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ANALYTICA CHIMICA ACTA

An international journal devoted to all branches of analytical chemistry

New Methods and Strategies in Environmental Analysis

Papers by Young Analytical Chemists

Presented at Euroanalysis VIII, Edinburgh, UK, September 5–11, 1993

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ANALYTICA CHIMICA ACTA

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SPECIAL ISSUE

NEW METHODS AND STRATEGIES IN ENVIRONMENTAL ANALYSIS

PAPERS BY YOUNG ANALYTICAL CHEMISTS

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PREFACE

Analytica Chimica Acta sponsored a one day session of lecture and poster presentations at Euroanalysis VIII which was held in Edinburgh, UK, 5–11 September 1993. The two primary objectives of the session were to promote discussion on "New Methods and Strategies in Environmental Analysis" and to encourage the participation of young analytical chemists by awarding travel bursaries.

The contributions covered a wide range of environmental matrices, most notably natural waters (marine and freshwater), polluted waters (including industrial effluents), sediments and global and occupational atmospheres. It is clear from the papers contained in this special issue that the complete armoury of analytical techniques is being used to address the challenge presented by environmental analytical problems. There are however a number of themes of generic interest that were also highlighted during the session. These include the necessity for reliable preconcentration techniques (particularly in situ methods that overcome problems associated with sample collection and storage), methods for the physico-chemical speciation and fractionation of environmental samples and the validation and quality assurance of analytical data relating to environmental samples.

All the young scientists who participated in the session are to be congratulated on the quality of the work undertaken and the manner in which the presentations were made. The size of the audience throughout the day and the lively nature of the discussion periods is testimony to the enthusiasm of researchers in the environmental analytical chemistry field and to the timeliness of such a meeting.

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In-line ultraviolet-digestion of natural water samples for trace metal determination using an automated voltammetric system

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Abstract

The use of in-line UV-digestion for breakdown of dissolved organic matter (DOM), prior to voltammetric determination of trace metals in natural samples, is discussed in this paper. Destruction of DOM is necessary to free trace metals that are organically complexed and to remove interfering organic surfactants. Complete breakdown of DOM in natural water samples is achieved by in-line UV-digestion using a 100 W medium pressure mercury vapour lamp and a silica coil, at a sample digestion time of 4.5 min. The efficiency of the system is tested with destruction of humic acid, conversion of Cr(III) to Cr(VI), and the release of Ni and Cu from organic complexes in sea water. The in-line application of UV is illustrated by automated voltammetry of nanomolar levels of Cu and Ni in samples from oceanic origin.

Key words: Voltammetry; Humic acid; Metals; Trace metals; Waters; UV-digestion, in-line

1. Introduction

1.1. Digestion of dissolved organic matter

Many trace metals, including Cu, Ni, Co, Cd and Zn, are known to occur organically complexed in sea water [1–5]. The organic complexation causes these trace metals to be partially non-reactive (non-labile) during voltammetric analysis unless the dissolved organic matter (DOM) is destroyed. The organic material also acts as a surfactant, causing interferences and reducing the accessibility to the mercury drop for the metal, hence decreasing the voltammetric signal.

Wet digestion is a commonly used method for the destruction of DOM prior to metal analysis. Wet digestion methods use chemical oxidants like sodium persulphate, nitric acid, sulphuric acid, perchloric acid and hydrogen peroxide to destroy organic matter [6]. The high levels of oxidants added by these methods often introduce contamination to the samples, which is unacceptable for analysis of ocean waters containing trace metals at nanomolar levels.

UV-digestion is a method that is often used

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for destruction of DOM prior to voltammetric trace metal analysis [7,8]. It is a clean method, as it does not generally require added oxidants. In addition, it is effective and can easily be incorporated in an automated trace metal analyser, and therefore is a more preferable method for destruction of DOM prior to voltammetric trace metal determination.

