (122) 75
Ito, T. (122) 75, (125) 155
Itoh, H. (128) 269
Ivaska, A. (121) 51
Iwasaki, M. (126) 237

Jacintho, A.O. (123) 221, (125) 37, (130) 243, (130) 361, (130) 409
Jackson, K.W. (128) 45, (128) 67
Jagner, D. (127) 147, (128) 155, (129) 153
James, D.L. (126) 125
Janauer, G.E. (124) 365
Janshekar, H. (130) 81
Jarbawi, T.B. (126) 57
Jezorek, J.R. (129) 29
Jiao-Mai, P. (124) 177
Jin, Q. (123) 329
Johansson, G. (128) 141
Johnson, D.C. (124) 303, (124) 315, (129) 87, (129) 101
Johnson, S.M. (129) 57
Jones, R.D. (121) 1
Jongejan, P.A.C. (130) 1
Jordan, C. (129) 133, (129) 145
Josefson, M. (127) 147, (128) 155, (129) 153

Kaiser, G. (128) 9
Kaji, H. (130) 345
Kałowska, H. (123) 279
Kanda, Y. (121) 157
Kankare, J.J. (130) 415
Kano, K. (127) 195
Karadakov, B. (125) 149
Karasawa, I. (126) 241

Karlberg, B. (125) 199
Karlsson, A. (128) 135
Karm, L. (124) 365
Karube, I. (127) 245
Kashima, J. (124) 267, (124) 275
Kato, M. (126) 95
Kaufman, L. (122) 347
Kawaguchi, H. (122) 75
Keliher, P.N. (128) 251
Kellö, V. (122) 47
Kendall, D.S. (126) 207, (129) 19
Khalil, S.E.A. (126) 175
Kiekens, P. (127) 251
Kilpiö, J.O. (125) 131
Kimura, A. (127) 93
Kimura, K. (125) 203
King, S.W. (128) 221
Kirchnerová, J. (123) 83
Kissinger, P.T. (130) 295
Kitazume, E. (125) 101, (128) 229
Knowles, D.J. (121) 175
Kobos, R.K. (121) 111
Kohashi, K. (121) 289
Kolihová, D. (127) 109
Konjović, M. (130) 419
Korečková, J. (130) 267
Kostadinov, K. (123) 303
Kouimtzis, T.A. (123) 315
Kowalski, B.R. (122) 215, (130) 243
Koziol, M.J. (128) 195
Krishnamurthy, M.V. (124) 211
Krug, F.J. (123) 221, (125) 29, (125) 37, (130) 243, (130) 361, (130) 409
Kryger, L. (121) 71, (122) 179
Kuan, S.S. (124) 169
Kubešová-Svobodová, H. (124) 91
Kublik, Z. (123) 201, (130) 133
Kuhnhardt, C. (124) 237
Kunitake, M. (125) 161
Kurokawa, Y. (121) 281
Kuwamoto, T. (130) 289, (130) 401
Kvitek, R.J. (124) 229, (129) 269
Kwan, W.-O. (122) 215

Landy, M.P. (121) 39
Langeweg, F. (124) 15
Langmyhr, F.J. (128) 23
Larsen, E. (124) 1
Laserna, J.J. (121) 295
Lásztity, A. (121) 219
Lee, G.L. (123) 213
Lee, M.L. (127) 55
Legault, D. (123) 75
Leggett, D.J. (128) 163
Levi, S. (127) 103
Levitt, L.S. (125) 219
Leyden, D.E. (126) 117, (126) 207, (129) 19
Lo, J.M. (126) 191
Lochmüller, C.H. (130) 31
Lodmell, J.C. (129) 49
Louvrier, J. (129) 213
Lovell, M.W. (127) 203
Lub, T.T. (122) 267
Luciano, V.J. (125) 183
Luecke, G.R. (124) 315
Lund, W. (130) 257
Lundbäck, H. (128) 141
Lundberg, E. (128) 1, (130) 267
Lyle, S.J. (121) 341

McAlister, J.J. (128) 257
Macášek, F. (121) 249
McCormick, M.J. (121) 233
McDevitt, R.J. (122) 223
McElvany, S.W. (123) 351
Mach, V. (127) 165
McKown, D. (123) 65
Madsen, B.C. (124) 437
Maeder, M. (122) 303
Maessen, F.J.M.J. (127) 181
Mahadevappa, D.S. (128) 163
Maher, W.A. (126) 157
Majer, J.R. (126) 175
Majkić-Singh, N. (130) 419
Malinowski, E.R. (122) 327
Manahan, S.E. (123) 65
Maney, J.P. (125) 183
Marczenko, Z. (123) 271, (123) 279
Marino, D.F. (123) 247, (124) 23
Marriott, P.J. (121) 175
Marshall, D.B. (130) 31
Marshall, G.B. (126) 135, (126) 147
Martinez, P.R. (125) 13
Masaki, T. (124) 403
Massart, D.L. (122) 347
Massee, R. (127) 181
Matsumoto, K. (123) 297
Matsunaga, S. (130) 353
Matsunaga, T. (127) 245
Matsuno, S. (124) 403
Matsusaki, K. (124) 163
Mattera, V.D. Jr. (124) 409
Meister, P. (121) 205
Meites, L. (126) 105
Meranger, J.C. (124) 131
Mercer-Smith, J.A. (122) 171
Merciny, E. (129) 113
Meschi, P.L. (124) 303, (124) 315
Messer, B. (126) 229
Mickler, W. (130) 177
Miller, H.C. (124) 225
Miller, J.N. (124) 221
Milne, G.W.A. (122) 117
Milne, P.J. (123) 263
Mirzoyan, F.V. (124) 185
Miyaishi, K. (124) 381, (125) 161
Miyamoto, S. (121) 289
Miyashita, Y. (122) 241
Miyata, S. (130) 353
Miyazaki, A. (127) 93
Miyoshi, F. (127) 195
Mizuike, A. (122) 75, (124) 427
Mlynárik, V. (122) 47
Moenke-Bankenburg, L. (127) 71
Moesta, B. (130) 193
Möls, J.J. (130) 1
Montgomery, E.L. Jr. (121) 85
Morishige, K. (121) 301
Mortatti, J. (123) 221, (125) 29
Mortensen, J. (121) 71

Mortier, R. (121) 147
Moselhy, M.M. (130) 157
Motomizu, S. (128) 185
Mottola, H.A. (127) 39
Müller, J. (125) 175
Mulligan, K.J. (130) 227
Muñoz Leyva, J.A. (130) 183
Munshower, F.F. (123) 325
Murata, A. (125) 155
Murphy, R.J. (125) 73

Nagai, H. (130) 149
Naganuma, K. (124) 267, (124) 275
Nagy, V.Y. (128) 85
Nakagawa, G. (121) 265, (124) 193
Nakajima, K. (121) 197
Nakamura, H. (125) 203, (126) 185
Nakamura, T. (128) 269
Nakayama, E. (130) 289, (130) 401
Narasaki, H. (125) 187
Nash, C.F. (121) 101
Navarrete, G.A. (130) 209
Navas, A. (121) 295
Nembrini, G. (130) 385
Nestrick, T.J. (121) 345
Neuman, D.R. (123) 325
Nielsen, T. (124) 1
Nikolelis, D.P. (126) 43
Nishibe, J. (125) 109
Niwaguchi, T. (121) 157
Noirfalise, A. (121) 337
Nojiri, Y. (123) 57
Nomura, T. (124) 81, (126) 241
Nonova, D. (123) 289
Nord, L. (125) 199
Northcott, S.E. (126) 117
Norwitz, G. (128) 251
Novotny, M. (127) 55
Nullens, H. (122) 373
Nygaard, D.D. (127) 257, (130) 391
Nylén, P. (125) 65

Ogawa, T. (125) 161, (127) 195
Ögren, L. (125) 45, (130) 45
Ohashi, S. (121) 281
Ohkura, Y. (121) 289, (126) 237
Ohshita, K. (124) 193
Oikawa, T. (121) 157
Okazaki, S. (121) 119
Opekar, F. (126) 15, (127) 239
Osborn, T.W. (128) 213
Oshima, T. (122) 95
Osteryoung, J. (123) 97
Ottaway, J.M. (124) 281, (128) 57
Otto, M. (126) 65, (128) 177

Padron, J.L. (129) 247
Pakalns, P. (127) 263
Palmisano, F. (124) 143
Panak, J. (130) 369
Pankow, J.F. (124) 357
Pantel, S. (129) 231
Papadoyannis, I.N. (123) 315
Papastathopoulos, D.S. (126) 43, (128) 239
Parczewski, A. (130) 221
Pardue, H.L. (124) 39, (124) 65, (127) 23
Patriarche, G.J. (126) 57
Patsalides, E. (128) 101, (128) 109
Patterson, J.E. (125) 193
Pattyn-Fauville, G. (129) 113
Paulik, F. (124) 341
Paulik, J. (124) 341
Paulucci, J.B. (123) 347
Pavlova, S. (123) 289
Peaden, P.A. (127) 55
Peake, B.M. (123) 339
Pegon, Y. (124) 113, (129) 189, (130) 405
Peitryga, T. (122) 279
Pelizzetti, E. (126) 253, (128) 273
Penca, M. (122) 103
Pereira, W.E. (129) 57
Perlman, I. (121) 327
Pern, F.-J. (129) 19
Personov, R.I. (125) 1
Persson, B. (123) 115
Persson, J.-Å. (128) 1, (130) 267
Pessenda, L.C.R. (123) 221, (125) 29, (125) 37, (130) 361, (130) 409
Petik, P.A. (122) 323
Petránek, J. (128) 129
Petrukhin, O.M. (128) 85
Phillips, G.O. (130) 369
Phillips, R.J. (121) 225
Pietri, C.E. (129) 125
Pietsch, R. (127) 219
Pik, A.J. (124) 351
Pilate, A. (122) 57
Piljac, I. (122) 395
Pinedo, H.M. (127) 47, (130) 23
Pomernacki, C.L. (122) 403
Poppe, H. (123) 229, (126) 1, (130) 9
Posey, R.S. (124) 107
Pramauro, E. (126) 253, (128) 273
Pungor, E. (121) 93
Purdy, W.C. (123) 83, (127) 103
Püschel, P. (127) 109

Rakiás, F. (121) 93
Ramasamy, S.M. (127) 39
Ramsey, T.A. (121) 111
Ramsing, A.U. (129) 1
Ramstad, T. (121) 345
Rasmussen, G.T. (122) 171
Rasmussen, J. (121) 71
Rasmussen, L. (125) 117
Razinger, M. (122) 103
Rechnitz, G.A. (123) 135, (126) 35, (128) 121
Reich, G. (122) 139
Reijn, J.M. (123) 229, (126) 1
Reis, B.F. (123) 221, (125) 37, (130) 243, (130) 361, (130) 409
Remeš, M. (124) 91
Riley, J.P. (130) 199
Robberecht, H. (130) 167
Robinson, R.A. (130) 93
Román Ceba, M. (124) 201, (130) 183
Romanovskii, Y.V. (125) 1
Roolvink, W.B. (122) 81
Rosén, L. (123) 115
Roy, R.N. (129) 247

Růžička, J. (129) 1
Ryan, D.E. (124) 373
Ryba, O. (128) 129

Sabbatini, L. (124) 143
Saeed, K. (130) 281
Safavi, A. (128) 75
Safford, H.W. (130) 329
Saito, K. (122) 95
Sakharieva, M. (125) 149
Salinas, F. (130) 337
Salmela, S. (125) 131
Samuelsson, R. (123) 97
Sanemasa, I. (130) 149
Sano, M. (122) 241
Sanui, Y. (126) 185
Sanzolone, R.F. (128) 225
Sarbar, M. (130) 93, (130) 103
Sasaki, S. (122) 87, (122) 95
Sasaki, S.-I. (122) 241
Sasaki, Y. (127) 209
Sasuga, H. (128) 269
Sato, J. (128) 269
Savage, R.N. (123) 319, (124) 245
Savory, J. (128) 221
Schabron, J.F. (129) 243
Schiffel, B. (124) 237
Schlipf, J. (121) 257
Schmuckler, G. (126) 229
Schroll, G. (124) 1
Schulman, S.G. (127) 203
Schwartz, L.M. (122) 291
Scott, W.J. (126) 71, (126) 259
Seegopaul, P. (121) 61, (125) 55
Sexton, E.D. (121) 125
Shabushnig, J.G. (121) 165, (126) 167
Shahbaz, N.A. (125) 215
Sharma, Y. (126) 233
Shaw, P.G. (123) 65
Shepherd, R.J. (130) 55
Sherwood, G.A. (129) 101
Sherwood, G.A. Jr. (129) 87
Shete, S.D. (125) 165
Shinde, V.M. (125) 165
Shukla, S. (126) 105
Siebert, R.J. (123) 335
Sieper, H.P. (127) 71
Silver, H.F. (126) 225
Skov, H.J. (122) 179
Slanina, J. (130) 1
Slovák, Z. (129) 263, (130) 203
Smit, H.C. (122) 1, (122) 151, (122) 201, (122) 267
Smit, J.C. (122) 1, (122) 151
Smith, J.D. (123) 263
Smith, J.E. (124) 225
Smith, J.F. (123) 213
Smith, M.E. (130) 215
Smits, J. (123) 9
Smyth, W.F. (121) 51, (123) 175
Snook, R.D. (123) 329
Sofoniou, M.C. (123) 315
Sohr, H. (121) 309
Solsky, R.L. (123) 135
Spasić, S. (130) 419
Stacey, C.I. (127) 213
Steinheimer, T.R. (129) 57
Stock, J.T. (124) 85
Stojanov, M. (130) 419
Stojanovic, D.D. (124) 295
Strelow, F.W.E. (127) 63
Subramanian, K.S. (124) 131
Sugimae, A. (121) 331
Sugisaki, H. (125) 203
Sulaiman, S.T. (125) 215
Sullivan, J.V. (124) 259
Sunahara, H. (121) 197
Suzuki, N. (125) 139, (126) 199, (129) 221
Suzuki, S. (127) 245, (129) 237
Svehla, G. (125) 73, (129) 133, (129) 145
Swennen, L. (129) 113
Sychra, V. (127) 109
Szakács, O. (121) 219
Székely, G.G. (122) 257
Szepesváry, P. (122) 257

Taddia, M. (129) 259
Takagi, E. (130) 149
Takagi, M. (124) 403, (126) 185
Takahashi, Y. (122) 241
Talley, C.P. (127) 223
Tallman, D.E. (124) 321
Talmi, Y. (127) 71
Tamizi, M. (121) 341
Tandy, S. (121) 327
Tarayan, V.M. (124) 185
Tarutani, T. (130) 345
Tazaki, M. (124) 403
Temmerman, E. (127) 251
Teraoka, N. (127) 245
Thakrar, H. (124) 221
Thomassen, Y. (128) 23, (130) 281
Tiffon, B. (124) 415
Tkalčec, M. (122) 395
Todorovsky, D. (123) 303
Tôei, K. (128) 185
Tokoro, H. (130) 289
Tölg, G. (128) 9
Toman, J.J. (123) 187
Tomellini, S.A. (124) 409
Tonelli, D. (124) 215
Torsi, G. (124) 143
Tóth, K. (121) 93
Townshend, A. (127) 199, (127) 227, (128) 75
Trojánek, A. (121) 23, (126) 15, (127) 239
Tseng, C.L. (126) 191
Tsujii, K. (125) 101, (128) 229
Tsurubo, S. (130) 289, (130) 401
Tujino, R. (125) 109
Turcotte, J.G. (123) 41

Uchida, H. (123) 57
Ueno, K. (124) 403, (126) 185
Uhlemann, E. (130) 177
Umezaki, Y. (127) 93
Umland, F. (121) 275
Uścińska, J. (123) 271

Vajda, F. (128) 31
Valcarcel, M. (125) 21, (130) 73
Vallon, J.J. (124) 113, (129) 189, (130) 405

Van den Bosch, P.A. (127) 121
Van der Linden, W.E. (123) 229, (126) 1
Van der Maas, J.H. (122) 357, (122) 363
Van der Wiel, P.F.A. (122) 421
Van Dyck, P. (130) 167
Van Espen, P. (122) 373
Van Grieken, R. (123) 9, (128) 207, (130) 167
Van Lamoen, L. (130) 1
Van Nieuwkerk, H.J. (121) 13
Van Oort, W.J. (127) 47, (130) 23, (130) 111
Van Rooijen, H.W. (130) 9
Vančo, D. (121) 249
Vandecasteele, C. (121) 147
Vandeginste, B.G.M. (122) 435
Vanderborght, B. (128) 207
Vaneesorn, Y. (121) 51
Vansant, E.F. (122) 373
Varmuza, K. (122) 227
Vaughn, L. (124) 155
Verbeeck, J. (128) 207
Verbeeck, R.M.H. (127) 251
Veress, G.E. (124) 341
Vernon, F. (123) 309
Viczián, M. (122) 323
Vida, M. (122) 41, (122) 47
Vijan, P.N. (130) 157
Visser, T. (122) 357, (122) 363
Vittori, O. (121) 315
Vloon, W.J. (122) 267
Vo-Dinh, T. (125) 13
Voigt, B. (127) 87
Voinovitch, I.A. (129) 213
Volodarskii, L.B. (128) 85
Vos, L. (130) 167
Vuori, E. (125) 131
Vytřas, K. (124) 91, (127) 165

Wada, H. (121) 265, (124) 193
Waddell, T.G. (126) 207
Wang, J. (127) 157, (128) 147, (129) 253
Warren, J. (126) 125
Watanabe, Y. (130) 377
Watson, M.W. (129) 269
Webb, D.C. (128) 67
Webster, I.J. (123) 355
Wedlock, D. (130) 369
Weibust, G. (128) 23
Weiss, A.D. (124) 245
Weiss, J.R. (129) 125
Weisshaar, D.E. (124) 321
Weisz, H. (121) 257, (123) 239, (130) 193
Welch, D.K. (123) 41
Werner, G. (126) 65, (128) 177
West, T.S. (129) 69
Westerlund, S. (127) 147, (128) 155, (129) 153
Whitten, D.G. (122) 171
Wickramanayake, P.P. (125) 175
Wienhold, K. (121) 309
Wilder, D.R. (130) 31
Wilkinson, J.R. (128) 45
Williams, K.L. (124) 351
Williams, T.R. (123) 351
Wills, M.R. (128) 221
Winefordner, J.D. (124) 155, (124) 295
Wyn-Jones, E. (130) 369
Wynn, T.F. (124) 155

Xi-Ping, J. (124) 177

Yagi, K. (128) 229
Yamada, S. (127) 195
Yamada, T. (124) 275, (125) 179
Yamaguchi, E. (130) 345
Yamaguchi, M. (121) 289
Yamamoto, Y. (124) 163
Yamane, T. (130) 65
Yamasaki, T. (122) 87
Yarnitzky, C. (126) 229
Yeh, S.J. (126) 191
Yellin, J. (121) 327
Yoshida, K. (129) 221
Yoshimura, K. (130) 345
Yoshino, T. (124) 163
Yotsui, Y. (122) 241
Yoza, N. (121) 281
Yuan, C.-L. (124) 169

Zagatto, E.A.G. (123) 221, (125) 29, (125) 37, (130) 243, (130) 361, (130) 409
Zaitsu, K. (126) 237
Ziegler, E. (122) 315
Zin, W.M. (123) 309
Zolotov, Y.A. (128) 85
Zuman, P. (126) 71, (126) 259, (130) 111
Zupan, J. (122) 103, (122) 337

SUBJECT INDEX

VOLUMES 121—130

The volume number is placed in parentheses before the page number

A

Adsorption of ions on surfaces
At. abs. spectrometry; Calcium; Magnesium (126) 175

Air
Emission spectrometry; Hydrocarbons; Chemiluminescence; Reaction with oxygen atoms (124) 15

Gas chromatography; Mass spectrometry; Isotope dilut. analysis; Tetramethyllead; Tetraethyllead; Two-step enrichment method; Single ion monitoring (124) 1

Gas chromatography; Polychlorinated biphenyls; Trace organic compounds; Collection on Amberlite XAD-2 resins (124) 31

Piezoelectric sensing; Toluene diisocyanate; Coating materials (129) 163

Piezoelectric sensing; Toluene diisocyanate; Personal monitoring (129) 175

Air particulates
Spectrophotometry; Vanadium; Extraction; With *N*-benzoyl-*N*-phenylhydroxylamine (130) 353

Aldehydes
Polarography; Ketones; Hydration-dehydration equilibria; *α,α,α*-Trifluoroacetophenone (126) 71

Algorithms
Gas chromatography; High-level data processing; Computer processing; Optimal separation conditions (122) 279

Aliphatic carboxylates
Ion exchange; Gas chromatography; Direct derivatization; Lipophilic straight-chain aliphatic carboxylate ions (124) 403

Aliquat-336
Gas chromatography; Quaternary ammonium components (123) 213

Alkaline phosphatase
Fluorimetry; Sorbitol dehydrogenase; Peroxidase; Pseudocholinesterase; Fluoristic technique (129) 231

Alkaloids
Potentiometry; Picrate-selective electrode; Insoluble alkaloid picrate salts (123) 341

Alloys
Bismuth; Extraction; Pharmaceuticals; With N-benzoyl-*α*-butanoic acid into chloroform (127) 219

Aluminium
Electrotherm. atomization; At. abs. spectrometry; Plasma; Serum (128) 57

Electrotherm. atomization; At. abs. spectrometry; Serum; Aerosol generation system (128) 221

Emission spectrometry; Copper alloys; Glow lamp; Sputtering and emission intensity (124) 275

Flow system; Spectrophotometry; At. abs. spectrometry; Iron; Plants; Flow injection; Zone-sampling (125) 37

Fluorimetry; Gallium; Indium; Scandium; Beryllium; Alkyl derivatives of aromatic Schiff bases (121) 301

Aluminium oxide
Electrotherm. atomization; At. abs. spectrometry; Trace metals; Slurry atomization (129) 263

Aluminium salts
Electrotherm. atomization; At. abs. spectrometry; Copper; Iron; Involatile matrices (130) 203

Amines
Emission spectrometry; Molecular emission cavity analysis; Indirect determination; Formaldehyde-sulphite addition compound (128) 75

Amino acids
Fluorimetry; Proline; Secondary; Hydroxyproline; Sarcosine (130) 377

Amphetamines
N.m.r. spectrometry; Mono- and dimethylamphetamines; ^{13}C-n.m.r. (123) 75

Amplification
Ion exchange; Inversion tube; Definition (130) 193

Aniline
Coulometry; Titrimetry; End-point anticipation; Electronically controlled dual-intermediate titrator (124) 85

Anions
Gel perm. chromatography; Semi-automatic (126) 229

H.p.l.c.; Ion chromatography; Waters; Selective detectors (130) 1

Antibiotics
Pattern recognition; Biological activity; Cluster analysis; Structure-activity relationships; Hierarchal clustering; Nonlinear mapping (122) 241

Antibody-selective membrane electrode
Potentiometry; Polyvinyl chloride membrane (123) 135

Antimony
At. fluor. spectrometry; Silicon; Hydride generation; Depth profiles; Non-dispersive; Semiconductor (128) 229

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Selenium; Extraction; With ammonium pyrrolidinedithiocarbamate-methyl isobutyl ketone (124) 131

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Selenium; Tellurium; Spectral interferences from phosphate (130) 281

Arenediazonium salts
Potentiometry; Titrimetry; Coated-wire electrode; Azo dyestuffs; With sodium tetraphenylborate; Ion-pair formation (124) 91

Aromatic carboxamides
Spectrophotometry; Potentiometry; Hammett acidity function; Prototropic reaction isotherms; Hydration requirements (127) 203

Aromatics
Mass spectrometry; T.l.c.; Petroleum; η^6-Arene-η^5-cyclopentadienyliron cations; Ligand-exchange reaction (125) 171

Arrhenius model
Thermom. analysis; Reaction kinetic calculations (124) 341

Arsenic
At. fluor. spectrometry; Semiconductor silicon; Hydride generation; Depth profiles; Chemical etching; Non-dispersive (125) 101

Electrotherm. atomization; At. abs. spectrometry; Antimony; Selenium; Extraction; With ammonium pyrrolidinedithiocarbamate-methyl isobutyl ketone (124) 131

Electrotherm. atomization; At. abs. spectrometry; Selenium; Matrix modification with silver (128) 225

Electrotherm. atomization; At. abs. spectrometry; Surface reactions; High-temperature equilibrium calculations; Platform technique (130) 267

Electrotherm. atomization; At. abs. spectrometry; Antimony; Selenium; Tellurium; Spectral interferences from phosphate (130) 281

Arsenic species
Ion exchange; Electrotherm. atomization; At. abs. spectrometry; Marine organisms; Sediments; Hydride generation; Inorganic and methylated (126) 157

Artificial intelligence systems
Molecular spectroscopy; Polyatomic molecules (122) 249

Atomization with controlled atmosphere and temperature
Electrotherm. atomization; At. abs. spectrometry; Volatile elements; Tungsten wire; Quartz tube (128) 1

Atropine
Potentiometry; Novatropine; Pharmaceuticals; Liquid-membrane electrodes (128) 239

Automated system
At. abs. spectrometry; Flow system; Extraction (125) 199

Azo dyestuffs
Potentiometry; Titrimetry; Coated-wire electrode; Arenediazonium salts; With sodium tetraphenylborate; Ion-pair formation (124) 91

B

Background correction
At. abs. spectrometry; Electrotherm. atomization; Line absorption of matrix; Tables of bandwidths (128) 31

Benzoate-sensitive liquid membrane electrode
Potentiometry; Enhancement of selectivity by alkylphenol (121) 119

Benzoic acids
H.p.l.c.; Foods; Sorbic acids; Soft drinks (121) 337

Benzoquinone
Flow system; Amperometry; Renewable stationary mercury electrode; Detector (123) 107

Benzylpenicilloic acid
D.c. polarography; Diffl. pulse polarography; Cyclic voltammetry (128) 135

Beryllium
Fluorimetry; Aluminium; Gallium; Indium; Scandium; Alkyl derivatives of aromatic Schiff bases (121) 301

Binary tree-based heuristics
Clustering methods; Computer (122) 337

Biological materials
At. abs. spectrometry; Diffl. pulse voltammetry; Rocks; Soils; Selenium; Hydride generation; Combustion in oxygen; Cathodic stripping (128) 9

Phosphorimetry; Glucuronidase; *p*-Nitrophenol formed (121) 289

Potentiometry; Stripping analysis; Cadmium; Lead; Copper; Zinc; Computerized (127) 147

Bismuth
At. abs. spectrometry; Electrotherm. atomization; Tellurium; Copper alloys; Lead; Solid samples; Induction furnace (125) 93

Spectrophotometry; Bismuth oxide; Flotation; As tetraiodobismuthate(III) (123) 297

Spectrophotometry; Copper; Diethyldithiocarbamates; Extraction; Into chloroform (125) 149

Extraction; Alloys; Pharmaceuticals; With N-benzoyl-α-butanoic acid into chloroform (127) 219

Bismuth oxide
Spectrophotometry; Bismuth; Flotation; As tetraiodobismuthate(III) (123) 297

Blood
A.c. polarography; Paracetamol; Serum; Urine; Nitro derivative (124) 113

At. abs. spectrometry; Electrotherm. atomization; Gel filtration; Zinc; Copper; Serum; Speciation; Protein-bound; Immunonephelometry (124) 281

Emission spectrometry; Plasmas; Serum; Trace elements; Inductively coupled; Microsampling technique; Internal standardization (123) 57

Spectrophotometry; Creatine kinase-MB isoenzyme; Serum; Immobilized immunostirrer (124) 169

Boron
Activ. analysis; In zirconium and zircaloy; Proton activation; Anion-exchange (121) 147

Emission spectrometry; Molecular emission cavity analysis; Improved procedure (127) 227

Spectrophotometry; Flow system; Plants; Flow injection; With azomethine-H (125) 29

Boron shield
Activ. analysis; Choice; Epithermal neutron (124) 373

Bovine liver
Anod. stripping voltammetry; Selenium; Gold-plated glassy carbon disk electrode (124) 107

Brines
X-ray fluores. spectrometry; Vanadium; Molybdenum; Tungsten; Chloralkali electrolysis (128) 207

Butadiene-styrene copolymers
H.p.l.c.; Tris(nonylphenyl) phosphite; Nonylphenol; Ultraviolet detector (129) 243

C

Cadmium
Anod. stripping voltammetry; Electrotherm. atomization; At. abs. spectrometry; Lead; Sea water; In situ deposition; Mercury-coated graphite tube (124) 121

Anod. stripping voltammetry; Thallium; Lead; Influence of surfactant in perfluorinated emulsion; Differential pulse (127) 233

Anod. stripping voltammetry; Mercury film carbon fibre electrode; Differential pulse (130) 323

At. abs. spectrometry; Copper; Lead; Thallium; Liver tissue; Kidney; Enzymatic digestion (125) 209

At. abs. spectrometry; Extraction; Masking zinc; With ethylxanthate into chloroform (127) 209

Diffl. pulse voltammetry; Anod. stripping voltammetry; Copper; Lead; Zinc; Snow; Rotating glassy carbon electrode (121) 39

Electrotherm. atomization; At. abs. spectrometry; Urine; Matrix modification (130) 209

Flow system; Emission spectrometry; Ion exchange; Zinc; Chemiluminescence; Flow injection (127) 199

Ion exchange; Zinc; Hexacyanoferrate(II) salts on gel and macroporous resins; In situ precipitation (124) 365

Potentiometry; Stripping analysis; Lead; Copper; Zinc; Biological materials; Computerized (127) 147

Potentiometry; Lead; Urine; Computerized; Stripping analysis (128) 155

Potentiometry; Zinc; Lead; Copper; Sea water; Computerized; Stripping analysis (129) 153

Spectrophotometry; Extraction; With 4-(2-pyridylazo)resorcinol; And cetyldimethylbenzylammonium chloride (123) 289

Voltammetry; Pulse polarography; Microcomputer-controlled; Staircase voltammetry (122) 67

Calcium
Activ. analysis; Titanium; From ^{47}Sc (121) 327

At. abs. spectrometry; Adsorption of ions on surfaces; Magnesium (126) 175

At. abs. spectrometry; Oil, lubricating; Ionization suppressor (130) 395

Flow system; Spectrophotometry; Waters; Soils; Plants; Merging zones; Flow injection; With glyoxal bis(2-hydroxyanil) (130) 361

Ion exchange; Quantitative separation; Macroporous resin (127) 63

Titrimetry; Spectrophotometry; Metallochromic indicators (121) 265

Calcium-selective electrode
Potentiometry; Macrocyclic polyether diamides; Tetra(4-chlorophenyl)borate (128) 129

Capillary columns
Gas chromatography; H.p.l.c.; Polynuclear aromatic hydrocarbons (127) 55

Carbon
Emission spectrometry; Irons; Glow lamp; Sputtering and emission intensity; White, gray and malleable cast (124) 267

I.r. spectrometry; Steels; Plutonium; Carbon dioxide (130) 215

Carbonate
Potentiometry; Titrimetry; Hydroxide; Sodium chloride solutions; Computer method (122) 81

Potentiometry; Titrimetry; Presence of hydroxide; First-derivative (130) 93

Potentiometry; Titrimetry; Computer simulation (130) 103

Carbon dioxide
I.r. spectrometry; Carbon; Steels; Plutonium (130) 215

Carbon disulphide reduction
H.p.l.c.; Mass spectrometry; In N,N-dimethylformamide (129) 49

Cerium
Ion exchange; Spectrophotometry; Scandium; Yttrium; Uranium; Xylenol orange complexes; Strong anion exchangers (125) 139

Cetyl-trimethylammonium bromide with bromocresol purple
Titrimetry; Computerized system; Kalousek-type waveforms; Determination of double-layer capacitance (122) 387

Chelating resin
Ion exchange; Gold; Silver; Polyhydroxamic acid (123) 309

Chemical information system
I.r. spectrometry; Mass spectrometry; N.m.r. spectrometry; Minicomputer-oriented system (122) 103

Structure elucidation; Development of NIH-EPA system (122) 117

Chemiluminescence
Emission spectrometry; Chlorine; Waters; Hydrogen peroxide-hypochlorite reaction; Tap water; Interferences (123) 247

Emission spectrometry; Hydrocarbons; Air; Reaction with oxygen atoms (124) 15

Emission spectrometry; Humic acid; Waters; With permanganate (124) 23

Emission spectrometry; Electrogenerated; Lucigenin (130) 415

Flow system; Emission spectrometry; Ion exchange; Zinc; Cadmium; Flow injection (127) 199

Chloride
Electrotherm. atomization; Molecular absorption spectrometry (121) 205

Emission spectrometry; Plasmas; Microwave helium; Hydrogen chloride generation (123) 329

Spectrophotometry; Plants; Displacement of thiocyanate (123) 347

Spectrophotometry; Flow system; Ethanol; Flow injection (130) 409

Chlorinated phenols
H.p.l.c.; Reverse-phase separation; Online microprocessor control; Correlation chromatography (122) 267

Chlorine
Emission spectrometry; Waters; Chemiluminescence; Hydrogen peroxide-hypochlorite reaction; Tap water; Interferences (123) 247

Gas chromatography; Derivatisation with 2,6-dimethylphenol (124) 431

Chlorophenoxyphenols
Mass spectrometry; Soils; Waters; Extraction; Field desorption (124) 357

Chlorpromazine
Diffl. pulse voltammetry; Thin-layer electrochemical cell; Plasma; Urine (126) 57

Cholesterol
Amperometry; Enzyme membranes; Galactose; Maltose; Bound to collagen films; Glucose electrode (126) 23

Cholinesterase
Kinetic analysis; Thermom. analysis; Enzyme activity; Enthalpimetric determination (121) 125

Chromium
At. abs. spectrometry; Electrotherm. atomization; Removal of chloride interference (124) 163

At. abs. spectrometry; Electrotherm. atomization; Iron; Nickel; Sodium salts; Metallic sodium (126) 147

Gas chromatography; Polymers; Water-soluble; Trifluoroacetylacetone (127) 223

Gravimetry; Sea water; Chemical speciation; Naturally occurring organic materials (130) 289

Spectrophotometry; Liver tissue; With diphenylcarbazide (124) 391

Spectrophotometry; Ion flotation; Diphenylcarbazone complex (129) 237

X-ray fluores. spectrometry; Waters; Speciation; Membrane filters (124) 351

Sea water; Chemical speciation; Redox processes (130) 401

Cigarette smoke
H.p.l.c.; In sera; Low-molecular-weight, u.v.-absorbing compounds (123) 33

Citric acid
Spectrophotometry; NADH absorbance; Dehydrogenase reactions; Tetrazolium reagent (124) 215

Coal liquefaction product
Fluorimetry; Phosphorimetry; Polynuclear aromatic hydrocarbons; Synchronous luminescence (125) 13

Coated-wire electrode
Potentiometry; Titrimetry; Arenediazonium salts; Azo dyestuffs; With sodium tetraphenylborate; Ion-pair formation (124) 91

Potentiometry; Propranolol-responsive electrodes; Didodecylnaphthalene sulfonic acid salt (125) 179

Potentiometry; Titrimetry; Surfactants; Ion-pair formation; Cationic and anionic (127) 165

Cobalt
Spectrophotometry; Flow system; Kinetic analysis; Catalysis of SPADNS oxidation; Flow injection (130) 65

Extraction; Diethyldithiocarbamates; Zinc; Iron; Liquid-liquid (121) 249

Cod liver oil
H.p.l.c.; Vitamins; Fat-soluble; Formulated feed; Stopped-flow scanning (127) 213

Computer-aided spectra interpretation
I.r. spectrometry; Raman spectrometry; CRISE computer program (122) 363

N.m.r. spectrometry; Hardware; Software; Artificial intelligence system (122) 41

N.m.r. spectrometry; Minicomputer; Chemical structure (122) 47

Vibrational spectra (122) 357

Computer control
D.c. polarography; 2-Hydroxypropanoate complexes; Stability constants; Lead(II) propanoate (122) 395

Computerization
Titrimetry; H.p.l.c.; Micro-mini-mainframe-computer network; Correlation chromatography (122) 201

Classical chemical analysis; Interactive systems (122) 193

Computerized system
Potentiometry; Voltammetry (122) 179

Titrimetry; Cetyl-trimethylammonium bromide with bromocresol purple; Kalousek-type waveforms; Determination of double-layer capacitance (122) 387

Human interface; Dialogue programs; Command languages (122) 315

Controlled temperature-gradient lamps
At. abs. spectrometry; Performance and application (124) 259

Copper
Anod. stripping voltammetry; Sea water; Negative errors (123) 335

Anod. stripping voltammetry; Tin; Lead; Plants; Tissue; Sediments; Alternating current; Digesting agent (130) 385

At. abs. spectrometry; Electrotherm. atomization; Gel filtration; Zinc; Blood; Serum; Speciation; Protein-bound; Immunonephelometry (124) 281

At. abs. spectrometry; Cadmium; Lead; Thallium; Liver tissue; Kidney; Enzymatic digestion (125) 209

Diffl. pulse polarography; Polymaleic acid; Ionisation constants; Stability constants (129) 69

Diffl. pulse voltammetry; Anod. stripping voltammetry; Cadmium; Lead; Zinc; Snow; Rotating glassy carbon electrode (121) 39

Electrotherm. atomization; At. abs. spectrometry; Zinc; Serum (127) 103

Electrotherm. atomization; At. abs. spectrometry; Iron; Aluminium salts; Involatile matrices (130) 203

Flow system; Spectrophotometry; Conductometry; "Stat" methods; Iron; Iodide; Hydrochloric acid (123) 239

Fluorimetry; Kinetic analysis; Autoxidation of 2,2'-dipyridyl ketone azine or hydrazone or phenyl-2-pyridyl ketone hydrazone (125) 21

Gas chromatography; Vanadium; Petroleum oils; Nickel; Extraction of chelate (128) 109

Kinetic analysis; Flow system; Serum; Flow injection; Iron(III)-thiosulfate indicator reaction (127) 39

Kinetic analysis; Simplex optimization; Numerical model; Catalyzed decomposition of hydrogen peroxide (128) 177

Potentiometry; Stripping analysis; Cadmium; Lead; Zinc; Biological materials; Computerized (127) 147

Potentiometry; Zinc; Cadmium; Lead; Sea water; Computerized; Stripping analysis (129) 153

Spectrophotometry; Bismuth; Diethyldithiocarbamates; Extraction; Into chloroform (125) 149

Zinc; Hair; Wash solutions (124) 409

Silica-bound complexing agents; Photoacoustic spectroscopy; Complexation (129) 19

Copper alloys
At. abs. spectrometry; Electrotherm. atomization; Bismuth; Tellurium; Lead; Solid samples; Induction furnace (125) 93

Emission spectrometry; Glow lamp; Aluminium; Sputtering and emission intensity (124) 275

Copper ions
Voltammetry; Thiocyanate; Cathodic stripping; Application (123) 201

Copper-selective electrode
Potentiometry; Chloride interference; Mechanisms (123) 151

Potentiometry; Chloride interference region (123) 161

Correlation
Emission spectrometry; Gas chromatography; Wine; Glass-capillary; Stepwise regression analysis; Subjective sensory evaluations (122) 215

Corticosteroids
Polarography; Mechanism; Prednisolone; Dexamethasone (130) 111

Creatine kinase-MB isoenzyme
Spectrophotometry; Blood; Serum; Immobilized immuno-stirrer (124) 169

Cross polarization
N.m.r. spectrometry; Magic angle spinning; Organosilanes; Carbon-13; Bonded to silica (126) 207

Crown ether complexes
Spectrophotometry; Sodium; Extraction; Serum; Monoanionic dyes; Ion-pair (126) 185

Cyanide
Piezoelectric sensing; Single-drop method (124) 81

Cyanide sensor
Potentiometry; Mercury; Indirect determination; Air-gap electrode (126) 221