1.2. Photochemistry

Processes leading to degradation of organic matter can be subdivided into primary and secondary photochemical processes. The primary processes are restricted to those compounds which are excited by the direct absorption of irradiation (chromophores), the excited species undergo chemical alteration, resulting in the formation of new ground state products. In certain instances the new product is reactive and proceeds either intramolecularly or intermolecularly to undergo further reactions in the system. Such reactions are referred to as secondary processes, and can involve all organic compounds [9].

Electronic energy deactivation may be an important source of secondary photochemistry, though it does not alter the chromophore initially excited. Oxygen is an element involved in electronic deactivation processes [9]. Oxygen is the dominant solute in water with a low-lying electronic energy gap (the $^1O_2$ singlet state lies 22 kcal above the triplet ground state $^3O_2$) and the production of singlet oxygen is possible from chromophores activated at wavelengths as long as 700 nm. During this process singlet oxygen is produced when a photo-excited organic substrate, ^3S, deactivates by energy transfer to oxygen [9]:

$$^3S + {}^3O_2 \rightarrow {}^1S + {}^1O_2 \quad (1)$$

Humic substances have been shown to transfer energy to oxygen in this manner [10]. Substantial generation rates of singlet oxygen have been measured in both freshwater and coastal sea water [11].

Singlet oxygen has been proposed to be involved in the breakdown of DOM. Organic reactants susceptible to singlet oxygen attack include phenols, compounds with electron-rich centres, and those containing easily oxidisable functional groups [12]. In aqueous solution, however, singlet oxygen is rapidly quenched by H_2O. Thus, singlet oxygen is expected to be a minor contribution to degradation of DOM in natural systems, but may very well play an important role during artificial UV-digestion processes.

Although detailed mechanisms involving secondary processes are complex, the high concentration of O_2 in sea water and its reactivity as an energy transfer acceptor (i.e. singlet oxygen formation) and with free radicals (odd or unpaired electron species) suggests that many secondary processes will yield partially oxidised products that are especially susceptible to further oxidation.

The free radical reactions are diverse, numerous pathways are leading to the production of a single product: the superoxide radical. Swallow [13] calculated that hydrated electrons (formed by charge transfer reactions between DOM as a donor and water as an acceptor) in sea water form superoxide radicals:

$$e^-_{aq} + O_2 \leftrightarrow O_2^{\cdot -} \quad (2)$$

while electrons caught by CO_2 also end up as superoxide [14]:

$$CO_2^{\cdot -} + O_2 \leftrightarrow CO_2 + O_2^{\cdot -} \quad (3)$$

Superoxide can act both as oxidising and reducing agent in reaction with various organic compounds [15]. However in the absence of sufficient quantities of reactive substrates it should rapidly disproportionate:

$$2O_2^{\cdot -} + 2H^+ \leftrightarrow H_2O_2 + O_2 \quad (4)$$

The existence of such processes are supported by significant concentrations of H_2O_2 measured in sea water [9].

Finally, free radical auto-oxidation is believed to be an important mechanism responsible for natural photo-degradation of DOM in both freshwater and sea water [14]. Free radicals are generated by direct photolytic cleavage and by photo-induced charge transfer reactions of organic and inorganic species. A major product of these reac-

tions in water is the hydroxy radical, which in turn initiates organic auto-oxidation [14,16,17].

$$OH^{\cdot} + RH \rightarrow R^{\cdot} + HOH \quad (5)$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (6)$$

$$Ar^{\cdot} + O_2 \rightarrow ArOO^{\cdot} \quad (7)$$

$$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot} \quad (8)$$

Auto-oxidation processes result in the net oxidation of organic compounds and the reduction of oxygen. Especially when structures such as aromatic rings are interrupted by a radical addition, subsequent reactions may almost completely oxidise the molecule, often generating more radicals in the process. It can therefore be speculated that organic radicals lead to their own photo-catalysed destruction by way of secondary processes [9].

In this study experiments are described showing the effectiveness of in-line UV-digestion. The effects of the initial DOM concentration, sample digestion time, presence of H_2O_2 and oxygen on the efficiency of the breakdown of organic material are illustrated. The results of in-line UV-digestion are compared with conventional batch-wise UV-digestion.