Cysteine
Voltammetry; Selenocystine; Cystine; Cathodic stripping (124) 333

Cystine
Voltammetry; Selenocystine; Cysteine; Cathodic stripping (124) 333

D

Diamine oxidase
Kinetic analysis; Spectrophotometry (130) 419

Diethyldithiocarbamates
Spectrophotometry; Bismuth; Copper; Extraction; Into chloroform (125) 149

Extraction; Cobalt; Zinc; Iron; Liquid-liquid (121) 249

Digital integrator
Coulometry; Plutonium; Controlled-potential (129) 125

Dihydroxytartaric acid
Cyclic voltammetry; Diffl. pulse polarography; Osazone; Tautomeric equilibrium (129) 133

Diffl. pulse polarography; Osazone; Kinetic measurements (129) 145

Dinitrosalicylate-selective electrode
Potentiometry; Titrimetry; Liquid-membrane (126) 51

Dioxin
Gas chromatography; Active carbon; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Environmental samples (123) 25

Diphenylcarbazones
Spectrophotometry; Asymmetrically substituted; Chelate formers (121) 275

Dispatching rules
I.r. spectrometry; N.m.r. spectrometry; Mass spectrometry; Structural analysis; Digital simulation (122) 435

Dispersion phenomena
Flow system; Transport; Flow injection; Single bead string reactor (126) 1

Dissociation constant
Conductometry; Equivalent conductance; Weak electrolyte (125) 219

Potentiometry; *N*-(2-Acetamido)iminodiacetic acid (129) 247

Dithizone
Spectrophotometry; Effect of temperature (128) 261

Drugs
H.p.l.c.; Mitomycin C; Plasma; Human blood; U.v. detection (127) 47

H.p.l.c.; Plasma; Urine; Anti-neoplastic agents (130) 23

Voltammetry; Flow system; 2-Thiobarbituric acid; Thioamide; Mercury-coated glassy carbon electrode (121) 51

Dry-column chromatography
Fluorimetry; T.l.c.; Polynuclear aromatic hydrocarbons; Standardization of aluminium oxide (126) 225

Dusts
Quartz; X-ray diffractometry; Sample preparation (128) 269

Dye binding
Spectrophotometry; Ionic to a polyelectrolyte in solution; Dialysis membrane (130) 369

E

Edible oils
H.p.l.c.; Phenolic anti-oxidants (128) 235

Eigenvector analysis
Spectrophotometry; Data reduction; Equilibrium and kinetic studies; Desk computer (122) 303

Electrochemical detector
Flow system; Amperometry; Kel-F-graphite electrode (124) 321

Flow system; Amperometry; Theoretical treatment; Open tubular electrode (127) 157

Electrochemical digital simulation
Point method (122) 331

Electrochemical reactivation
H.p.l.c.; Flow system; For solid electrodes (130) 9

Electrodialysis
Quantitative enrichment of trace levels (130) 313

Electroplating baths
Diffl. pulse polarography; A.c. polarography; Saccharin; After extraction; Masking with cyanide (124) 237

Element-selective detection
Gas chromatography; H.p.l.c.; Emission spectrometry; Plasmas; Review (130) 227

Enzymatic analysis
Potentiometry; Flow system; Kinetic analysis; Glucose; Sulphur dioxide probe; Glucose oxidase; Oxidation of hydrogensulphite (125) 55

Enzymatic digestion
At. abs. spectrometry; Cadmium; Copper; Lead; Thallium; Liver tissue; Kidney (125) 209

Enzyme activity
Kinetic analysis; Thermom. analysis; Cholinesterase; Enthalpimetric determination (121) 125

Enzyme membranes
Amperometry; Galactose; Cholesterol; Maltose; Bound to collagen films; Glucose electrode (126) 23

Enzyme reactor
Flow system; Spectrophotometry; Potentiometry; Glucose; Urea; On-line dialysis (130) 45

Potentiometry; Mercury; Waters; On-site samping; Immobilized urease; Ammonia electrode (125) 45

Enzyme sensor
Potentiometry; Oxalate; Immobilized oxalate decarboxylase; Carbon dioxide gas-sensing electrode; Urine (121) 111

Equivalent conductance
Conductometry; Dissociation constant; Weak electrolyte (125) 219

Ethanol
A.c. polarography; Tensammetry; In vodka (126) 213

Spectrophotometry; Flow system; Chloride; Flow injection (130) 409

Voltammetry; Flow system; Immobilized alcohol oxidase; Oxygen electrode; Blood alcohol (123) 125

Europium
Fluorimetry; With 1,1,1-trifluoro-4-(2-thienyl)-2,4-butanedione; Nitrogen laser; Photon counter (127) 195

Explosive mixtures
H.p.l.c.; Gunshot residue; Nitro aromatics; Nitrate esters; Nitramines (130) 295

Extraction
At. abs. spectrometry; Ion exchange; Electrotherm. atomization; Trace metals; Sea water; Ammonium pyrrolidinedithiocarbamate/diethylammonium diethyldithiocarbamate into freon; Chelex-100 resin (125) 117

At. abs. spectrometry; Flow system; Automated system (125) 199

At. abs. spectrometry; Cadmium; Masking zinc; With ethylxanthate into chloroform (127) 209

Diffl. pulse polarography; Flurazepam; Metabolites (123) 175

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Antimony; Selenium; With ammonium pyrrolidinedithiocarbamate-methyl isobutyl ketone (124) 131

Emission spectrometry; Sea water; Plasmas; Trace metals; Inductively coupled; Sodium diethyldithiocarbamate into chloroform (121) 331

Emission spectrometry; Phosphorus; Waters; Plasmas; Sea water; Inductively coupled; Molybdoantimonylphosphoric acid into diisobutyl ketone (127) 93

Fluorimetry; Flow system; Gallium; Flow injection; With lumogallion; Laser excitation (129) 195

Isotope dilut. analysis; Uranium; Substoichiometric; Synergic system (129) 221

Mass spectrometry; Chlorophenoxyphenols; Soils; Waters; Field desorption (124) 357

Spectrophotometry; Cadmium; With 4-(2-pyridylazo)resorcinol; And cetyldimethylbenzylammonium chloride (123) 289

Spectrophotometry; Non-ionic surfactants; Waters; Sea water; Potassium tetrathiocyanatozincate(II); Zinc(II) (123) 355

Spectrophotometry; Thermal lens effect; Iron; Ion-pair; With 4,7-diphenyl-1,10-phenanthroline disulfonic acid (124) 381

Spectrophotometry; Bismuth; Copper; Diethyldithiocarbamates; Into chloroform (125) 149

Spectrophotometry; Vanadium; Niobium; From succinate solution; Using tri-n-octylamine (125) 165

Spectrophotometry; Sodium; Crown ether complexes; Serum; Monoanionic dyes; Ion-pair (126) 185

Spectrophotometry; Ion-pairs; Triphenylmethane dye cations (128) 185

Spectrophotometry; Gas chromatography; Oxalic acid; Urine; Ion-pair formation (129) 189

Spectrophotometry; Vanadium; Air particulates; With *N*-benzoyl-*N*-phenylhydroxylamine (130) 353

Diethyldithiocarbamates; Cobalt; Zinc; Iron; Liquid-liquid (121) 249

Simplex optimization; Synergic extraction; Copper(II) acetylacetonate into cyclohexane (122) 223

Gold diethyldithiocarbamate dichloride; Extraction constant; Chloroform (126) 191

Bismuth; Alloys; Pharmaceuticals; With N-benzoyl-*α*-butanoic acid into chloroform (127) 219

Gallium; Bidentate ligands; Radiochemistry (130) 177

Extractors
Unconventional; Standard Soxhlet (125) 175

F

Flotation
Spectrophotometry; Osmium; With thiocyanate and methylene blue; Ion associate precipitate (123) 271

Spectrophotometry; Bismuth oxide; Bismuth; As tetraiodobismuthate(III) (123) 297

Fluoride
Potentiometry; Microcomputer-based system; Ion-selective electrodes; In toothpaste (125) 65

Flurazepam
Diffl. pulse polarography; Extraction; Metabolites (123) 175

Foods
H.p.l.c.; Sorbic acids; Benzoic acids; Soft drinks (121) 337

Free acid
Potentiometry; Titrimetry; Addition of potassium oxalate; Hydrolyzable cations (127) 263

G

Galactose
Amperometry; Enzyme membranes; Cholesterol; Maltose; Bound to collagen films; Glucose electrode (126) 23

Gallium
Fluorimetry; With benzyl-2-pyridylketone-2-pyridylhydrazone (121) 295

Fluorimetry; Aluminium; Indium; Scandium; Beryllium; Alkyl derivatives of aromatic Schiff bases (121) 301

Fluorimetry; Flow system; Extraction; Flow injection; With lumogallion; Laser excitation (129) 195

Extraction; Bidentate ligands; Radiochemistry (130) 177

Gas-sheathed atom cell
At. fluor. spectrometry; Mercury; Plants; Cold-vapour (128) 45

Gel filtration
At. abs. spectrometry; Electrotherm. atomization; Zinc; Copper; Blood; Serum; Speciation; Protein-bound; Immunonephelometry (124) 281

Geological materials
Ion exchange; Emission spectrometry; Yttrium; Rare earth elements; Plasmas; Inductively-coupled (124) 421

Germanium
Spectrophotometry; As methylene blue 12-molybdogermanate (124) 185

Glass
Activ. analysis; Trace elements; Instrumental photon activation (121) 157

Glass surfaces
Titrimetry; Potentiometry; Thermom. analysis; Modified with N-2-aminoethyl-3-aminopropyltrimethoxysilane; Titrated with copper(II) (124) 229

Glow lamp
Emission spectrometry; Irons; Carbon; Sputtering and emission intensity; White, gray and malleable cast (124) 267

Emission spectrometry; Copper alloys; Aluminium; Sputtering and emission intensity (124) 275

Glucose
Flow system; Spectrophotometry; Potentiometry; Urea; Enzyme reactor; On-line dialysis (130) 45

Potentiometry; Flow system; Kinetic analysis; Enzymatic analysis; Sulphur dioxide probe; Glucose oxidase; Oxidation of hydrogensulphite (125) 55

Glucose oxidase
Potentiometry; Flow system; Gas-sensing sulphur dioxide probe (121) 61

Glucuronidase
Phosphorimetry; Biological materials; *p*-Nitrophenol formed (121) 289

Gold
Ion exchange; Silver; Chelating resin; Polyhydroxamic acid (123) 309

Gold diethyldithiocarbamate dichloride
Extraction; Extraction constant; Chloroform (126) 191

Gradient chamber
Kinetic analysis; Flow system; Titrimetry; Kinetic treatment; Flow-injection (124) 39

Kinetic analysis; Flow system; Titrimetry; Kinetic treatment; Flow-injection (124) 65

Guanine enzyme electrode
Potentiometry; Serum; Ammonia gas sensor; Immobilization (126) 43

Gunshot residue
H.p.l.c.; Explosive mixtures; Nitro aromatics; Nitrate esters; Nitramines (130) 295

H

Hafnium
Fluorimetry; With 3-hydroxychromone (125) 155

Hair
At. abs. spectrometry; Electrotherm. atomization; Trace metals; Flame; Cold-vapor (123) 45

Electrotherm. atomization; At. abs. spectrometry; Trace elements; Washing procedures (125) 131

Copper; Zinc; Wash solutions (124) 409

Hammett acidity function
Spectrophotometry; Potentiometry; Aromatic carboxamides; Prototropic reaction isotherms; Hydration requirements (127) 203

High flow-rate cells
Flow system; Voltammetry; Static mercury drop electrode (127) 121

Homogeneity of solids
Empirical mathematical models (130) 221

Human interface
Computerized system; Dialogue programs; Command languages (122) 315

Humic acid
Emission spectrometry; Chemiluminescence; Waters; With permanganate (124) 23

Humic substances
D.c. polarography; Waters; Potable; Tri-n-butylphosphate layer (121) 309

Hydride generation
At. abs. spectrometry; Diffl. pulse voltammetry; Biological materials; Rocks; Soils; Selenium; Combustion in oxygen; Cathodic stripping (128) 9

At. fluor. spectrometry; Arsenic; Semiconductor silicon; Depth profiles; Chemical etching; Non-dispersive (125) 101

At. fluor. spectrometry; Antimony; Silicon; Depth profiles; Non-dispersive; Semiconductor (128) 229

Emission spectrometry; Lead; Plasmas; Inductively-coupled (125) 109

Ion exchange; Electrotherm. atomization; At. abs. spectrometry; Arsenic species; Marine organisms; Sediments; Inorganic and methylated (126) 157

Hydrocarbons
Emission spectrometry; Air; Chemiluminescence; Reaction with oxygen atoms (124) 15

Hydrochloric acid
Flow system; Spectrophotometry; Conductometry; "Stat" methods; Copper; Iron; Iodide (123) 239

Hydrogen peroxide
Voltammetry; Pickling baths; For copper alloys; Linear sweep (128) 141

Hydroxide
Potentiometry; Titrimetry; Carbonate; Sodium chloride solutions; Computer method (122) 81

I

Immobilized alcohol oxidase
Voltammetry; Flow system; Ethanol; Oxygen electrode; Blood alcohol (123) 125

Immobilized immuno-stirrer
Spectrophotometry; Creatine kinase-MB isoenzyme; Blood; Serum (124) 169

Indium
Fluorimetry; Aluminium; Gallium; Scandium; Beryllium; Alkyl derivatives of aromatic Schiff bases (121) 301

Industrial hygiene samples
At. abs. spectrometry; Tungsten compounds; Soluble and insoluble; Nitrous oxide-acetylene flame (121) 187

Inhibition release titration
At. abs. spectrometry; Magnesium; Phosphate (124) 295

Emission spectrometry; Titanium; Calcium atomization (129) 259

Interference of sulphite
Spectrophotometry; Phenol; 4-Aminoantipyrine (128) 251

Iodide
At. abs. spectrometry; Electrotherm. atomization; Use of mercury (126) 241

Flow system; Kinetic analysis; Thermom. analysis; Catalyzed cerium(IV)-arsenic(III) reaction (121) 135

Flow system; Spectrophotometry; Conductometry; "Stat" methods; Copper; Iron; Hydrochloric acid (123) 239

Ion chromatography
H.p.l.c.; Sulphur; Oil, fuel; Oxygen flask (121) 233

H.p.l.c.; Anions; Waters; Selective detectors (130) 1

Ion flotation
Spectrophotometry; Chromium; Diphenylcarbazone complex (129) 237

X-ray fluores. spectrometry; Platinum; Iridium; Separation; Anionic chlorocomplexes; With cationic surfactants (123) 1

Rhodium; Iridium; Separation (121) 239

Ion-selective electrodes
Potentiometry; Microcomputer-based system; Fluoride; In toothpaste (125) 65

Iridium
X-ray fluores. spectrometry; Platinum; Ion flotation; Separation; Anionic chlorocomplexes; With cationic surfactants (123) 1

Rhodium; Ion flotation; Separation (121) 239

Iron
At. abs. spectrometry; Electrotherm. atomization; Nickel; Chromium; Sodium salts; Metallic sodium (126) 147

Diffl. pulse polarography; Silicon; Solar-grade (126) 247

Electrotherm. atomization; At. abs. spectrometry; Copper; Aluminium salts; Involatile matrices (130) 203

Flow system; Spectrophotometry; Conductometry; "Stat" methods; Copper; Iodide; Hydrochloric acid (123) 239

Flow system; Spectrophotometry; At. abs. spectrometry; Aluminium; Plants; Flow injection; Zone-sampling (125) 37

Kinetic analysis; Fluorimetry; Thallium; Oxidation of 1,4-diamino-2,3-dihydroanthraquinone (130) 337

Spectrophotometry; Chrome azurol S; Eriochrome cyanine R; Cationic surfactants; Ternary complexes (123) 279

Spectrophotometry; Extraction; Thermal lens effect; Ion-pair; With 4,7-diphenyl-1,10-phenanthroline disulfonic acid (124) 381

Extraction; Diethyldithiocarbamates; Cobalt; Zinc; Liquid-liquid (121) 249

Irons
Emission spectrometry; Glow lamp; Carbon; Sputtering and emission intensity; White, gray and malleable cast (124) 267

Isopropylmethylphosphonofluoridate
Gas chromatography; Waters; Preconcentration; Direct thermal desorption (124) 225

Isosorbide dinitrate
Flow system; Polarography; Flow injection analysis (123) 115

K

Ketones
Polarography; Aldehydes; Hydration-dehydration equilibria; α,α,α-Trifluoro-acetophenone (126) 71

Kidney
At. abs. spectrometry; Cadmium; Copper; Lead; Thallium; Liver tissue; Enzymatic digestion (125) 209

L

Lactate dehydrogenase
Kinetic analysis; Regression data processing; Subunit determinations; Catalytic reaction (127) 23

Lactate electrode
Phenylalanine; Serum; Immobilized *Leuconostoc mesenteroides* (127) 245

Laser excitation
Fluorimetry; Organic materials; Polynuclear aromatic hydrocarbons; Narrow vibrational lines; Selective excitation (125) 1

Fluorimetry; Detection limits; Nitrogen laser-pumped dye laser (125) 161

Laser microprobe
Emission spectrometry; Multichannel detection system; Imaging detectors (127) 71

Lead
Anod. stripping voltammetry; Electrotherm. atomization; At. abs. spectrometry; Cadmium; Sea water; In situ deposition; Mercury-coated graphite tube (124) 121

Anod. stripping voltammetry; Thallium; Cadmium; Influence of surfactant in perfluorinated emulsion; Differential pulse (127) 233

Anod. stripping voltammetry; Tin; Copper; Plants; Tissue; Sediments; Alternating current; Digesting agent (130) 385

At. abs. spectrometry; Electrotherm. atomization; Bismuth; Tellurium; Copper alloys; Solid samples; Induction furnace (125) 93

At. abs. spectrometry; Cadmium; Copper; Thallium; Liver tissue; Kidney; Enzymatic digestion (125) 209

At. abs. spectrometry; Microsampling-cup procedure; Plants; Solid sample (128) 67

Diffl. pulse voltammetry; Anod. stripping voltammetry; Cadmium; Copper; Zinc; Snow; Rotating glassy carbon electrode (121) 39

Electrotherm. atomization; At. abs. spectrometry; Sea water; Electrolytic accumulation on glassy carbon (124) 143

Electrotherm. atomization; At. abs. spectrometry; Rocks; Yield monitoring; Extraction with dithizone (125) 203

Emission spectrometry; Plasmas; Hydride generation; Inductively-coupled (125) 109

Potentiometry; Stripping analysis; Cadmium; Copper; Zinc; Biological materials; Computerized (127) 147

Potentiometry; Cadmium; Urine; Computerized; Stripping analysis (128) 155

Potentiometry; Zinc; Cadmium; Copper; Sea water; Computerized; Stripping analysis (129) 153

Titrimetry; Thiocyanate amplification; Precipitation as lead hexathiocyanato-chromate(III) (125) 215

Least-squares fitting
Mossbauer spectroscopy (122) 373

Lecithin-cholesterol acyltransferase
Fluorimetry; Plasma; Enzyme reaction (126) 237

Lignin
Spectrophotometry; Biodegraded (130) 81

Limiting current in electrochemical flow-through cells
Flow system; Voltammetry; Survey of the equations; Tubular, thin-layer, wall-jet and disk electrodes (121) 13

Liver tissue
At. abs. spectrometry; Cadmium; Copper; Lead; Thallium; Kidney; Enzymatic digestion (125) 209

Spectrophotometry; Chromium; With diphenylcarbazide (124) 391

Lucigenin
Voltammetry; Spectrophotometry; Electrochemical reduction (125) 73

M

Magic angle spinning
N.m.r. spectrometry; Cross polarization; Organosilanes; Carbon-13; Bonded to silica (126) 207

Magnesium
At. abs. spectrometry; Adsorption of ions on surfaces; Calcium (126) 175

Fluorimetry; Ternary complex (130) 73

Maltose
Amperometry; Enzyme membranes; Galactose; Cholesterol; Bound to collagen films; Glucose electrode (126) 23

Manganese
Coulometry; Voltammetry; Flow system; Cathodic stripping (126) 15

Electrotherm. atomization; At. abs. spectrometry; Serum; Urine (129) 205

Flow system; Voltammetry; Peristaltic pump pulsation; Synchronization of signal sampling; Cathodic stripping (127) 239

Marine organisms
Ion exchange; Electrotherm. atomization; At. abs. spectrometry; Arsenic species; Sediments; Hydride generation; Inorganic and methylated (126) 157

Measurement theory
Spectrometry; Weighting functions; Shot noise; Poisson statistics (122) 27

Mercury
At. abs. spectrometry; Waters; Decomposition of organic mercury; Bromine monochloride; Cold-vapour (121) 219

At. abs. spectrometry; Milk products; Plastics; Oxygen bomb; Cold-vapour technique (125) 187

At. fluor. spectrometry; Gas-sheathed atom cell; Plants; Cold-vapour (128) 45

Ion exchange; At. abs. spectrometry; Waters; Sea water; Cold-vapour; Anion-exchange resin (130) 149

Piezoelectric sensing; Waters; Gold-coated (130) 141

Potentiometry; Enzyme reactor; Waters; On-site samping; Immobilized urease; Ammonia electrode (125) 45

Potentiometry; Cyanide sensor; Indirect determination; Air-gap electrode (126) 221

Mercury film electrode
Anod. stripping voltammetry; Trace metals; Sea water; Physically-coated (130) 199

Metal complexes
Anod. stripping voltammetry; Voltammetry; Convolution techniques (123) 187

Metal ions
Ion exchange; Attaching N-phenylhydroxamic acid groups; Amberlite XAD-4 (121) 225

Titrimetry; Kinetic analysis; Gaseous catalysts for end-point indication; Hydrogen sulphide (121) 257

Metals
E.s.r.; Stable free-radical complexing reagents; Spin-labelled iminooxime (128) 85

Metal speciation
Polarography; Derivative normal pulse; Microcomputer (129) 79

Methyl esters
H.p.l.c.; Phosphatidic acid dimethyl esters; Silver nitrate-impregnated silica gel column (123) 41

Micellar systems
Spectrophotometry; Polymethylphenols; Acidity and binding constants in sodium dodecyl sulphate (126) 253

Microcomputer-based system
Potentiometry; Ion-selective electrodes; Fluoride; In toothpaste (125) 65

Microdensitometer-minicomputer system
Mass spectrometry; Semiautomatic; Spark-source (122) 323

Microsampling-cup procedure
At. abs. spectrometry; Lead; Plants; Solid sample (128) 67

Milk products
At. abs. spectrometry; Mercury; Plastics; Oxygen bomb; Cold-vapour technique (125) 187

Mitomycin C
H.p.l.c.; Plasma; Drugs; Human blood; U.v. detection (127) 47

Modelling
Chromatography; Computer implementation; Simulation; Linear or non-linear isotherms (122) 1

Chromatography; Computer implementation; Simulation; Mass transport; Non-linear isotherm (122) 151

Structural analysis; Algebraic; Theory of semi-lattices; Boolean equations (122) 257

Models, multiparameter
Potentiometry; Titrimetry; Statistical uncertainties; Multiple regression (122) 291

Modified silica surfaces
Fluorimetry; Derivatized dansylated silica (130) 31

Molecular absorption spectrometry
Electrotherm. atomization; Chloride (121) 205

Molecular emission cavity analysis
Emission spectrometry; Sulphur; Variation in cavity position; Phosphate addition (121) 175

Emission spectrometry; Waters; Selenium; After coprecipitation (123) 315

Emission spectrometry; Boron; Improved procedure (127) 227

Emission spectrometry; Amines; Indirect determination; Formaldehyde-sulphite addition compound (128) 75

Molecular spectroscopy
Artificial intelligence systems; Polyatomic molecules (122) 249

Molybdenum
Diffl. pulse polarography; Silicon; Solar-grade; Molybdate catalytic wave (124) 233

Electrotherm. atomization; At. abs. spectrometry; Plants (123) 325

Ion exchange; H.p.l.c.; Uranium; Controlled-pore glass; Immobilized diamine (126) 117

Potentiometry; Titrimetry; Ion exchange; Nickel alloys; With cerium(IV) solutions (121) 85

X-ray fluores. spectrometry; Vanadium; Tungsten; Brines; Chloralkali electrolysis (128) 207

Monohydrogenphosphate-sensing electrode
Potentiometry; Phosphate; Formulations (121) 101

Multichannel detection system
Emission spectrometry; Laser microprobe; Imaging detectors (127) 71

N

Neptunium
Spectrophotometry (124) 443

Nickel
At. abs. spectrometry; Electrotherm. atomization; Iron; Chromium; Sodium salts; Metallic sodium (126) 147

Gas chromatography; Vanadium; Petroleum oils; Copper; Extraction of chelate (128) 109

Spectrophotometry; 2-(2-Pyridylazo)-5-alkoxyphenol derivatives; Solvent extraction (124) 193

Nickel alloys
Potentiometry; Titrimetry; Ion exchange; Molybdenum; With cerium(IV) solutions (121) 85

Nicotine-sensitive electrode
Potentiometry; Tobacco products; Liquid membrane (127) 173

Niobium
Spectrophotometry; Vanadium; Extraction; From succinate solution; Using tri-n-octylamine (125) 165

Nitrate
Flow system; Spectrophotometry; Waters; Flow injection; Hydrazine reduction; Rain (124) 437

H.p.l.c.; Amperometry; Flow-through electrode (129) 101

Kinetic analysis; Amperometry; Electrocatalysis of reduction; By electrodeposited copper (129) 87

Nitrite; Pneumatoamperometry (130) 391

Nitrite
Nitrate; Pneumatoamperometry (130) 391

Nitrogen
Flow system; Spectrophotometry; Sea water; Alkaline peroxodisulphate (130) 55

Nitrosamines
Gas chromatography; Emission spectrometry; Electronic noise filter; Chemiluminescent detector (126) 125

H.p.l.c.; Voltammetry; Electrochemical detection (123) 97

Non-ionic surfactants
Spectrophotometry; Extraction; Waters; Sea water; Potassium tetrathiocyanatozincate(II); Zinc(II) (123) 355

Nonylphenol
H.p.l.c.; Tris(nonylphenyl) phosphite; Butadiene-styrene copolymers; Ultraviolet detector (129) 243

Novatropine
Potentiometry; Atropine; Pharmaceuticals; Liquid-membrane electrodes (128) 239

O

Oil
At. fluor. spectrometry; Electrotherm. atomization; Wear metals; Wavelength-modulated, continuum-source excited; Jet engine lubricating; Filament in a flame (124) 155

Oil, fuel
H.p.l.c.; Sulphur; Ion chromatography; Oxygen flask (121) 233

Oil, lubricating
At. abs. spectrometry; Calcium; Ionization suppressor (130) 395

Organic compounds
Gas chromatography; Mass spectrometry; Sediments; Computer techniques; Capillary column (129) 57

I.r. spectrometry; N.m.r. spectrometry; Structure elucidation; Computer program system; New CHEMICS (122) 87

I.r. spectrometry; N.m.r. spectrometry; Structure elucidation; CHEMICS program (122) 95

Organic materials
Fluorimetry; Laser excitation; Polynuclear aromatic hydrocarbons; Narrow vibrational lines; Selective excitation (125) 1

Organosilanes
N.m.r. spectrometry; Cross polarization; Magic angle spinning; Carbon-13; Bonded to silica (126) 207

Organosilanes, reactivity
Ethylenediamine vs. glycidoxy side-chains (129) 269

Organotin compounds
At. abs. spectrometry; Spectrophotometry; Tin; Substoichiometric determination; Complexation with salicylideneamino-2-thiophenol (126) 199

Osmium
Spectrophotometry; Flotation; With thiocyanate and methylene blue; Ion associate precipitate (123) 271

Oxalate
Potentiometry; Enzyme sensor; Immobilized oxalate decarboxylase; Carbon dioxide gas-sensing electrode; Urine (121) 111

Oxalic acid
Spectrophotometry; Gas chromatography; Extraction; Urine; Ion-pair formation (129) 189

Oxygen
I.r. spectrometry; Selenium (127) 87

Oxygen, continuous removal
Flow system; Semipermeable membrane (121) 23

P

Paracetamol
A.c. polarography; Blood; Serum; Urine; Nitro derivative (124) 113

Gas chromatography; Plasma; Rapid extraction (130) 405

Pattern recognition
Gas chromatography; Chemical structure; From retention data (122) 139

Review (122) 227

Antibiotics; Biological activity; Cluster analysis; Structure-activity relationships; Hierarchal clustering; Nonlinear mapping (122) 241

Operational research model; Clustering (122) 347

Peristaltic pump pulsation
Flow system; Voltammetry; Manganese; Synchronization of signal sampling; Cathodic stripping (127) 239

Peroxidase
Fluorimetry; Alkaline phosphatase; Sorbitol dehydrogenase; Pseudocholinesterase; Fluoristic technique (129) 231

Petroleum
Mass spectrometry; T.l.c.; Aromatics; η^6-Arene-η^5-cyclopentadienyliron cations; Ligand-exchange reaction (125) 171

Petroleum oils
Gas chromatography; Vanadium; Fluorinated β-diketonates (128) 101

Gas chromatography; Vanadium; Copper; Nickel; Extraction of chelate (128) 109

pH
Potentiometry; Standardisation; Measurement (127) 1

Pharmaceuticals
Potentiometry; Titrimetry; Containing covalently bound halogen; Ion-selective electrodes; Combustion; pH adjustment (121) 93

Potentiometry; Atropine; Novatropine; Liquid-membrane electrodes (128) 239

Bismuth; Extraction; Alloys; With N-benzoyl-α-butanoic acid into chloroform (127) 219

Phase solubility analysis
Ion-pair formation (126) 105

Phenol
Spectrophotometry; Interference of sulphite; 4-Aminoantipyrine (128) 251

Phenolic anti-oxidants
H.p.l.c.; Edible oils (128) 235

Phenylalanine
Lactate electrode; Serum; Immobilized *Leuconostoc mesenteroides* (127) 245

Phosphate
Potentiometry; Monohydrogenphosphate-sensing electrode; Formulations (121) 101

Spectrophotometry; Soils (128) 257

Phosphatidic acid dimethyl esters
H.p.l.c.; Methyl esters; Silver nitrate-impregnated silica gel column (123) 41

Phosphorus
Diffl. pulse polarography; Semiconductor silicon; Molybdophosphoric acid; Catalytic molybdenum wave (124) 99

Emission spectrometry; Waters; Plasmas; Inductively coupled (121) 197

Emission spectrometry; Waters; Extraction; Plasmas; Sea water; Inductively coupled; Molybdoantimonylphosphoric acid into diisobutyl ketone (127) 93

Photoacoustic spectroscopy
Copper; Silica-bound complexing agents; Complexation (129) 19

Pickling baths
Voltammetry; Hydrogen peroxide; For copper alloys; Linear sweep (128) 141

Plants
Anod. stripping voltammetry; Tin; Lead; Copper; Tissue; Sediments; Alternating current; Digesting agent (130) 385

At. abs. spectrometry; Lead; Microsampling-cup procedure; Solid sample (128) 67

At. fluor. spectrometry; Mercury; Gas-sheathed atom cell; Cold-vapour (128) 45

Electrotherm. atomization; At. abs. spectrometry; Molybdenum (123) 325

Emission spectrometry; Flow system; Plasmas; Rocks; Flow injection; Inductively-coupled (130) 243

Flow system; Spectrophotometry; At. abs. spectrometry; Aluminium; Iron; Flow injection; Zone-sampling (125) 37

Flow system; Spectrophotometry; Calcium; Waters; Soils; Merging zones; Flow injection; With glyoxal bis(2-hydroxyanil) (130) 361

Spectrophotometry; Chloride; Displacement of thiocyanate (123) 347

Spectrophotometry; Flow system; Boron; Flow injection; With azomethine-H (125) 29

Spectrophotometry; Sugars; Reducing; Alkaline *p*-hydroxybenzoic acid hydrazide (128) 195

Plasma
Diffl. pulse voltammetry; Chlorpromazine; Thin-layer electrochemical cell; Urine (126) 57

Electrotherm. atomization; At. abs. spectrometry; Aluminium; Serum (128) 57

Fluorimetry; Lecithin-cholesterol acyltransferase; Enzyme reaction (126) 237

Gas chromatography; Paracetamol; Rapid extraction (130) 405

H.p.l.c.; Mitomycin C; Drugs; Human blood; U.v. detection (127) 47

H.p.l.c.; Urine; Drugs; Anti-neoplastic agents (130) 23

Plasmas
Emission spectrometry; Phosphorus; Waters; Inductively coupled (121) 197

Emission spectrometry; Sea water; Trace metals; Extraction; Inductively coupled; Sodium diethyldithiocarbamate into chloroform (121) 331

Emission spectrometry; Programmable monochromator; Data processing; Intensity measurements (122) 75

Emission spectrometry; Serum; Blood; Trace elements; Inductively coupled; Micro-sampling technique; Internal standardization (123) 57

Emission spectrometry; Miniature inductively-coupled; Background radiation; Torch (123) 319

Emission spectrometry; Chloride; Microwave helium; Hydrogen chloride generation (123) 329

Emission spectrometry; Miniature inductively-coupled; Torch (124) 245

Emission spectrometry; Lead; Hydride generation; Inductively-coupled (125) 109

Emission spectrometry; Phosphorus; Waters; Extraction; Sea water; Inductively coupled; Molybdoantimonylphosphoric acid into diisobutyl ketone (127) 93

Emission spectrometry; Trace metals; Sewage; Inductively coupled (130) 157

Emission spectrometry; Flow system; Plants; Rocks; Flow injection; Inductively-coupled (130) 243

Gas chromatography; H.p.l.c.; Emission spectrometry; Element-selective detection; Review (130) 227

Ion exchange; Emission spectrometry; Yttrium; Rare earth elements; Geological materials; Inductively-coupled (124) 421

Plastics
At. abs. spectrometry; Mercury; Milk products; Oxygen bomb; Cold-vapour technique (125) 187

Platinum
X-ray fluores. spectrometry; Iridium; Ion flotation; Separation; Anionic chloro-complexes; With cationic surfactants (123) 1

Plutonium
Coulometry; Digital integrator; Controlled-potential (129) 125

I.r. spectrometry; Carbon; Steels; Carbon dioxide (130) 215

Spectrophotometry; Oxidation with cerium(IV) (124) 449

Pneumatoamperometry
Sulphide; Sulphite (127) 257

Nitrite; Nitrate (130) 391

Point method
Electrochemical digital simulation (122) 331

Polyatomic molecules
Artificial intelligence systems; Molecular spectroscopy (122) 249

Polychlorinated biphenyls
Gas chromatography; Air; Trace organic compounds; Collection on Amberlite XAD-2 resins (124) 31

Polymaleic acid
Diffl. pulse polarography; Copper; Ionisation constants; Stability constants (129) 69

Polymers
Gas chromatography; Chromium; Water-soluble; Trifluoroacetylacetone (127) 223

Polymethylphenols
Spectrophotometry; Micellar systems; Acidity and binding constants in sodium dodecyl sulphate (126) 253

Polynuclear aromatic hydrocarbons
Fluorimetry; Principal component and decomposition analysis; Correlation (122) 171

Fluorimetry; Organic materials; Laser excitation; Narrow vibrational lines; Selective excitation (125) 1

Fluorimetry; Phosphorimetry; Coal liquefaction product; Synchronous luminescence (125) 13

Fluorimetry; T.l.c.; Dry-column chromatography; Standardization of aluminium oxide (126) 225

Gas chromatography; H.p.l.c.; Capillary columns (127) 55

Polyphosphates
Flow system; Spectrophotometry; Flow injection; Complexes of xylenol orange and methylthymol blue (121) 281

Potentiometric stripping analysis
Potentiometry; Amalgamated metals as reducing agents; Selenium; Sulphur; Halides (121) 71

Preparation of standard solutions
Optimization of calculations (123) 303

Progestogens
Diffl. pulse polarography; Removal of interference; Oral contraceptives (127) 135

Programmable monochromator
Emission spectrometry; Plasmas; Data processing; Intensity measurements (122) 75

Proline
Fluorimetry; Amino acids; Secondary; Hydroxyproline; Sarcosine (130) 377

Propranolol-responsive electrodes
Potentiometry; Coated-wire electrode; Didodecylnaphthalene sulfonic acid salt (125) 179

Pseudocholinesterase
Fluorimetry; Alkaline phosphatase; Sorbitol dehydrogenase; Peroxidase; Fluoristic technique (129) 231

Pulsed-stirring voltammetry
Voltammetry; Evaluation (129) 253

Pyridoxal-5'-phosphate
Potentiometry; Ammonia gas-sensing electrode; Coenzyme (126) 35

Q

Quartz
Dusts; X-ray diffractometry; Sample preparation (128) 269

Quenching errors
Fluorimetry; Correction through time resolution (123) 255

R

Rare earth elements
Ion exchange; Emission spectrometry; Yttrium; Geological materials; Plasmas; Inductively-coupled (124) 421

Spectrophotometry; Chlorophosphonazo-*m*-NO_2; Cerium sub-group; In presence of yttrium sub-group (124) 177

Redox reactions, stoichiometry
Spectrophotometry; Equilibrium constants (130) 183

Regression data processing
Kinetic analysis; Lactate dehydrogenase; Subunit determinations; Catalytic reaction (127) 23

Rhodium
Iridium; Ion flotation; Separation (121) 239

Rocks
At. abs. spectrometry; Diffl. pulse voltammetry; Biological materials; Soils; Selenium; Hydride generation; Combustion in oxygen; Cathodic stripping (128) 9

Electrotherm. atomization; At. abs. spectrometry; Lead; Yield monitoring; Extraction with dithizone (125) 203

Emission spectrometry; Flow system; Plasmas; Plants; Flow injection; Inductively-coupled (130) 243

Spectrophotometry; Vanadium; Waters; Adsorption on Sephadex gel; With 4-(2-pyridylazo)resorcinol (130) 345

Rotary sample comparison valve
At. abs. spectrometry; Motor-driven (125) 193

S

Saccharin
Diffl. pulse polarography; A.c. polarography; Electroplating baths; After extraction; Masking with cyanide (124) 237

Sample dispenser
Electronic control (126) 167

Scandium
Fluorimetry; Aluminium; Gallium; Indium; Beryllium; Alkyl derivatives of aromatic Schiff bases (121) 301

Ion exchange; Spectrophotometry; Yttrium; Cerium; Uranium; Xylenol orange complexes; Strong anion exchangers (125) 139

Sea water
Anod. stripping voltammetry; Copper; Negative errors (123) 335

Anod. stripping voltammetry; Electrotherm. atomization; At. abs. spectrometry; Lead; Cadmium; In situ deposition; Mercury-coated graphite tube (124) 121

Anod. stripping voltammetry; Mercury film electrode; Trace metals; Physically-coated (130) 199

At. abs. spectrometry; Ion exchange; Electrotherm. atomization; Trace metals; Extraction; Ammonium pyrrolidinedithiocarbamate/diethylammonium diethyldithiocarbamate into freon; Chelex-100 resin (125) 117

Electrotherm. atomization; At. abs. spectrometry; Lead; Electrolytic accumulation on glassy carbon (124) 143

Emission spectrometry; Plasmas; Trace metals; Extraction; Inductively coupled; Sodium diethyldithiocarbamate into chloroform (121) 331

Emission spectrometry; Phosphorus; Waters; Extraction; Plasmas; Inductively coupled; Molybdoantimonylphosphoric acid into diisobutyl ketone (127) 93

Flow system; Spectrophotometry; Nitrogen; Alkaline peroxodisulphate (130) 55

Gravimetry; Chromium; Chemical speciation; Naturally occurring organic materials (130) 289

Ion exchange; At. abs. spectrometry; Mercury; Waters; Cold-vapour; Anion-exchange resin (130) 149

Potentiometry; Zinc; Cadmium; Lead; Copper; Computerized; Stripping analysis (129) 153