The investigations involved monitoring of breakdown of humic acid using fluorescence detection [18]. Humic acid was chosen as an indicator compound for UV digestion because it is a good example of a complex organic chromophore and is well known for its refractory nature [19]. These workers have shown that humic acids consist of a three dimensional array of aromatic rings that have numerous functional groups and side chains. Hence, humic substances are a mixture of polydisperse molecules, of which a significant portion fluoresce, which means that upon excitation these compounds emit light at a different wavelength (~ 420–460 nm) [20].

Furthermore, experiments are presented to test the release of Cu and Ni from organic complexes in sea water, the breakdown of humic acids interfering with Cr analysis and the conversion of Cr(III) to Cr(VI). In addition, the use of in-line UV-digestion in an automated voltammetric system is described and examples of oceanographic

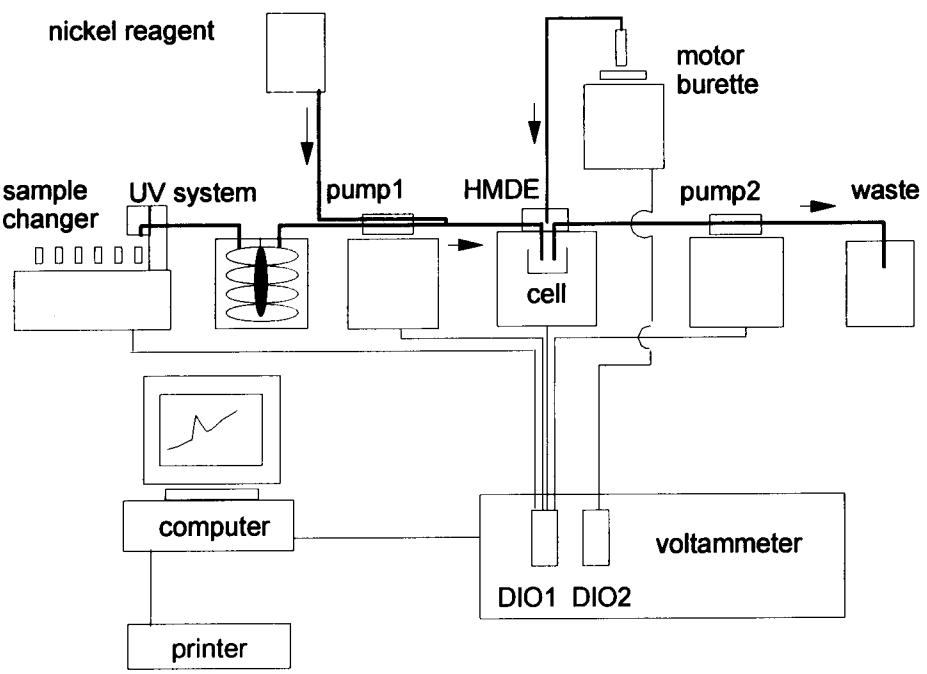

Fig. 1. Scheme of the automated voltammetric system with in-line UV-digestion unit.

depth profiles showing nanomolar levels of Cu and Ni, determined with such a system are presented.

2. Experimental

2.1. Equipment

The apparatus used for in-line UV-digestion consisted of a medium pressure mercury discharge tube (100 W; Hanovia) and a silica coil (i.d., 1.0 mm; length, 4.4 m; volume, 3.47 ml), both placed in an aluminium housing (width × depth × height: 0.1 × 0.1 × 0.2 m; home-built). The mercury lamp emitted the entire mercury line spectrum from 185 nm, including an emission peak at 254 nm. The lamp was cooled by a fan (RS, 12 W), and both fan and lamp were run from a home-built power supply. During the investigations in the effectiveness of in-line UV-digestion, sample aliquots were pumped through the silica coil using a peristaltic pump (Gilson, Minipuls 3).

Voltammetric instruments consisted of an Autolab voltammeter (Eco Chemie) and a Metrohm hanging mercury drop electrode (HMDE, 663 VA Stand, drop surface area: 0.38 mm^2). The volume of the voltammetric cell of the HMDE was 10 ml. In the case of automated voltammetric determination of trace metals using in-line UV-digestion, the UV lamp was placed in between the sample changer and the peristaltic pump (Fig. 1). Details about the components and working of the automated voltammetric system can be found in elsewhere [21].

The silica coil was replaced by a stand for 5 home-made 30 ml silica tubes for batch-wise UV-digestions. The UV system was upgraded using a 1000 W medium pressure mercury arc tube (Hanovia), placed in a home-built aluminium housing (0.25 × 0.25 × 0.6 m) and cooled by a Parvalux fan for comparative batch-wise irradiation. The 1000 W mercury lamp emitted a similar light spectrum as the 100 W lamp, with a higher intensity. Up to 10 large and 8 small silica tubes, holding respectively 100 and 30 ml could be irradiated simultaneously in this system.

A Perkin Elmer LS-5 luminescence spectrometer was used with a 1 cm quartz cell for the determination of the fluorescence of the humic acid. The C, H and N composition of the humic acid was determined using a Carlo Erba elemental analyzer (Model 1106).

2.2. Chemicals

Water purified by reverse osmosis (Milli-RO, Millipore) and de-ionisation (Milli-Q (MQ), Millipore) was used for rinsing and to prepare reagents. All chemicals were purchased from BDH (AnalaR quality), unless indicated differently. Acidified samples were neutralised prior to voltammetric analysis using 9 M NH_3 (quartz-distilled).

A stock solution of 0.1 M tropolone (Aldrich) was prepared in methanol (quartz distilled) for the manual voltammetric determination of Cu. A pH buffer for Cu determinations contained 1 M boric acid (Aristar grade) and 0.35 M ammonia, giving rise to a pH of 8.35 upon 100-fold dilution with sea water. An aqueous stock solution of 0.01 M oxine was prepared in 0.25 M HCl. An aqueous stock solution of 1 M HEPES–0.5 M ammonia was prepared, giving rise to a pH of 7.8 upon 100 fold dilution with sea water.

A mixed aqueous reagent was prepared containing 0.2 mM oxine, 200 mM HEPES buffer, 10 mM hydroxylammonium chloride and 0.15 M ammonia for determination of total Cu using the automated voltammetric system with in-line UV-digestion. A high level of ammonia was used to neutralise acidified sea water samples and the hydroxylammonium chloride was used to reduce hypochlorite, which was formed during the UV-digestion of acidified sea water. Addition of 0.625 ml of this solution to 10 ml sample gave the required oxine concentrations (12.5 μM) and pH (pH 7.7), whereas Cu contamination due to the addition was < 0.08 nM. Standard solutions of Cu were prepared by dilution of an atomic absorption standard solution and were acidified to pH 2.5 using 6 M HCl (quartz-distilled).

For the manual determination of Ni, a stock solution of 0.1 M DMG (dimethyl glyoxime) was prepared in methanol (quartz distilled). The pH

buffer contained 1 M boric acid (Aristar grade) and 0.35 M ammonia, giving rise to a pH of 8.3 upon 100-fold dilution with sea water. Ni contamination due to addition of DMG and buffer was < 0.06 nM.

For fully automatic determinations of total Ni a mixed aqueous reagent was prepared containing 3.2 mM DMG and 0.18 M ammonia. Addition of 0.625 ml of this solution to 10 ml of acidified (pH 2) sea water gave the required DMG concentration (0.2 mM) and pH (pH 9), whereas Ni contamination due to the addition was < 0.08 nM. Standard solutions of Ni were prepared by dilution of an atomic absorption solution and were acidified to pH 2.5 using 6 M HCl (quartz-distilled).