Spectrophotometry; Extraction; Non-ionic surfactants; Waters; Potassium tetrathiocyanatozincate(II); Zinc(II) (123) 355

Sorption on surfaces; Waters; Trace metal losses (127) 181

Chromium; Chemical speciation; Redox processes (130) 401

Sediments
Anod. stripping voltammetry; Tin; Lead; Copper; Plants; Tissue; Alternating current; Digesting agent (130) 385

Gas chromatography; Mass spectrometry; Organic compounds; Computer techniques; Capillary column (129) 57

Ion exchange; Electrotherm. atomization; At. abs. spectrometry; Arsenic species; Marine organisms; Hydride generation; Inorganic and methylated (126) 157

Selenium
Anod. stripping voltammetry; Bovine liver; Gold-plated glassy carbon disk electrode (124) 107

At. abs. spectrometry; Diffl. pulse voltammetry; Biological materials; Rocks; Soils; Hydride

generation; Combustion in oxygen; Cathodic stripping (128) 9

At. abs. spectrometry; Argon-hydrogen flame; Electrochemical preconcentration (130) 257

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Antimony; Extraction; With ammonium pyrrolidinedithiocarbamate-methyl isobutyl ketone (124) 131

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Matrix modification with silver (128) 225

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Antimony; Tellurium; Spectral interferences from phosphate (130) 281

Emission spectrometry; Waters; Molecular emission cavity analysis; After coprecipitation (123) 315

I.r. spectrometry; Oxygen (127) 87

Potentiometry; Kinetic analysis; Catalytic determination; Picrate-selective electrode; Picrate-sulfide reaction (123) 143

Selenocystine
Voltammetry; Cystine; Cysteine; Cathodic stripping (124) 333

Semiconductor silicon
At. fluor. spectrometry; Arsenic; Hydride generation; Depth profiles; Chemical etching; Non-dispersive (125) 101

Diffl. pulse polarography; Phosphorus; Molybdophosphoric acid; Catalytic molybdenum wave (124) 99

Semidifferential electroanalysis
Voltammetry; Stationary solid working electrode (126) 95

Serine-selective membrane
Potentiometry; Immobilized anaerobic bacteria; Ammonia gas sensor (128) 121

Serum
A.c. polarography; Paracetamol; Blood; Urine; Nitro derivative (124) 113

At. abs. spectrometry; Electrotherm. atomization; Gel filtration; Zinc; Copper; Blood; Speciation; Protein-bound; Immunonephelometry (124) 281

Electrotherm. atomization; At. abs. spectrometry; Zinc; Copper (127) 103

Electrotherm. atomization; At. abs. spectrometry; Aluminium; Plasma (128) 57

Electrotherm. atomization; At. abs. spectrometry; Aluminium; Aerosol generation system (128) 221

Electrotherm. atomization; At. abs. spectrometry; Manganese; Urine (129) 205

Emission spectrometry; Plasmas; Blood; Trace elements; Inductively coupled; Micro-sampling technique; Internal standardization (123) 57

Kinetic analysis; Flow system; Copper; Flow injection; Iron(III)-thiosulfate indicator reaction (127) 39

Potentiometry; Guanine enzyme electrode; Ammonia gas sensor; Immobilization (126) 43

Spectrophotometry; Creatine kinase-MB isoenzyme; Blood; Immobilized immunostirrer (124) 169

Spectrophotometry; Sodium; Extraction; Crown ether complexes; Monoanionic dyes; Ion-pair (126) 185

Spectrophotometry; Flow system; Urine; Thiocyanate; Chloramine-T (128) 169

Phenylalanine; Lactate electrode; Immobilized *Leuconostoc mesenteroides* (127) 245

Sewage
Emission spectrometry; Trace metals; Plasmas; Inductively coupled (130) 157

Shale oil products
Activ. analysis; Trace elements (123) 65

Silica
Gravimetry; Silicates; Distillation; Quinoline 12-molybdosilicate (129) 213

Silica-bound complexing agents
I.r. spectrometry; Thermom. analysis; Synthetic aspects; Characterization (129) 29

Copper; Photoacoustic spectroscopy; Complexation (129) 19

Silicate
Spectrophotometry; Waters; Formation of α-molybdosilicic acid and reduction (123) 263

Silicates
Gravimetry; Silica; Distillation; Quinoline 12-molybdosilicate (129) 213

Silicon
At. fluor. spectrometry; Antimony; Hydride generation; Depth profiles; Non-dispersive; Semiconductor (128) 229

Diffl. pulse polarography; Molybdenum; Solar-grade; Molybdate catalytic wave (124) 233

Diffl. pulse polarography; Iron; Solar-grade (126) 247

Silver
Ion exchange; Gold; Chelating resin; Polyhydroxamic acid (123) 309

Simplex optimization
Kinetic analysis; Copper; Numerical model; Catalyzed decomposition of hydrogen peroxide (128) 177

Extraction; Synergic extraction; Copper(II) acetylacetonate into cyclohexane (122) 223

Super-modified procedure; Modifications (122) 421

Single bead string reactor
Flow system; Dispersion process; Flow injection analysis (123) 229

Slurry atomization
Electrotherm. atomization; At. abs. spectrometry; Trace metals; Aluminium oxide (129) 263

Smith predictor controller
Sensitivity to variations in system parameters (122) 403

Snow
Diffl. pulse voltammetry; Anod. stripping voltammetry; Cadmium; Copper; Lead; Zinc; Rotating glassy carbon electrode (121) 39

Sodium
Spectrophotometry; Extraction; Crown ether complexes; Serum; Monoanionic dyes; Ion-pair (126) 185

Sodium chloride solutions
Potentiometry; Titrimetry; Hydroxide; Carbonate; Computer method (122) 81

Sodium contamination, prevention
Mass spectrometry; Surface treatment of borosilicate glass (124) 427

Sodium salts
At. abs. spectrometry; Electrotherm. atomization; Metallic sodium; Volatilization characteristics; Graphite rod (126) 135

At. abs. spectrometry; Electrotherm. atomization; Iron; Nickel; Chromium; Metallic sodium (126) 147

Soils
At. abs. spectrometry; Diffl. pulse voltammetry; Biological materials; Rocks; Selenium; Hydride generation; Combustion in oxygen; Cathodic stripping (128) 9

Flow system; Spectrophotometry; Calcium; Waters; Plants; Merging zones; Flow injection; With glyoxal bis(2-hydroxyanil) (130) 361

Mass spectrometry; Chlorophenoxyphenols; Waters; Extraction; Field desorption (124) 357

Spectrophotometry; Phosphate (128) 257

Sorbic acids
H.p.l.c.; Foods; Benzoic acids; Soft drinks (121) 337

Sorbitol dehydrogenase
Fluorimetry; Alkaline phosphatase; Peroxidase; Pseudocholinesterase; Fluoristic technique (129) 231

Sorption on surfaces
Sea water; Waters; Trace metal losses (127) 181

Spectral-line modulation
Emission spectrometry; Spectral interferences; Flame emission (121) 165

Spin-spin relaxation times
N.m.r. spectrometry; Carbon-13; Spin-echo Fourier transform; Carbon disulfide (124) 415

Stability constants, evaluation
Spectrophotometry; Mono- and poly-nuclear complexes (124) 201

Static mercury drop electrodes
D.c. polarography; Diffl. pulse polarography; Pulse polarography (121) 1

Statistical uncertainties
Potentiometry; Titrimetry; Models, multi-parameter; Multiple regression (122) 291

"Stat" methods
Flow system; Spectrophotometry; Conductometry; Copper; Iron; Iodide; Hydrochloric acid (123) 239

Steels
I.r. spectrometry; Carbon; Plutonium; Carbon dioxide (130) 215

Spectrophotometry; Vanadium; *p*-Sulphobenzeneazo-4-(2,3-dihydroxy-5-chloropyridine) (126) 233

Stripping analysis
Potentiometry; Cadmium; Lead; Copper; Zinc; Biological materials; Computerized (127) 147

Structural analysis
I.r. spectrometry; N.m.r. spectrometry; Mass spectrometry; Dispatching rules; Digital simulation (122) 435

Modelling; Algebraic; Theory of semi-lattices; Boolean equations (122) 257

Structure elucidation
I.r. spectrometry; N.m.r. spectrometry; Organic compounds; Computer program system; New CHEMICS (122) 87

I.r. spectrometry; N.m.r. spectrometry; Organic compounds; CHEMICS program (122) 95

Chemical information system; Development of NIH-EPA system (122) 117

Sugars
Spectrophotometry; Plants; Reducing; Alkaline *p*-hydroxybenzoic acid hydrazide (128) 195

Sulphate
Spectrophotometry; Waters; Reduction (128) 263

Sulphide
Flow system; Spectrophotometry; Flow injection; Methylene blue method (128) 163

Pneumatoamperometry; Sulphite (127) 257

Sulphite
Pneumatoamperometry; Sulphide (127) 257

Sulphonamides
Titrimetry; Cationic micellar systems (128) 273

Sulphur
Emission spectrometry; Molecular emission cavity analysis; Variation in cavity position; Phosphate addition (121) 175

H.p.l.c.; Ion chromatography; Oil, fuel; Oxygen flask (121) 233

Sulphur dioxide
Flow system; Titrimetry; Pyridinium bromide perbromide; Flow injection (123) 351

Surfactants
Potentiometry; Titrimetry; Coated-wire electrode; Ion-pair formation; Cationic and anionic (127) 165

T

Target factor analysis
Gas chromatography; Theory of error (122) 327

Tellurium
At. abs. spectrometry; Electrotherm. atomization; Bismuth; Copper alloys; Lead; Solid samples; Induction furnace (125) 93

Electrotherm. atomization; At. abs. spectrometry; Thermal stabilization; Inorganic and organic (128) 23

Electrotherm. atomization; At. abs. spectrometry; Arsenic; Antimony; Selenium; Spectral interferences from phosphate (130) 281

Polarography; Hanging and dropping mercury electrodes; Adsorbed monolayer (121) 315

Tensammetry
A.c. polarography; Ethanol; In vodka (126) 213

Tetraethyllead
Gas chromatography; Mass spectrometry; Isotope dilut. analysis; Tetramethyllead; Air; Two-step enrichment method; Single ion monitoring (124) 1

Tetramethylammonium and tetraethylammonium ions
Potentiometry; Liquid membrane electrodes; Quaternary ammonium tetrakis(m-trifluoromethylphenyl)borates (126) 217

Tetramethyllead
Gas chromatography; Mass spectrometry; Isotope dilut. analysis; Tetraethyllead; Air; Two-step enrichment method; Single ion monitoring (124) 1

Thallium
Anod. stripping voltammetry; Lead; Cadmium; Influence of surfactant in perfluorinated emulsion; Differential pulse (127) 233

At. abs. spectrometry; Cadmium; Copper; Lead; Liver tissue; Kidney; Enzymatic digestion (125) 209

Kinetic analysis; Fluorimetry; Iron; Oxidation of 1,4-diamino-2,3-dihydroanthraquinone (130) 337

Theory of error
Gas chromatography; Target factor analysis (122) 327

Thermal lens effect
Spectrophotometry; Extraction; Iron; Ion-pair; With 4,7-diphenyl-1,10-phenanthroline disulfonic acid (124) 381

Thin-layer electrochemical cell
Diffl. pulse voltammetry; Chlorpromazine; Plasma; Urine (126) 57

Thiocyanate
Spectrophotometry; Flow system; Serum; Urine; Chloramine-T (128) 169

Voltammetry; Copper ions; Cathodic stripping; Application (123) 201

Thiocyanate amplification
Titrimetry; Lead; Precipitation as lead hexathiocyanatochromate(III) (125) 215

Thioethers
Titrimetry; Potentiometry; With mercury(II) perchlorate (121) 321

Thiourea
Cyclic voltammetry; Coulometry; Mechanism of electrochemical oxidation (123) 83

Thyronine derivatives
Fluorimetry; Tyrosine derivatives; *o*-Phthalaldehyde derivatives of iodinated amino acids (124) 221

Time resolution of interferences
At. abs. spectrometry; Electrotherm. atomization; Graphite tube surfaces; Matrix components (125) 183

Tin
Anod. stripping voltammetry; Presence of lead; Rotating mercury-film disc-ring electrode (127) 251

Anod. stripping voltammetry; Lead; Copper; Plants; Tissue; Sediments; Alternating current; Digesting agent (130) 385

At. abs. spectrometry; Spectrophotometry; Organotin compounds; Substoichiometric determination; Complexation with salicylideneamino-2-thiophenol (126) 199

Polarography; Stabilizing agents; Antioxidant (130) 133

Tissue
Activ. analysis; Zirconium (128) 213

Anod. stripping voltammetry; Tin; Lead; Copper; Plants; Sediments; Alternating current; Digesting agent (130) 385

Titanium
Activ. analysis; Calcium; From ^{47}Sc (121) 327

Emission spectrometry; Inhibition release titration; Calcium atomization (129) 259

Tobacco products
Potentiometry; Nicotine-sensitive electrode; Liquid membrane (127) 173

Toluene diisocyanate
Piezoelectric sensing; Air; Coating materials (129) 163

Piezoelectric sensing; Air; Personal monitoring (129) 175

Trace anions
X-ray fluores. spectrometry; Ion exchange; Waters; 2,2'-Diaminodiethylamine cellulose filters (123) 9

Trace elements
Activ. analysis; Glass; Instrumental photon activation (121) 157

Activ. analysis; Shale oil products (123) 65

Electrotherm. atomization; At. abs. spectrometry; Hair; Washing procedures (125) 131

Emission spectrometry; Plasmas; Serum; Blood; Inductively coupled; Micro-sampling technique; Internal standardization (123) 57

Trace metals
Anod. stripping voltammetry; Different convection rates; Subtractive (128) 147

Anod. stripping voltammetry; Mercury film electrode; Sea water; Physically-coated (130) 199

At. abs. spectrometry; Electrotherm. atomization; Hair; Flame; Cold-vapor (123) 45

At. abs. spectrometry; Ion exchange; Electrotherm. atomization; Sea water; Extraction; Ammonium pyrrolidinedithiocarbamate/diethylammonium diethyldithiocarbamate into freon; Chelex-100 resin (125) 117

Electrotherm. atomization; At. abs. spectrometry; Aluminium oxide; Slurry atomization (129) 263

Emission spectrometry; Sea water; Plasmas; Extraction; Inductively coupled; Sodium diethyldithiocarbamate into chloroform (121) 331

Emission spectrometry; Sewage; Plasmas; Inductively coupled (130) 157

Ion exchange; 8-Hydroxyquinoline-5-sulfonic acid; Anionic complexes (123) 19

X-ray fluores. spectrometry; Urine; Energy-dispersive (130) 167

Trace organic compounds
Gas chromatography; Polychlorinated biphenyls; Air; Collection on Amberlite XAD-2 resins (124) 31

Trifluoroacetophenone
Polarography; Reaction with ammonia (126) 259

Tris(nonylphenyl) phosphite
H.p.l.c.; Butadiene-styrene copolymers; Nonylphenol; Ultraviolet detector (129) 243

Tungsten
E.s.r.; Paramagnetic resonance; Tungsten(V)-thiocyanate complex (124) 211

X-ray fluores. spectrometry; Vanadium; Molybdenum; Brines; Chloralkali electrolysis (128) 207

Tungsten compounds
At. abs. spectrometry; Industrial hygiene samples; Soluble and insoluble; Nitrous oxide-acetylene flame (121) 187

Tungsten-tube
Electrotherm. atomization; At. abs. spectrometry; Design and performance (127) 109

Tyrosine derivatives
Fluorimetry; Thyronine derivatives; *o*-Phthalaldehyde derivatives of iodinated amino acids (124) 221

U

Uranium
Coulometry; Constant-current (129) 113

Ion exchange; Spectrophotometry; Scandium; Yttrium; Cerium; Xylenol orange complexes; Strong anion exchangers (125) 139

Ion exchange; H.p.l.c.; Molybdenum; Controlled-pore glass; Immobilized diamine (126) 117

Isotope dilut. analysis; Extraction; Substoichiometric; Synergic system (129) 221

Spectrophotometry; Flow system; Extraction; With 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (121) 341

Urea
Flow system; Spectrophotometry; Potentiometry; Glucose; Enzyme reactor; On-line dialysis (130) 45

Urine
A.c. polarography; Paracetamol; Blood; Serum; Nitro derivative (124) 113

Diffl. pulse voltammetry; Chlorpromazine; Thin-layer electrochemical cell; Plasma (126) 57

Electrotherm. atomization; At. abs. spectrometry; Manganese; Serum (129) 205

Electrotherm. atomization; At. abs. spectrometry; Cadmium; Matrix modification (130) 209

H.p.l.c.; Plasma; Drugs; Anti-neoplastic agents (130) 23

Potentiometry; Cadmium; Lead; Computerized; Stripping analysis (128) 155

Spectrophotometry; Flow system; Serum; Thiocyanate; Chloramine-T (128) 169

Spectrophotometry; Gas chromatography; Extraction; Oxalic acid; Ion-pair formation (129) 189

X-ray fluores. spectrometry; Trace metals; Energy-dispersive (130) 167

V

Vanadium
Cyclic voltammetry; Kinetic analysis; Redox catalyst in non-aqueous media; V(V)/V(IV) couple (126) 65

Gas chromatography; Petroleum oils; Fluorinated β-diketonates (128) 101

Gas chromatography; Petroleum oils; Copper; Nickel; Extraction of chelate (128) 109

Spectrophotometry; Niobium; Extraction; From succinate solution; Using tri-n-octylamine (125) 165

Spectrophotometry; Steels; *p*-Sulphobenzene-azo-4-(2,3-dihydroxy-5-chloropyridine) (126) 233

Spectrophotometry; Waters; Rocks; Adsorption on Sephadex gel; With 4-(2-pyridylazo)resorcinol (130) 345

Spectrophotometry; Air particulates; Extraction; With *N*-benzoyl-*N*-phenylhydroxylamine (130) 353

X-ray fluores. spectrometry; Molybdenum; Tungsten; Brines; Chloralkali electrolysis (128) 207

Vibrational spectra
Computer-aided spectra interpretation (122) 357

Vitamin K_1
Diffl. pulse polarography; Cyclic voltammetry; Hanging mercury electrode (128) 245

Vitamin K_3
Amperometry; Spectrophotometry; H.p.l.c.; Detectors (125) 85

Vitamins
H.p.l.c.; Cod liver oil; Fat-soluble; Formulated feed; Stopped-flow scanning (127) 213

Volatile organics determination
Gas chromatography; Mass spectrometry; Purge vessel design (121) 345

W

Waters
At. abs. spectrometry; Mercury; Decomposition of organic mercury; Bromine monochloride; Cold-vapour (121) 219

D.c. polarography; Humic substances; Potable; Tri-n-butylphosphate layer (121) 309

Emission spectrometry; Phosphorus; Plasmas; Inductively coupled (121) 197

Emission spectrometry; Chlorine; Chemiluminescence; Hydrogen peroxide-hypochlorite reaction; Tap water; Interferences (123) 247

Emission spectrometry; Selenium; Molecular emission cavity analysis; After coprecipitation (123) 315

Emission spectrometry; Humic acid; Chemiluminescence; With permanganate (124) 23

Emission spectrometry; Phosphorus; Extraction; Plasmas; Sea water; Inductively coupled; Molybdoantimonylphosphoric acid into diisobutyl ketone (127) 93

Flow system; Spectrophotometry; Nitrate; Flow injection; Hydrazine reduction; Rain (124) 437

Flow system; Spectrophotometry; Calcium; Soils; Plants; Merging zones; Flow injection; With glyoxal bis(2-hydroxyanil) (130) 361

Gas chromatography; Isopropylmethyl-phosphonofluoridate; Preconcentration; Direct thermal desorption (124) 225

H.p.l.c.; Anions; Ion chromatography; Selective detectors (130) 1

Ion exchange; At. abs. spectrometry; Mercury; Sea water; Cold-vapour; Anion-exchange resin (130) 149

Mass spectrometry; Chlorophenoxyphenols; Soils; Extraction; Field desorption (124) 357