An aqueous solution containing 25 mM DTPA (Sigma), 5 M $NaNO_3$ and 0.5 M CH_3COONa was prepared to determine Cr. Cr contamination (comprising Cr(VI) and Cr(III)) was removed from a solution containing 5 M $NaNO_3$ and 0.5 M CH_3COONa by co-precipitation with iron(III) hydroxide added as iron(II) chloride (10^{-4} M) prior to the addition of the DTPA. The precipitate was removed from the solution by filtration using a 0.45 μm cellulose acetate membrane filter [22]. Addition of 1 ml of the Cr reagent to 10 ml of sample aliquot (not acidified) gave the required pH (pH 5.3 in sea water and pH 5.7 in MQ) and reagent concentration, whereas Cr(VI) contamination due to the addition was < 0.01 nM.

A suspension of 100 g l^{-1} LiChrosorb Si 60 silica (particle size 5 μm, surface area 490 m^2 g^{-1}; Merck) was used to remove Cr(III) from sample aliquots prior to the determination of Cr(VI) [21]. A quantity of 100 μl of this suspension was added to 25 ml of an aliquot at least 20 min before analysis. Cr(III) contamination was removed from the LiChrosorb silica by soaking overnight in 5 M HCl, followed by rinsing with MQ and four-fold UV irradiation and centrifugation. Cr(VI) contamination due to addition of the silica at a level of 0.4 g l^{-1} was < 0.01 nM.

Standard solutions of Cr(VI) were prepared by dissolving K_2CrO_4 in water. Standard solutions of Cr(III) were prepared by dilution of an atomic absorption standard solution and were acidified to pH 2.5 using 6 M HCl (quartz – distilled). Fresh standard solutions of Cr(III) were prepared daily. Measurements of Cr(VI) in diluted aqueous solutions of 1.6 nM Cr(III) indicated the presence of less than 0.01 nM Cr(VI), i.e. less than 0.5%.

A stock solution of 1 g l^{-1} humic acid (EGA Chemie, soil type) was prepared by dissolution in water and was stored in the refrigerator. This humic acid had the following elemental composition: 0.38% N, 39.4% C, 4.0% H. Dilutions were prepared daily (pH 7.2).

2.3. Experimental procedures for breakdown of humic acid by UV-digestion

The effectiveness of batch-wise UV-digestion was tested using a 100 W as well as a 1000 W UV system. Humic acid solutions of 3.8, 7.75 and 15.5 mg C l^{-1} in MQ were irradiated during these experiments and fractions were collected at certain time intervals. Residual humic acid was detected by fluorescence at excitation and emission wavelengths of 310 and 460 nm, respectively.

Humic acid solutions (3.8, 7.75 and 15.5 mg C l^{-1}, respectively) in MQ and sea water were treated by in-line UV-digestion at different pumping speeds, and fractions were collected. Comparative in-line UV-digestions were carried out in the presence of H_2O_2, which was added to the humic acid solutions prior to UV-digestion in order to improve the breakdown process [8]. In addition, in-line UV-digestion experiments were carried out at different temperatures and oxygen levels.

2.4. Experimental procedures for breakdown of trace metal complexing ligands by UV-digestion

Sea water sampled (using clean sampling procedures) in the Menai Straits, the North Atlantic Ocean (RV Challenger cruise, 1991, station 10, 1000 m) and Western Mediterranean (FS Valdivia cruise, 1992, station 9, 800 m) was used to investigate the effectiveness of the in-line UV-digestion. Metal concentrations were determined in the sea water samples by voltammetry after the UV-digestion at various conditions with respect to digestion time and pH.

Sea water aliquots were pumped through the digestion coil at different rates, collected and subsequently analysed manually for Cu, Ni or Cr. Aliquots for Cr and Cu (to be analysed using tropolone) were irradiated at neutral pH (pH 8.1), whereas aliquots for Ni and Cu (to be analysed using oxine) were irradiated at pH ~ 2.5.