Piezoelectric sensing; Mercury; Gold-coated (130) 141

Potentiometry; Mercury; Enzyme reactor; On-site samping; Immobilized urease; Ammonia electrode (125) 45

Spectrophotometry; Silicate; Formation of α-molybdosilicic acid and reduction (123) 263

Spectrophotometry; Extraction; Non-ionic surfactants; Sea water; Potassium tetrathiocyanatozincate(II); Zinc(II) (123) 355

Spectrophotometry; Sulphate; Reduction (128) 263

Spectrophotometry; Vanadium; Rocks; Adsorption on Sephadex gel; With 4-(2-pyridylazo)resorcinol (130) 345

X-ray fluores. spectrometry; Ion exchange; Trace anions; 2,2'-Diaminodiethylamine cellulose filters (123) 9

X-ray fluores. spectrometry; Chromium; Speciation; Membrane filters (124) 351

Sea water; Sorption on surfaces; Trace metal losses (127) 181

Wear metals
At. fluor. spectrometry; Electrotherm. atomization; Oil; Wavelength-modulated, continuum-source excited; Jet engine lubricating; Filament in a flame (124) 155

Wine
Emission spectrometry; Gas chromatography; Correlation; Glass-capillary; Stepwise regression analysis; Subjective sensory evaluations (122) 215

X

X-ray diffractometry
Quartz; Dusts; Sample preparation (128) 269

Y

Yield monitoring
Electrotherm. atomization; At. abs. spectrometry; Lead; Rocks; Extraction with dithizone (125) 203

Yttrium
Ion exchange; Emission spectrometry; Rare earth elements; Geological materials; Plasmas; Inductively-coupled (124) 421

Ion exchange; Spectrophotometry; Scandium; Cerium; Uranium; Xylenol orange complexes; Strong anion exchangers (125) 139

Z

Zinc
At. abs. spectrometry; Electrotherm. atomization; Gel filtration; Copper; Blood; Serum; Speciation; Protein-bound; Immuno-nephelometry (124) 281

Diffl. pulse voltammetry; Anod. stripping voltammetry; Cadmium; Copper; Lead; Snow; Rotating glassy carbon electrode (121) 39

Electrotherm. atomization; At. abs. spectrometry; Copper; Serum (127) 103

Flow system; Emission spectrometry; Ion exchange; Cadmium; Chemiluminescence; Flow injection (127) 199

Ion exchange; Cadmium; Hexacyanoferrate(II) salts on gel and macroporous resins; In situ precipitation (124) 365

Potentiometry; Stripping analysis; Cadmium; Lead; Copper; Biological materials; Computerized (127) 147

Potentiometry; Cadmium; Lead; Copper; Sea water; Computerized; Stripping analysis (129) 153

Extraction; Diethyldithiocarbamates; Cobalt; Iron; Liquid-liquid (121) 249

Copper; Hair; Wash solutions (124) 409

Zirconium
Activ. analysis; Tissue (128) 213

Zone-sampling
Spectrophotometry; Flow system; At. abs. spectrometry; Potassium in plant digests (123) 221

TECHNIQUE INDEX

VOLUMES 121—130

The volume number is placed in parentheses before the page number

Activation analysis

Boron; In zirconium and zircaloy; Proton activation; Anion-exchange (121) 147

Trace elements; Glass; Instrumental photon activation (121) 157

Calcium; Titanium; From ^{47}Sc (121) 327

Shale oil products; Trace elements (123) 65

Boron shield; Choice; Epithermal neutron (124) 373

Zirconium; Tissue (128) 213

A.c. polarography

Paracetamol; Blood; Serum; Urine; Nitro derivative (124) 113

Diffl. pulse polarography; Saccharin; Electroplating baths; After extraction; Masking with cyanide (124) 237

Ethanol; Tensammetry; In vodka (126) 213

Amperometry

Flow system; Benzoquinone; Renewable stationary mercury electrode; Detector (123) 107

Flow system; Tubular electrodes; Flow-injection; Dispersion of mass injected (124) 303

Flow system; Electrochemical detector; Kel-F-graphite electrode (124) 321

Spectrophotometry; H.p.l.c.; Vitamin K_3; Detectors (125) 85

Enzyme membranes; Galactose; Cholesterol; Maltose; Bound to collagen films; Glucose electrode (126) 23

Flow system; Electrochemical detector; Theoretical treatment; Open tubular electrode (127) 157

Kinetic analysis; Nitrate; Electrocatalysis of reduction; By electrodeposited copper (129) 87

H.p.l.c.; Nitrate; Flow-through electrode (129) 101

Coulometry; Titrimetry; Manganese electrode; EDTA (130) 329

Anodic stripping voltammetry

Diffl. pulse voltammetry; Cadmium; Copper; Lead; Zinc; Snow; Rotating glassy carbon electrode (121) 39

Voltammetry; Metal complexes; Convolution techniques (123) 187

Copper; Sea water; Negative errors (123) 335

Selenium; Bovine liver; Gold-plated glassy carbon disk electrode (124) 107

Electrotherm. atomization; At. abs. spectrometry; Lead; Cadmium; Sea water; In situ deposition; Mercury-coated graphite tube (124) 121

Thallium; Lead; Cadmium; Influence of surfactant in perfluorinated emulsion; Differential pulse (127) 233

Tin; Presence of lead; Rotating mercury-film disc-ring electrode (127) 251

Trace metals; Different convection rates; Subtractive (128) 147

Mercury film electrode; Trace metals; Sea water; Physically-coated (130) 199

Cadmium; Mercury film carbon fibre electrode; Differential pulse (130) 323

Tin; Lead; Copper; Plants; Tissue; Sediments; Alternating current; Digesting agent (130) 385

Atomic absorption spectrometry

Tungsten compounds; Industrial hygiene samples; Soluble and insoluble; Nitrous oxide-acetylene flame (121) 187

Mercury; Waters; Decomposition of organic mercury; Bromine monochloride; Cold-vapour (121) 219

Electrotherm. atomization; Trace metals; Hair; Flame; Cold-vapor (123) 45

Spectrophotometry; Flow system; Zone-sampling; Potassium in plant digests (123) 221

Electrotherm. atomization; Molybdenum; Plants (123) 325

Anod. stripping voltammetry; Electrotherm. atomization; Lead; Cadmium; Sea water; In

situ deposition; Mercury-coated graphite tube (124) 121

Electrotherm. atomization; Arsenic; Antimony; Selenium; Extraction; With ammonium pyrrolidinedithiocarbamate-methyl isobutyl ketone (124) 131

Electrotherm. atomization; Lead; Sea water; Electrolytic accumulation on glassy carbon (124) 143

Electrotherm. atomization; Chromium; Removal of chloride interference (124) 163

Controlled temperature-gradient lamps; Performance and application (124) 259

Electrotherm. atomization; Gel filtration; Zinc; Copper; Blood; Serum; Speciation; Protein-bound; Immunonephelometry (124) 281

Inhibition release titration; Magnesium; Phosphate (124) 295

Flow system; Spectrophotometry; Aluminium; Iron; Plants; Flow injection; Zone-sampling (125) 37

Electrotherm. atomization; Bismuth; Tellurium; Copper alloys; Lead; Solid samples; Induction furnace (125) 93

Ion exchange; Electrotherm. atomization; Trace metals; Sea water; Extraction; Ammonium pyrrolidinedithiocarbamate/ diethylammonium diethyldithiocarbamate into freon; Chelex-100 resin (125) 117

Electrotherm. atomization; Trace elements; Hair; Washing procedures (125) 131

Electrotherm. atomization; Time resolution of interferences; Graphite tube surfaces; Matrix components (125) 183

Mercury; Milk products; Plastics; Oxygen bomb; Cold-vapour technique (125) 187

Rotary sample comparison valve; Motor-driven (125) 193

Flow system; Automated system; Extraction (125) 199

Electrotherm. atomization; Lead; Rocks; Yield monitoring; Extraction with dithizone (125) 203

Cadmium; Copper; Lead; Thallium; Liver tissue; Kidney; Enzymatic digestion (125) 209

Electrotherm. atomization; Sodium salts; Metallic sodium; Volatilization characteristics; Graphite rod (126) 135

Electrotherm. atomization; Iron; Nickel; Chromium; Sodium salts; Metallic sodium (126) 147

Ion exchange; Electrotherm. atomization; Arsenic species; Marine organisms; Sediments; Hydride generation; Inorganic and methylated (126) 157

Adsorption of ions on surfaces; Calcium; Magnesium (126) 175

Spectrophotometry; Tin; Organotin compounds; Substoichiometric determination; Complexation with salicylideneamino-2-thiophenol (126) 199

Electrotherm. atomization; Iodide; Use of mercury (126) 241

Electrotherm. atomization; Zinc; Copper; Serum (127) 103

Electrotherm. atomization; Tungsten-tube; Design and performance (127) 109

Cadmium; Extraction; Masking zinc; With ethylxanthate into chloroform (127) 209

Electrotherm. atomization; Atomization with controlled atmosphere and temperature; Volatile elements; Tungsten wire; Quartz tube (128) 1

Diffl. pulse voltammetry; Biological materials; Rocks; Soils; Selenium; Hydride generation; Combustion in oxygen; Cathodic stripping (128) 9

Electrotherm. atomization; Tellurium; Thermal stabilization; Inorganic and organic (128) 23

Electrotherm. atomization; Background correction; Line absorption of matrix; Tables of bandwidths (128) 31

Electrotherm. atomization; Aluminium; Plasma; Serum (128) 57

Lead; Microsampling-cup procedure; Plants; Solid sample (128) 67

Electrotherm. atomization; Aluminium; Serum; Aerosol generation system (128) 221

Electrotherm. atomization; Arsenic; Selenium; Matrix modification with silver (128) 225

Electrotherm. atomization; Manganese; Serum; Urine (129) 205

Electrotherm. atomization; Trace metals; Aluminium oxide; Slurry atomization (129) 263

Ion exchange; Mercury; Waters; Sea water; Cold-vapour; Anion-exchange resin (130) 149

Electrotherm. atomization; Copper; Iron; Aluminium salts; Involatile matrices (130) 203

Electrotherm. atomization; Cadmium; Urine; Matrix modification (130) 209

Selenium; Argon-hydrogen flame; Electrochemical preconcentration (130) 257

Electrotherm. atomization; Arsenic; Surface reactions; High-temperature equilibrium calculations; Platform technique (130) 267

Electrotherm. atomization; Arsenic; Antimony; Selenium; Tellurium; Spectral interferences from phosphate (130) 281

Calcium; Oil, lubricating; Ionization suppressor (130) 395

Atomic fluorescence spectrometry
Electrotherm. atomization; Wear metals; Oil; Wavelength-modulated, continuum-source excited; Jet engine lubricating; Filament in a flame (124) 155

Arsenic; Semiconductor silicon; Hydride generation; Depth profiles; Chemical etching; Non-dispersive (125) 101

Mercury; Gas-sheathed atom cell; Plants; Cold-vapour (128) 45

Antimony; Silicon; Hydride generation; Depth profiles; Non-dispersive; Semiconductor (128) 229

Chromatography
see also Gas chromatography, Gel permeation chromatography, High-performance liquid chromatography, Thin-layer chromatography

Modelling; Computer implementation; Simulation; Linear or non-linear isotherms (122) 1

Modelling; Computer implementation; Simulation; Mass transport; Non-linear isotherm (122) 151

Conductometry
Flow system; Spectrophotometry; "Stat" methods; Copper; Iron; Iodide; Hydrochloric acid (123) 239

Dissociation constant; Equivalent conductance; Weak electrolyte (125) 219

Coulometry
Cyclic voltammetry; Thiourea; Mechanism of electrochemical oxidation (123) 83

Titrimetry; Aniline; End-point anticipation; Electronically controlled dual-intermediate titrator (124) 85

Flow system; Tubular electrodes; Flow-injection; Equation (124) 315

Voltammetry; Flow system; Manganese; Cathodic stripping (126) 15

Uranium; Constant-current (129) 113

Digital integrator; Plutonium; Controlled-potential (129) 125

Titrimetry; Amperometry; Manganese electrode; EDTA (130) 329

Cyclic voltammetry
Coulometry; Thiourea; Mechanism of electrochemical oxidation (123) 83

Kinetic analysis; Vanadium; Redox catalyst in non-aqueous media; V(V)/V(IV) couple (126) 65

D.c. polarography; Diffl. pulse polarography; Benzylpenicilloic acid (128) 135

Diffl. pulse polarography; Vitamin K_1; Hanging mercury electrode (128) 245

Diffl. pulse polarography; Dihydroxytartaric acid; Osazone; Tautomeric equilibrium (129) 133

Differential pulse polarography
D.c. polarography; Pulse polarography; Static mercury drop electrodes (121) 1

Extraction; Flurazepam; Metabolites (123) 175

Phosphorus; Semiconductor silicon; Molybdophosphoric acid; Catalytic molybdenum wave (124) 99

Molybdenum; Silicon; Solar-grade; Molybdate catalytic wave (124) 233

A.c. polarography; Saccharin; Electroplating baths; After extraction; Masking with cyanide (124) 237

Iron; Silicon; Solar-grade (126) 247

Progestogens; Removal of interference; Oral contraceptives (127) 135

D.c. polarography; Cyclic voltammetry; Benzylpenicilloic acid (128) 135

Cyclic voltammetry; Vitamin K_1; Hanging mercury electrode (128) 245

Polymaleic acid; Copper; Ionisation constants; Stability constants (129) 69

Cyclic voltammetry; Dihydroxytartaric acid; Osazone; Tautomeric equilibrium (129) 133

Dihydroxytartaric acid; Osazone; Kinetic measurements (129) 145

Differential pulse voltammetry
Anod. stripping voltammetry; Cadmium; Copper; Lead; Zinc; Snow; Rotating glassy carbon electrode (121) 39

Chlorpromazine; Thin-layer electrochemical cell; Plasma; Urine (126) 57

At. abs. spectrometry; Biological materials; Rocks; Soils; Selenium; Hydride generation; Combustion in oxygen; Cathodic stripping (128) 9

D.c. polarography
Diffl. pulse polarography; Pulse polarography; Static mercury drop electrodes (121) 1

Humic substances; Waters; Potable; Tri-n-butylphosphate layer (121) 309

Computer control; 2-Hydroxypropanoate complexes; Stability constants; Lead(II) propanoate (122) 395

Diffl. pulse polarography; Cyclic voltammetry; Benzylpenicilloic acid (128) 135

Electron spin resonance
Flow system; Simple flow cell (123) 339

Tungsten; Paramagnetic resonance; Tungsten(V)-thiocyanate complex (124) 211

Metals; Stable free-radical complexing reagents; Spin-labelled iminooxime (128) 85

Electrothermal atomization

Chloride; Molecular absorption spectrometry (121) 205

At. abs. spectrometry; Trace metals; Hair; Flame; Cold-vapor (123) 45

At. abs. spectrometry; Molybdenum; Plants (123) 325

Anod. stripping voltammetry; At. abs. spectrometry; Lead; Cadmium; Sea water; In situ deposition; Mercury-coated graphite tube (124) 121

At. abs. spectrometry; Arsenic; Antimony; Selenium; Extraction; With ammonium pyrrolidinedithiocarbamate-methyl isobutyl ketone (124) 131

At. abs. spectrometry; Lead; Sea water; Electrolytic accumulation on glassy carbon (124) 143

At. fluor. spectrometry; Wear metals; Oil; Wavelength-modulated, continuum-source excited; Jet engine lubricating; Filament in a flame (124) 155

At. abs. spectrometry; Chromium; Removal of chloride interference (124) 163

At. abs. spectrometry; Gel filtration; Zinc; Copper; Blood; Serum; Speciation; Protein-bound; Immunonephelometry (124) 281

At. abs. spectrometry; Bismuth; Tellurium; Copper alloys; Lead; Solid samples; Induction furnace (125) 93

At. abs. spectrometry; Ion exchange; Trace metals; Sea water; Extraction; Ammonium pyrrolidinedithiocarbamate/diethylammonium diethyldithiocarbamate into freon; Chelex-100 resin (125) 117

At. abs. spectrometry; Trace elements; Hair; Washing procedures (125) 131

At. abs. spectrometry; Time resolution of interferences; Graphite tube surfaces; Matrix components (125) 183

At. abs. spectrometry; Lead; Rocks; Yield monitoring; Extraction with dithizone (125) 203

At. abs. spectrometry; Sodium salts; Metallic sodium; Volatilization characteristics; Graphite rod (126) 135

At. abs. spectrometry; Iron; Nickel; Chromium; Sodium salts; Metallic sodium (126) 147

Ion exchange; At. abs. spectrometry; Arsenic species; Marine organisms; Sediments; Hydride generation; Inorganic and methylated (126) 157

At. abs. spectrometry; Iodide; Use of mercury (126) 241

At. abs. spectrometry; Zinc; Copper; Serum (127) 103

At. abs. spectrometry; Tungsten-tube; Design and performance (127) 109

At. abs. spectrometry; Atomization with controlled atmosphere and temperature; Volatile elements; Tungsten wire; Quartz tube (128) 1

At. abs. spectrometry; Tellurium; Thermal stabilization; Inorganic and organic (128) 23

At. abs. spectrometry; Background correction; Line absorption of matrix; Tables of bandwidths (128) 31

At. abs. spectrometry; Aluminium; Plasma; Serum (128) 57

At. abs. spectrometry; Aluminium; Serum; Aerosol generation system (128) 221

At. abs. spectrometry; Arsenic; Selenium; Matrix modification with silver (128) 225

At. abs. spectrometry; Manganese; Serum; Urine (129) 205

At. abs. spectrometry; Trace metals; Aluminium oxide; Slurry atomization (129) 263

At. abs. spectrometry; Copper; Iron; Aluminium salts; Involatile matrices (130) 203

At. abs. spectrometry; Cadmium; Urine; Matrix modification (130) 209

At. abs. spectrometry; Arsenic; Surface reactions; High-temperature equilibrium calculations; Platform technique (130) 267

At. abs. spectrometry; Arsenic; Antimony; Selenium; Tellurium; Spectral interferences from phosphate (130) 281

Emission spectrometry

Spectral-line modulation; Spectral interferences; Flame emission (121) 165

Sulphur; Molecular emission cavity analysis; Variation in cavity position; Phosphate addition (121) 175

Phosphorus; Waters; Plasmas; Inductively coupled (121) 197

Sea water; Plasmas; Trace metals; Extraction; Inductively coupled; Sodium diethyldithiocarbamate into chloroform (121) 331

Programmable monochromator; Plasmas; Data processing; Intensity measurements (122) 75

Gas chromatography; Correlation; Wine; Glass-capillary; Stepwise regression analysis; Subjective sensory evaluations (122) 215

Plasmas; Serum; Blood; Trace elements; Inductively coupled; Micro-sampling technique; Internal standardization (123) 57

Chlorine; Waters; Chemiluminescence; Hydrogen peroxide-hypochlorite reaction; Tap water; Interferences (123) 247

Waters; Selenium; Molecular emission cavity analysis; After coprecipitation (123) 315

Plasmas; Miniature inductively-coupled; Background radiation; Torch (123) 319

Chloride; Plasmas; Microwave helium; Hydrogen chloride generation (123) 329

Hydrocarbons; Air; Chemiluminescence; Reaction with oxygen atoms (124) 15

Humic acid; Chemiluminescence; Waters; With permanganate (124) 23

Plasmas; Miniature inductively-coupled; Torch (124) 245

Irons; Glow lamp; Carbon; Sputtering and emission intensity; White, gray and malleable cast (124) 267

Copper alloys; Glow lamp; Aluminium; Sputtering and emission intensity (124) 275

Ion exchange; Yttrium; Rare earth elements; Geological materials; Plasmas; Inductively-coupled (124) 421

Lead; Plasmas; Hydride generation; Inductively-coupled (125) 109

Gas chromatography; Nitrosamines; Electronic noise filter; Chemiluminescent detector (126) 125

Multichannel detection system; Laser microprobe; Imaging detectors (127) 71

Phosphorus; Waters; Extraction; Plasmas; Sea water; Inductively coupled; Molybdoantimonyl-phosphoric acid into diisobutyl ketone (127) 93

Flow system; Ion exchange; Zinc; Cadmium; Chemiluminescence; Flow injection (127) 199

Molecular emission cavity analysis; Boron; Improved procedure (127) 227

Molecular emission cavity analysis; Amines; Indirect determination; Formaldehyde-sulphite addition compound (128) 75

Titanium; Inhibition release titration; Calcium atomization (129) 259

Trace metals; Sewage; Plasmas; Inductively coupled (130) 157

Gas chromatography; H.p.l.c.; Element-selective detection; Plasmas; Review (130) 227

Flow system; Plasmas; Plants; Rocks; Flow injection; Inductively-coupled (130) 243

Chemiluminescence; Electrogenerated; Lucigenin (130) 415

Flow system

Voltammetry; Limiting current in electrochemical flow-through cells; Survey of the equations; Tubular, thin-layer, wall-jet and disk electrodes (121) 13

Oxygen, continuous removal; Semipermeable membrane (121) 23

Voltammetry; Drugs; 2-Thiobarbituric acid; Thioamide; Mercury-coated glassy carbon electrode (121) 51

Potentiometry; Glucose oxidase; Gas-sensing sulphur dioxide probe (121) 61

Kinetic analysis; Thermom. analysis; Iodide; Catalyzed cerium(IV)-arsenic(III) reaction (121) 135

Spectrophotometry; Polyphosphates; Flow injection; Complexes of xylenol orange and methylthymol blue (121) 281

Spectrophotometry; Uranium; Extraction; With 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (121) 341

Amperometry; Benzoquinone; Renewable stationary mercury electrode; Detector (123) 107

Polarography; Isosorbide dinitrate; Flow injection analysis (123) 115

Voltammetry; Immobilized alcohol oxidase; Ethanol; Oxygen electrode; Blood alcohol (123) 125

Spectrophotometry; At. abs. spectrometry; Zone-sampling; Potassium in plant digests (123) 221

Single bead string reactor; Dispersion process; Flow injection analysis (123) 229

Spectrophotometry; Conductometry; "Stat" methods; Copper; Iron; Iodide; Hydrochloric acid (123) 239

E.s.r.; Simple flow cell (123) 339

Titrimetry; Sulphur dioxide; Pyridinium bromide perbromide; Flow injection (123) 351

Kinetic analysis; Titrimetry; Gradient chamber; Kinetic treatment; Flow-injection (124) 39

Kinetic analysis; Titrimetry; Gradient chamber; Kinetic treatment; Flow-injection (124) 65

Amperometry; Tubular electrodes; Flow-injection; Dispersion of mass injected (124) 303

Coulometry; Tubular electrodes; Flow-injection; Equation (124) 315

Amperometry; Electrochemical detector; Kel-F-graphite electrode (124) 321

Spectrophotometry; Nitrate; Waters; Flow injection; Hydrazine reduction; Rain (124) 437

Spectrophotometry; Boron; Plants; Flow injection; With azomethine-H (125) 29

Spectrophotometry; At. abs. spectrometry; Aluminium; Iron; Plants; Flow injection; Zone-sampling (125) 37

Potentiometry; Kinetic analysis; Enzymatic analysis; Glucose; Sulphur dioxide probe; Glucose oxidase; Oxidation of hydrogensulphite (125) 55

At. abs. spectrometry; Automated system; Extraction (125) 199

Dispersion phenomena; Transport; Flow injection; Single bead string reactor (126) 1

Coulometry; Voltammetry; Manganese; Cathodic stripping (126) 15

Kinetic analysis; Copper; Serum; Flow injection; Iron(III)-thiosulfate indicator reaction (127) 39

Voltammetry; High flow-rate cells; Static mercury drop electrode (127) 121

Amperometry; Electrochemical detector; Theoretical treatment; Open tubular electrode (127) 157

Emission spectrometry; Ion exchange; Zinc; Cadmium; Chemiluminescence; Flow injection (127) 199

Voltammetry; Peristaltic pump pulsation; Manganese; Synchronization of signal sampling; Cathodic stripping (127) 239

Spectrophotometry; Sulphide; Flow injection; Methylene blue method (128) 163

Spectrophotometry; Serum; Urine; Thiocyanate; Chloramine-T (128) 169

Titrimetry; Theory; High-speed; Flow injection (129) 1

Fluorimetry; Gallium; Extraction; Flow injection; With lumogallion; Laser excitation (129) 195

H.p.l.c.; Electrochemical reactivation; For solid electrodes (130) 9

Spectrophotometry; Potentiometry; Glucose; Urea; Enzyme reactor; On-line dialysis (130) 45

Spectrophotometry; Nitrogen; Sea water; Alkaline peroxodisulphate (130) 55

Spectrophotometry; Kinetic analysis; Cobalt; Catalysis of SPADNS oxidation; Flow injection (130) 65

Emission spectrometry; Plasmas; Plants; Rocks; Flow injection; Inductively-coupled (130) 243

Spectrophotometry; Calcium; Waters; Soils; Plants; Merging zones; Flow injection; With glyoxal bis(2-hydroxyanil) (130) 361

Spectrophotometry; Chloride; Ethanol; Flow injection (130) 409

Fluorimetry

Gallium; With benzyl-2-pyridylketone-2-pyridylhydrazone (121) 295

Aluminium; Gallium; Indium; Scandium; Beryllium; Alkyl derivatives of aromatic Schiff bases (121) 301

Polynuclear aromatic hydrocarbons; Principal component and decomposition analysis; Correlation (122) 171

Quenching errors; Correction through time resolution (123) 255

Tyrosine derivatives; Thyronine derivatives; *o*-Phthalaldehyde derivatives of iodinated amino acids (124) 221

Organic materials; Laser excitation; Polynuclear aromatic hydrocarbons; Narrow vibrational lines; Selective excitation (125) 1

Phosphorimetry; Polynuclear aromatic hydrocarbons; Coal liquefaction product; Synchronous luminescence (125) 13

Kinetic analysis; Copper; Autoxidation of 2,2'-dipyridyl ketone azine or hydrazone or phenyl-2-pyridyl ketone hydrazone (125) 21

Hafnium; With 3-hydroxychromone (125) 155

Laser excitation; Detection limits; Nitrogen laser-pumped dye laser (125) 161

T.l.c.; Polynuclear aromatic hydrocarbons; Dry-column chromatography; Standardization of aluminium oxide (126) 225

Plasma; Lecithin-cholesterol acyltransferase; Enzyme reaction (126) 237

Europium; With 1,1,1-trifluoro-4-(2-thienyl)-2,4-butanedione; Nitrogen laser; Photon counter (127) 195

Flow system; Gallium; Extraction; Flow injection; With lumogallion; Laser excitation (129) 195

Alkaline phosphatase; Sorbitol dehydrogenase; Peroxidase; Pseudocholinesterase; Fluoristic technique (129) 231

Modified silica surfaces; Derivatized dansylated silica (130) 31

Magnesium; Ternary complex (130) 73

Kinetic analysis; Iron; Thallium; Oxidation of 1,4-diamino-2,3-dihydroanthraquinone (130) 337

Amino acids; Proline; Secondary; Hydroxyproline; Sarcosine (130) 377

Gas chromatography

Mass spectrometry; Volatile organics determination; Purge vessel design (121) 345

Pattern recognition; Chemical structure; From retention data (122) 139

Emission spectrometry; Correlation; Wine; Glass-capillary; Stepwise regression analysis; Subjective sensory evaluations (122) 215

Algorithms; High-level data processing; Computer processing; Optimal separation conditions (122) 279

Theory of error; Target factor analysis (122) 327

Dioxin; Active carbon; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; Environmental samples (123) 25

Aliquat-336; Quaternary ammonium components (123) 213

Mass spectrometry; Isotope dilut. analysis; Tetramethyllead; Tetraethyllead; Air; Two-step enrichment method; Single ion monitoring (124) 1

Polychlorinated biphenyls; Air; Trace organic compounds; Collection on Amberlite XAD-2 resins (124) 31

Isopropylmethylphosphonofluoridate; Waters; Preconcentration; Direct thermal desorption (124) 225

Ion exchange; Aliphatic carboxylates; Direct derivatization; Lipophilic straight-chain aliphatic carboxylate ions (124) 403

Chlorine; Derivatisation with 2,6-dimethylphenol (124) 431

Emission spectrometry; Nitrosamines; Electronic noise filter; Chemiluminescent detector (126) 125

H.p.l.c.; Polynuclear aromatic hydrocarbons; Capillary columns (127) 55

Chromium; Polymers; Water-soluble; Trifluoroacetylacetone (127) 223

Vanadium; Petroleum oils; Fluorinated β-diketonates (128) 101

Vanadium; Petroleum oils; Copper; Nickel; Extraction of chelate (128) 109

Mass spectrometry; Sediments; Organic compounds; Computer techniques; Capillary column (129) 57

Spectrophotometry; Extraction; Oxalic acid; Urine; Ion-pair formation (129) 189

H.p.l.c.; Emission spectrometry; Element-selective detection; Plasmas; Review (130) 227

Paracetamol; Plasma; Rapid extraction (130) 405

Gel permeation chromatography

Anions; Semi-automatic (126) 229

Gravimetry

Silica; Silicates; Distillation; Quinoline 12-molybdosilicate (129) 213

Chromium; Sea water; Chemical speciation; Naturally occurring organic materials (130) 289

High-performance liquid chromatography

Sulphur; Ion chromatography; Oil, fuel; Oxygen flask (121) 233

Foods; Sorbic acids; Benzoic acids; Soft drinks (121) 337

Titrimetry; Computerization; Micro-mini-mainframe-computer network; Correlation chromatography (122) 201

Chlorinated phenols; Reverse-phase separation; Online microprocessor control; Correlation chromatography (122) 267

Cigarette smoke; In sera; Low-molecular-weight, u.v.-absorbing compounds (123) 33

Methyl esters; Phosphatidic acid dimethyl esters; Silver nitrate-impregnated silica gel column (123) 41

Voltammetry; Nitrosamines; Electrochemical detection (123) 97

Amperometry; Spectrophotometry; Vitamin K_3; Detectors (125) 85

Ion exchange; Uranium; Molybdenum; Controlled-pore glass; Immobilized diamine (126) 117

Mitomycin C; Plasma; Drugs; Human blood; U.v. detection (127) 47

Gas chromatography; Polynuclear aromatic hydrocarbons; Capillary columns (127) 55

Vitamins; Cod liver oil; Fat-soluble; Formulated feed; Stopped-flow scanning (127) 213

Phenolic anti-oxidants; Edible oils (128) 235

Mass spectrometry; Carbon disulphide reduction; In N,N-dimethylformamide (129) 49

Amperometry; Nitrate; Flow-through electrode (129) 101

Tris(nonylphenyl) phosphite; Butadiene-styrene copolymers; Nonylphenol; Ultraviolet detector (129) 243

Anions; Ion chromatography; Waters; Selective detectors (130) 1

Flow system; Electrochemical reactivation; For solid electrodes (130) 9

Plasma; Urine; Drugs; Anti-neoplastic agents (130) 23

Gas chromatography; Emission spectrometry; Element-selective detection; Plasmas; Review (130) 227

Explosive mixtures; Gunshot residue; Nitro aromatics; Nitrate esters; Nitramines (130) 295

Infrared spectrometry

N.m.r. spectrometry; Structure elucidation; Organic compounds; Computer program system; New CHEMICS (122) 87

N.m.r. spectrometry; Structure elucidation; Organic compounds; CHEMICS program (122) 95

Mass spectrometry; N.m.r. spectrometry; Chemical information system; Minicomputer-oriented system (122) 103

Raman spectrometry; Computer-aided spectra interpretation; CRISE computer program (122) 363

N.m.r. spectrometry; Mass spectrometry; Dispatching rules; Structural analysis; Digital simulation (122) 435

Oxygen; Selenium (127) 87

Thermom. analysis; Silica-bound complexing agents; Synthetic aspects; Characterization (129) 29

Carbon; Steels; Plutonium; Carbon dioxide (130) 215

Ion exchange

Potentiometry; Titrimetry; Molybdenum; Nickel alloys; With cerium(IV) solutions (121) 85

Metal ions; Attaching N-phenylhydroxamic acid groups; Amberlite XAD-4 (121) 225

X-ray fluores. spectrometry; Trace anions; Waters; 2,2'-Diaminodiethylamine cellulose filters (123) 9

Trace metals; 8-Hydroxyquinoline-5-sulfonic acid; Anionic complexes (123) 19

Gold; Silver; Chelating resin; Polyhydroxamic acid (123) 309

Cadmium; Zinc; Hexacyanoferrate(II) salts on gel and macroporous resins; In situ precipitation (124) 365

Gas chromatography; Aliphatic carboxylates; Direct derivatization; Lipophilic straight-chain aliphatic carboxylate ions (124) 403

Emission spectrometry; Yttrium; Rare earth elements; Geological materials; Plasmas; Inductively-coupled (124) 421

At. abs. spectrometry; Electrotherm. atomization; Trace metals; Sea water; Extraction; Ammonium pyrrolidinedithiocarbamate/diethylammonium diethyldithiocarbamate into freon; Chelex-100 resin (125) 117

Spectrophotometry; Scandium; Yttrium; Cerium; Uranium; Xylenol orange complexes; Strong anion exchangers (125) 139

H.p.l.c.; Uranium; Molybdenum; Controlled-pore glass; Immobilized diamine (126) 117

Electrotherm. atomization; At. abs. spectrometry; Arsenic species; Marine organisms; Sediments; Hydride generation; Inorganic and methylated (126) 157

Calcium; Quantitative separation; Macroporous resin (127) 63

Flow system; Emission spectrometry; Zinc; Cadmium; Chemiluminescence; Flow injection (127) 199

At. abs. spectrometry; Mercury; Waters; Sea water; Cold-vapour; Anion-exchange resin (130) 149

Amplification; Inversion tube; Definition (130) 193

Isotope dilution analysis

Gas chromatography; Mass spectrometry; Tetramethyllead; Tetraethyllead; Air; Two-step enrichment method; Single ion monitoring (124) 1

Uranium; Extraction; Substoichiometric; Synergic system (129) 221

Kinetic analysis

Thermom. analysis; Enzyme activity; Cholinesterase; Enthalpimetric determination (121) 125

Flow system; Thermom. analysis; Iodide; Catalyzed cerium(IV)-arsenic(III) reaction (121) 135

Titrimetry; Metal ions; Gaseous catalysts for end-point indication; Hydrogen sulphide (121) 257

Potentiometry; Selenium; Catalytic determination; Picrate-selective electrode; Picrate-sulfide reaction (123) 143

Flow system; Titrimetry; Gradient chamber; Kinetic treatment; Flow-injection (124) 39

Flow system; Titrimetry; Gradient chamber; Kinetic treatment; Flow-injection (124) 65

Fluorimetry; Copper; Autoxidation of 2,2'-dipyridyl ketone azine or hydrazone or phenyl-2-pyridyl ketone hydrazone (125) 21

Potentiometry; Flow system; Enzymatic analysis; Glucose; Sulphur dioxide probe; Glucose oxidase; Oxidation of hydrogensulphite (125) 55

Cyclic voltammetry; Vanadium; Redox catalyst in non-aqueous media; V(V)/V(IV) couple (126) 65

Regression data processing; Lactate dehydrogenase; Subunit determinations; Catalytic reaction (127) 23

Flow system; Copper; Serum; Flow injection; Iron(III)-thiosulfate indicator reaction (127) 39

Simplex optimization; Copper; Numerical model; Catalyzed decomposition of hydrogen peroxide (128) 177

Amperometry; Nitrate; Electrocatalysis of reduction; By electrodeposited copper (129) 87

Spectrophotometry; Flow system; Cobalt; Catalysis of SPADNS oxidation; Flow injection (130) 65

Fluorimetry; Iron; Thallium; Oxidation of 1,4-diamino-2,3-dihydroanthraquinone (130) 337

Spectrophotometry; Diamine oxidase (130) 419

Mass spectrometry

Gas chromatography; Volatile organics determination; Purge vessel design (121) 345

Spark-source; Data acquisition system; Processing software (122) 57

I.r. spectrometry; N.m.r. spectrometry; Chemical information system; Minicomputer-oriented system (122) 103

Microdensitometer-minicomputer system; Semiautomatic; Spark-source (122) 323

I.r. spectrometry; N.m.r. spectrometry; Dispatching rules; Structural analysis; Digital simulation (122) 435

Gas chromatography; Isotope dilut. analysis; Tetramethyllead; Tetraethyllead; Air; Two-step enrichment method; Single ion monitoring (124) 1

Chlorophenoxyphenols; Soils; Waters; Extraction; Field desorption (124) 357

Sodium contamination, prevention; Surface treatment of borosilicate glass (124) 427

T.l.c.; Aromatics; Petroleum; η^6-Arene-η^5-cyclopentadienyliron cations; Ligand-exchange reaction (125) 171

H.p.l.c.; Carbon disulphide reduction; In N,N-dimethylformamide (129) 49

Gas chromatography; Sediments; Organic compounds; Computer techniques; Capillary column (129) 57

Mossbauer spectroscopy

Least-squares fitting (122) 373

Nuclear magnetic resonance spectrometry

Computer-aided spectra interpretation; Hardware; Software; Artificial intelligence system (122) 41

Computer-aided spectra interpretation; Minicomputer; Chemical structure (122) 47

I.r. spectrometry; Structure elucidation; Organic compounds; Computer program system; New CHEMICS (122) 87

I.r. spectrometry; Structure elucidation; Organic compounds; CHEMICS program (122) 95

I.r. spectrometry; Mass spectrometry; Chemical information system; Minicomputer-oriented system (122) 103

I.r. spectrometry; Mass spectrometry; Dispatching rules; Structural analysis; Digital simulation (122) 435

Amphetamines; Mono- and dimethyl-amphetamines; ^{13}C-n.m.r. (123) 75

Spin-spin relaxation times; Carbon-13; Spin-echo Fourier transform; Carbon disulfide (124) 415

Cross polarization; Magic angle spinning; Organosilanes; Carbon-13; Bonded to silica (126) 207

Phosphorimetry

Glucuronidase; Biological materials; *p*-Nitrophenol formed (121) 289

Fluorimetry; Polynuclear aromatic hydrocarbons; Coal liquefaction product; Synchronous luminescence (125) 13

Piezoelectric sensing

Cyanide; Single-drop method (124) 81

Toluene diisocyanate; Air; Coating materials (129) 163

Toluene diisocyanate; Air; Personal monitoring (129) 175

Mercury; Waters; Gold-coated (130) 141

see also A.c. polarography, D.c. polarography, Differential pulse polarography, Pulse polarography

In Fluosol-43; Influence of Pluronic F-68 surfactant; Dropping mercury electrode (121) 29

Tellurium; Hanging and dropping mercury electrodes; Adsorbed monolayer (121) 315

Flow system; Isosorbide dinitrate; Flow injection analysis (123) 115

Aldehydes; Ketones; Hydration-dehydration equilibria; *α,α,α*-Trifluoroacetophenone (126) 71

Trifluoroacetophenone; Reaction with ammonia (126) 259

Metal speciation; Derivative normal pulse; Microcomputer (129) 79

Corticosteroids; Mechanism; Prednisolone; Dexamethasone (130) 111

Tin; Stabilizing agents; Antioxidant (130) 133

Potentiometry

Flow system; Glucose oxidase; Gas-sensing sulphur dioxide probe (121) 61

Potentiometric stripping analysis; Amalgamated metals as reducing agents; Selenium; Sulphur; Halides (121) 71

Titrimetry; Ion exchange; Molybdenum; Nickel alloys; With cerium(IV) solutions (121) 85

Titrimetry; Pharmaceuticals; Containing covalently bound halogen; Ion-selective electrodes; Combustion; pH adjustment (121) 93

Monohydrogenphosphate-sensing electrode; Phosphate; Formulations (121) 101

Oxalate; Enzyme sensor; Immobilized oxalate decarboxylase; Carbon dioxide gas-sensing electrode; Urine (121) 111

Benzoate-sensitive liquid membrane electrode; Enhancement of selectivity by alkylphenol (121) 119

Titrimetry; Thioethers; With mercury(II) perchlorate (121) 321

Titrimetry; Hydroxide; Carbonate; Sodium chloride solutions; Computer method (122) 81

Voltammetry; Computerized system (122) 179

Titrimetry; Models, multiparameter; Statistical uncertainties; Multiple regression (122) 291

Antibody-selective membrane electrode; Polyvinyl chloride membrane (123) 135

Kinetic analysis; Selenium; Catalytic determination; Picrate-selective electrode; Picrate-sulfide reaction (123) 143

Copper-selective electrode; Chloride interference; Mechanisms (123) 151

Copper-selective electrode; Chloride interference region (123) 161

Alkaloids; Picrate-selective electrode; Insoluble alkaloid picrate salts (123) 341

Titrimetry; Coated-wire electrode; Arenediazonium salts; Azo dyestuffs; With sodium tetraphenylborate; Ion-pair formation (124) 91

Titrimetry; Thermom. analysis; Glass surfaces; Modified with N-2-aminoethyl-3-aminopropyl-trimethoxysilane; Titrated with copper(II) (124) 229

Mercury; Enzyme reactor; Waters; On-site samping; Immobilized urease; Ammonia electrode (125) 45

Flow system; Kinetic analysis; Enzymatic analysis; Glucose; Sulphur dioxide probe; Glucose oxidase; Oxidation of hydrogensulphite (125) 55

Microcomputer-based system; Ion-selective electrodes; Fluoride; In toothpaste (125) 65

Coated-wire electrode; Propranolol-responsive electrodes; Didodecylnaphthalene sulfonic acid salt (125) 179

Pyridoxal-5'-phosphate; Ammonia gas-sensing electrode; Coenzyme (126) 35

Guanine enzyme electrode; Serum; Ammonia gas sensor; Immobilization (126) 43

Titrimetry; Dinitrosalicylate-selective electrode; Liquid-membrane (126) 51

Tetramethylammonium and tetraethyl-ammonium ions; Liquid membrane electrodes; Quaternary ammonium tetrakis(m-trifluoro-methylphenyl)borates (126) 217

Mercury; Cyanide sensor; Indirect determination; Air-gap electrode (126) 221

pH; Standardisation; Measurement (127) 1

Stripping analysis; Cadmium; Lead; Copper; Zinc; Biological materials; Computerized (127) 147

Titrimetry; Coated-wire electrode; Surfactants; Ion-pair formation; Cationic and anionic (127) 165

Tobacco products; Nicotine-sensitive electrode; Liquid membrane (127) 173

Spectrophotometry; Hammett acidity function; Aromatic carboxamides; Prototropic reaction isotherms; Hydration requirements (127) 203

Titrimetry; Free acid; Addition of potassium oxalate; Hydrolyzable cations (127) 263

Serine-selective membrane; Immobilized anaerobic bacteria; Ammonia gas sensor (128) 121

Calcium-selective electrode; Macrocyclic polyether diamides; Tetra(4-chlorophenyl)-borate (128) 129

Cadmium; Lead; Urine; Computerized; Stripping analysis (128) 155

Atropine; Novatropine; Pharmaceuticals; Liquid-membrane electrodes (128) 239

Zinc; Cadmium; Lead; Copper; Sea water; Computerized; Stripping analysis (129) 153

Dissociation constant; *N*-(2-Acetamido)imino-diacetic acid (129) 247

Flow system; Spectrophotometry; Glucose; Urea; Enzyme reactor; On-line dialysis (130) 45

Titrimetry; Carbonate; Presence of hydroxide; First-derivative (130) 93

Titrimetry; Carbonate; Computer simulation (130) 103

Pulse polarography

D.c. polarography; Diffl. pulse polarography; Static mercury drop electrodes (121) 1

Voltammetry; Cadmium; Microcomputer-controlled; Staircase voltammetry (122) 67

Raman spectrometry
I.r. spectrometry; Computer-aided spectra interpretation; CRISE computer program (122) 363

Spectrophotometry
Titrimetry; Calcium; Metallochromic indicators (121) 265

Diphenylcarbazones; Asymmetrically substituted; Chelate formers (121) 275

Flow system; Polyphosphates; Flow injection; Complexes of xylenol orange and methylthymol blue (121) 281

Flow system; Uranium; Extraction; With 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (121) 341

Eigenvector analysis; Data reduction; Equilibrium and kinetic studies; Desk computer (122) 303

Flow system; At. abs. spectrometry; Zone-sampling; Potassium in plant digests (123) 221

Flow system; Conductometry; "Stat" methods; Copper; Iron; Iodide; Hydrochloric acid (123) 239

Silicate; Waters; Formation of α-molybdosilicic acid and reduction (123) 263

Flotation; Osmium; With thiocyanate and methylene blue; Ion associate precipitate (123) 271

Iron; Chrome azurol S; Eriochrome cyanine R; Cationic surfactants; Ternary complexes (123) 279

Extraction; Cadmium; With 4-(2-pyridylazo)-resorcinol; And cetyldimethylbenzylammonium chloride (123) 289

Bismuth oxide; Bismuth; Flotation; As tetraiodobismuthate(III) (123) 297

Chloride; Plants; Displacement of thiocyanate (123) 347

Extraction; Non-ionic surfactants; Waters; Sea water; Potassium tetrathiocyanatozincate(II); Zinc(II) (123) 355

Creatine kinase-MB isoenzyme; Blood; Serum; Immobilized immuno-stirrer (124) 169

Rare earth elements; Chlorophosphonazo-*m*-NO_2; Cerium sub-group; In presence of yttrium sub-group (124) 177

Germanium; As methylene blue 12-molybdogermanate (124) 185

Nickel; 2-(2-Pyridylazo)-5-alkoxyphenol derivatives; Solvent extraction (124) 193

Stability constants, evaluation; Mono- and poly-nuclear complexes (124) 201

Citric acid; NADH absorbance; Dehydrogenase reactions; Tetrazolium reagent (124) 215

Extraction; Thermal lens effect; Iron; Ion-pair; With 4,7-diphenyl-1,10-phenanthroline disulfonic acid (124) 381

Chromium; Liver tissue; With diphenylcarbazide (124) 391

Flow system; Nitrate; Waters; Flow injection; Hydrazine reduction; Rain (124) 437

Neptunium (124) 443

Plutonium; Oxidation with cerium(IV) (124) 449

Flow system; Boron; Plants; Flow injection; With azomethine-H (125) 29

Flow system; At. abs. spectrometry; Aluminium; Iron; Plants; Flow injection; Zone-sampling (125) 37

Voltammetry; Lucigenin; Electrochemical reduction (125) 73

Amperometry; H.p.l.c.; Vitamin K_3; Detectors (125) 85

Ion exchange; Scandium; Yttrium; Cerium; Uranium; Xylenol orange complexes; Strong anion exchangers (125) 139

Bismuth; Copper; Diethyldithiocarbamates; Extraction; Into chloroform (125) 149

Vanadium; Niobium; Extraction; From succinate solution; Using tri-n-octylamine (125) 165

Sodium; Extraction; Crown ether complexes; Serum; Monoanionic dyes; Ion-pair (126) 185

At. abs. spectrometry; Tin; Organotin compounds; Substoichiometric determination; Complexation with salicylideneamino-2-thiophenol (126) 199

Vanadium; Steels; *p*-Sulphobenzeneazo-4-(2,3-dihydroxy-5-chloropyridine) (126) 233

Micellar systems; Polymethylphenols; Acidity and binding constants in sodium dodecyl sulphate (126) 253

Potentiometry; Hammett acidity function; Aromatic carboxamides; Prototropic reaction isotherms; Hydration requirements (127) 203

Flow system; Sulphide; Flow injection; Methylene blue method (128) 163

Flow system; Serum; Urine; Thiocyanate; Chloramine-T (128) 169

Extraction; Ion-pairs; Triphenylmethane dye cations (128) 185

Sugars; Plants; Reducing; Alkaline *p*-hydroxybenzoic acid hydrazide (128) 195

Phenol; Interference of sulphite; 4-Aminoantipyrine (128) 251

Phosphate; Soils (128) 257

Dithizone; Effect of temperature (128) 261

Sulphate; Waters; Reduction (128) 263

Gas chromatography; Extraction; Oxalic acid; Urine; Ion-pair formation (129) 189

Ion flotation; Chromium; Diphenylcarbazone complex (129) 237

Flow system; Potentiometry; Glucose; Urea; Enzyme reactor; On-line dialysis (130) 45

Flow system; Nitrogen; Sea water; Alkaline peroxodisulphate (130) 55

Flow system; Kinetic analysis; Cobalt; Catalysis of SPADNS oxidation; Flow injection (130) 65

Lignin; Biodegraded (130) 81

Redox reactions, stoichiometry; Equilibrium constants (130) 183

Vanadium; Waters; Rocks; Adsorption on Sephadex gel; With 4-(2-pyridylazo)resorcinol (130) 345

Vanadium; Air particulates; Extraction; With *N*-benzoyl-*N*-phenylhydroxylamine (130) 353

Flow system; Calcium; Waters; Soils; Plants; Merging zones; Flow injection; With glyoxal bis(2-hydroxyanil) (130) 361

Dye binding; Ionic to a polyelectrolyte in solution; Dialysis membrane (130) 369

Flow system; Chloride; Ethanol; Flow injection (130) 409

Kinetic analysis; Diamine oxidase (130) 419

Thermometric analysis

Kinetic analysis; Enzyme activity; Cholinesterase; Enthalpimetric determination (121) 125

Flow system; Kinetic analysis; Iodide; Catalyzed cerium(IV)-arsenic(III) reaction (121) 135

Titrimetry; Potentiometry; Glass surfaces; Modified with N-2-aminoethyl-3-aminopropyltrimethoxysilane; Titrated with copper(II) (124) 229

Arrhenius model; Reaction kinetic calculations (124) 341

I.r. spectrometry; Silica-bound complexing agents; Synthetic aspects; Characterization (129) 29

Thin-layer chromatography

Mass spectrometry; Aromatics; Petroleum; η^6-Arene-η^5-cyclopentadienyliron cations; Ligand-exchange reaction (125) 171

Fluorimetry; Polynuclear aromatic hydrocarbons; Dry-column chromatography; Standardization of aluminium oxide (126) 225

Titrimetry

Potentiometry; Ion exchange; Molybdenum; Nickel alloys; With cerium(IV) solutions (121) 85

Potentiometry; Pharmaceuticals; Containing covalently bound halogen; Ion-selective electrodes; Combustion; pH adjustment (121) 93

Kinetic analysis; Metal ions; Gaseous catalysts for end-point indication; Hydrogen sulphide (121) 257

Spectrophotometry; Calcium; Metallochromic indicators (121) 265

Potentiometry; Thioethers; With mercury(II) perchlorate (121) 321

Potentiometry; Hydroxide; Carbonate; Sodium chloride solutions; Computer method (122) 81

H.p.l.c.; Computerization; Micro-mini-mainframe-computer network; Correlation chromatography (122) 201

Potentiometry; Models, multiparameter; Statistical uncertainties; Multiple regression (122) 291

Computerized system; Cetyl-trimethyl-ammonium bromide with bromocresol purple; Kalousek-type waveforms; Determination of double-layer capacitance (122) 387

Flow system; Sulphur dioxide; Pyridinium bromide perbromide; Flow injection (123) 351

Kinetic analysis; Flow system; Gradient chamber; Kinetic treatment; Flow-injection (124) 39

Kinetic analysis; Flow system; Gradient chamber; Kinetic treatment; Flow-injection (124) 65

Coulometry; Aniline; End-point anticipation; Electronically controlled dual-intermediate titrator (124) 85

Potentiometry; Coated-wire electrode; Arenediazonium salts; Azo dyestuffs; With sodium tetraphenylborate; Ion-pair formation (124) 91

Potentiometry; Thermom. analysis; Glass surfaces; Modified with N-2-aminoethyl-3-aminopropyltrimethoxysilane; Titrated with copper(II) (124) 229

Lead; Thiocyanate amplification; Precipitation as lead hexathiocyanatochromate(III) (125) 215

Potentiometry; Dinitrosalicylate-selective electrode; Liquid-membrane (126) 51

Potentiometry; Coated-wire electrode; Surfactants; Ion-pair formation; Cationic and anionic (127) 165

Potentiometry; Free acid; Addition of potassium oxalate; Hydrolyzable cations (127) 263

Sulphonamides; Cationic micellar systems (128) 273

Flow system; Theory; High-speed; Flow injection (129) 1

Potentiometry; Carbonate; Presence of hydroxide; First-derivative (130) 93

Potentiometry; Carbonate; Computer simulation (130) 103

Coulometry; Amperometry; Manganese electrode; EDTA (130) 329

Voltammetry

see also Anodic stripping voltammetry, Cyclic voltammetry, Differential pulse voltammetry

Flow system; Limiting current in electrochemical flow-through cells; Survey of the equations; Tubular, thin-layer, wall-jet and disk electrodes (121) 13

Flow system; Drugs; 2-Thiobarbituric acid; Thioamide; Mercury-coated glassy carbon electrode (121) 51

Pulse polarography; Cadmium; Microcomputer-controlled; Staircase voltammetry (122) 67

Potentiometry; Computerized system (122) 179

H.p.l.c.; Nitrosamines; Electrochemical detection (123) 97

Flow system; Immobilized alcohol oxidase; Ethanol; Oxygen electrode; Blood alcohol (123) 125

Anod. stripping voltammetry; Metal complexes; Convolution techniques (123) 187

Thiocyanate; Copper ions; Cathodic stripping; Application (123) 201

Selenocystine; Cystine; Cysteine; Cathodic stripping (124) 333

Spectrophotometry; Lucigenin; Electrochemical reduction (125) 73

Coulometry; Flow system; Manganese; Cathodic stripping (126) 15

Semidifferential electroanalysis; Stationary solid working electrode (126) 95

Flow system; High flow-rate cells; Static mercury drop electrode (127) 121

Flow system; Peristaltic pump pulsation; Manganese; Synchronization of signal sampling; Cathodic stripping (127) 239

Hydrogen peroxide; Pickling baths; For copper alloys; Linear sweep (128) 141

Pulsed-stirring voltammetry; Evaluation (129) 253

X-ray fluorescence spectrometry

Platinum; Iridium; Ion flotation; Separation; Anionic chlorocomplexes; With cationic surfactants (123) 1

Ion exchange; Trace anions; Waters; 2,2'-Diaminodiethylamine cellulose filters (123) 9

Chromium; Waters; Speciation; Membrane filters (124) 351

Vanadium; Molybdenum; Tungsten; Brines; Chloralkali electrolysis (128) 207

Urine; Trace metals; Energy-dispersive (130) 167

(continued from back cover)

Short Communications

A novel approach for determination of tin, lead and copper in biological samples and sediments by alternating current anodic stripping voltammetry
S. Dogan, G. Nembrini and W. Haerdi (Geneva, Switzerland) 385
Electrochemical determination of nitrite and nitrate by pneumatoamperometry
D. D. Nygaard (Denver, CO, U.S.A.) 391
The use of potassium hydroxide in 2-ethylhexanoic acid as ionization suppressor in the determination of calcium in lubricating oils by atomic absorption spectrometry
J. S. Dits (Rotterdam, The Netherlands) 395
Chemical speciation of chromium in sea water. Part 2. Effects of manganese oxides and reducible organic materials on the redox processes of chromium
E. Nakayama, T. Kuwamoto, S. Tsurubo and T. Fujinaga (Kyoto, Japan) 401
Extraction du paracétamol par relargage de solvant et application au dosage par chromatographie en phase gazeuse dans le plasma
Y. Pegon et J. J. Vallon (Lyon, France) 405
Spectrophotometric flow injection determination of chloride in ethanol
F. J. Krug, L. C. R. Pessenda, E. A. G. Zagatto, A. O. Jacintho and B. F. Reis (S. Paulo, Brasil) 409
Electrogenerated chemiluminescence of lucigenin in aqueous alkaline solutions at a platinum electrode
K. E. Haapakka and J. J. Kankare (Turku, Finland) 415
Determination of diamine oxidase by a kinetic method with 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate)
N. Majkić-Singh, M. Konjović, M. Stojanov, S. Spasić and I. Berkeš (Belgrade, Yugoslavia) 419

Author Index 425

Cumulative Indexes Volumes 121–130 429

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CONTENTS

Review: Element-selective detection for chromatography by plasma emission spectrometry
J. W. Carnahan, K. J. Mulligan and J. A. Caruso (Cincinnati, OH, U.S.A.) 22
Flow injection systems with inductively-coupled argon plasma atomic emission spectrometry. Part 1. Fundamental considerations
A. O. Jacintho, E. A. G. Zagatto, H. Bergamin F°, F. J. Krug, B. F. Reis, R. E. Burns (S. Paulo, Brasil) and B. R. Kowalski (Seattle, WA, U.S.A.) 24
Atomic absorption spectrometry of selenium in an argon–hydrogen flame after electrochemical preconcentration
B. Holen, R. Bye and W. Lund (Oslo, Norway) 25
Investigations of reactions involved in electrothermal atomic absorption procedures. Part 10. Factors influencing the determination of arsenic
J. Korečková, W. Frech, E. Lundberg, J.-Å. Persson and A. Cedergren (Umeå, Sweden) 26
Spectral interferences from phosphate matrices in the determination of arsenic, antimony, selenium and tellurium by electrothermal atomic absorption spectrometry
K. Saeed and Y. Thomassen (Oslo, Norway) 28
Chemical speciation of chromium in sea water. Part 1. Effect of naturally occurring organic materials on the complex formation of chromium(III)
E. Nakayama, T. Kuwamoto, S. Tsurubo, H. Tokoro and T. Fujinaga (Kyoto, Japan) 28
Determination of nitro aromatic, nitramine, and nitrate ester explosive compounds in explosive mixtures and gunshot residue by liquid chromatography and reductive electrochemical detection
K. Bratin, P. T. Kissinger (West Lafayette, IN, U.S.A.) and R. C. Briner (Cape Girardeau, MO, U.S.A.) . . 29
Quantitative enrichment of trace levels of ions by electrodialysis
J. A. Cox and R. Carlson (Carbondale, IL, U.S.A.) 31
Electrochemistry at carbon fibers. Part 1. Characteristics of the mercury film carbon fiber electrode in differential pulse anodic stripping voltammetry
M. R. Cushman, B. G. Bennett and C. W. Anderson (Durham, NC, U.S.A.) 32
The coulometric generation of manganese(II) from a manganese electrode: the titration of EDTA
L. G. Irr and H. W. Safford (Pittsburgh, PA, U.S.A.) 32
Kinetic fluorimetric determination of iron and thallium based on oxidation transformation of 1,4-diamino-2,3-dihydroanthraquinone
F. Salinas, C. Genestar and F. Grases (Mallorca, Spain) 33
Spectrophotometric determination of vanadium in natural waters and rocks after selective adsorption on Sephadex gel
K. Yoshimura, H. Kaji, E. Yamaguchi and T. Tarutani (Fukuoka, Japan) 34
Rapid and sensitive determination of total vanadium in airborne particulates by an extraction–spectrometric method with *N*-benzoyl-*N*-phenylhydroxylamine
T. Fukasawa, S. Miyata and S. Matsunaga (Kofu-shi, Japan) 35
Merging zones in flow injection analysis. Part 6. Determination of calcium in natural waters, soil and plant materials with glyoxal bis(2-hydroxyanil)
A. O. Jacintho, E. A. G. Zagatto, B. F. Reis, L. C. R. Pessenda and F. J. Krug (S. Paulo, Brasil) 36
A new method of determining the extent of binding of an ionic dye to a polyelectrolyte in solution
J. Gormally, J. Panak, E. Wyn-Jones (Salford, Gt. Britain), A. Dawson (Leeds, Gt. Britain), D. Wedlock and G. O. Phillips (Clwyd, Gt. Britain) 36
Fluorimetric determination of secondary amino acids by 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole
K. Imai and Y. Watanabe (Tokyo, Japan) 37

(continued on inside page of cove