To obtain a measure of the in-line digestion efficiency, conventional batch-wise irradiation was also used for total metal determinations. In this case total Cr, Ni and Cu concentrations were determined manually after batch-wise UV-digestion (100 W system) for 4.5 h. UV-digestion of sample aliquots for Cr took place at neutral pH, whereas aliquots for Ni and Cu were digested at pH ~ 2.5 in order to avoid losses to the walls of the silica tubes which were acid-precleaned.

Labile metal concentrations were determined in sea water without prior UV-digestion to indicate the trace metal levels that can be complexed in the sample by the added ligand (i.e. tropolone and oxine for Cu, DMG for Ni and DTPA for Cr) in the presence of competing natural complexing matter.

2.5. Electrochemical procedures for manual determination of Ni, Cu, Cr and hypochlorite

The following electrochemical procedure was used for the manual total Ni determinations [23,24]. DMG and borate buffer were added to a sample aliquot resulting in a final concentration of 0.2 mM and 10 mM, respectively. The voltammetric cell was de-aerated for 3 min using water-saturated N_2, subsequently 4 new mercury drops were made and after the extrusion of the fifth mercury drop the adsorption period was initiated. Adsorption of the Ni–DMG_2 complex on the HMDE was carried out for a period of 60 s, whilst stirring the solution with the potential set at −0.8 V. Then the stirrer was stopped and a quiescence period of 8 s was allowed, followed by the potential scan carried out using square-wave modulation at a frequency of 300 Hz, a modulation amplitude of 25 mV and a step height of 2.4 mV. The scan direction was negative and the reduction peak corresponding with Ni appeared at −1.0 V.

Manual Cu determinations were performed as follows [25]: tropolone and borate buffer (or oxine and HEPES buffer) were added to an aliquot of 10 ml, resulting in final concentrations of 0.4 mM tropolone and 10 mM borate (20 μM oxine and 10 mM HEPES). Adsorption of the Cu-tropolone (Cu-oxine) complex on the HMDE was carried out for a period of 90 s at −0.9 V. A quiescence period of 15 s was used at −0.1 V. The potential scan was started at −0.1 V, using the square-wave modulation (200 Hz). The scan direction was negative and the reduction peak corresponding with Cu appeared at −0.3 V for tropolone and −0.42 V for oxine.

In the case of manual Cr determinations, 1 ml of Cr reagent was added to an aliquot of 10 ml, resulting in a final concentration of 2.5 mM DTPA, 0.5 M $NaNO_3$ and 50 mM CH_3COONa. Adsorption of the Cr–DTPA complex onto the HMDE was carried out at −1 V for 40 s, whilst stirring. A quiescence period of 15 s was used at −1 V. The potential scan was started at −1 V, using the square-wave modulation at a frequency of 100 Hz. The scan direction was negative and the reduction peak corresponding with Cr appeared at −1.2 V.

Hypochlorite was determined manually in sea water and MQ in the presence of 10 mM HEPES buffer. After 4 min de-aeration, a new drop was made and the potential was set to −1 V for 2 s, whilst the solution was stirred. A quiescence period of 8 s was used at −0.1 V. The potential scan was started at −0.1 V, using the square-wave modulation (100 Hz). The scan direction was negative and the reduction peak corresponding with hypochlorite appeared at −0.4 V.

2.6. Procedures for automated determination of Ni and Cu

Total dissolved Cu and Ni were measured fully automatically in acidified samples from the North Atlantic and Western Mediterranean using in-line UV-digestion. A pumping speed of ca. 0.65 ml min^{-1} was applied, resulting in a digestion time of the sample in the UV coil of 320 s. Mixing of the sample with Ni and Cu reagent flows took

place after the UV lamp, at a rate of 16:1 (sample:reagent) resulting in a DMG concentration of 0.2 mM (for Ni), and an oxine concentration of 12.5 μM and a hydroxylammonium chloride concentration of 0.63 mM (for Cu). The electrochemical conditions used for the automated Ni and Cu determinations are similar to those described above for their manual analysis.

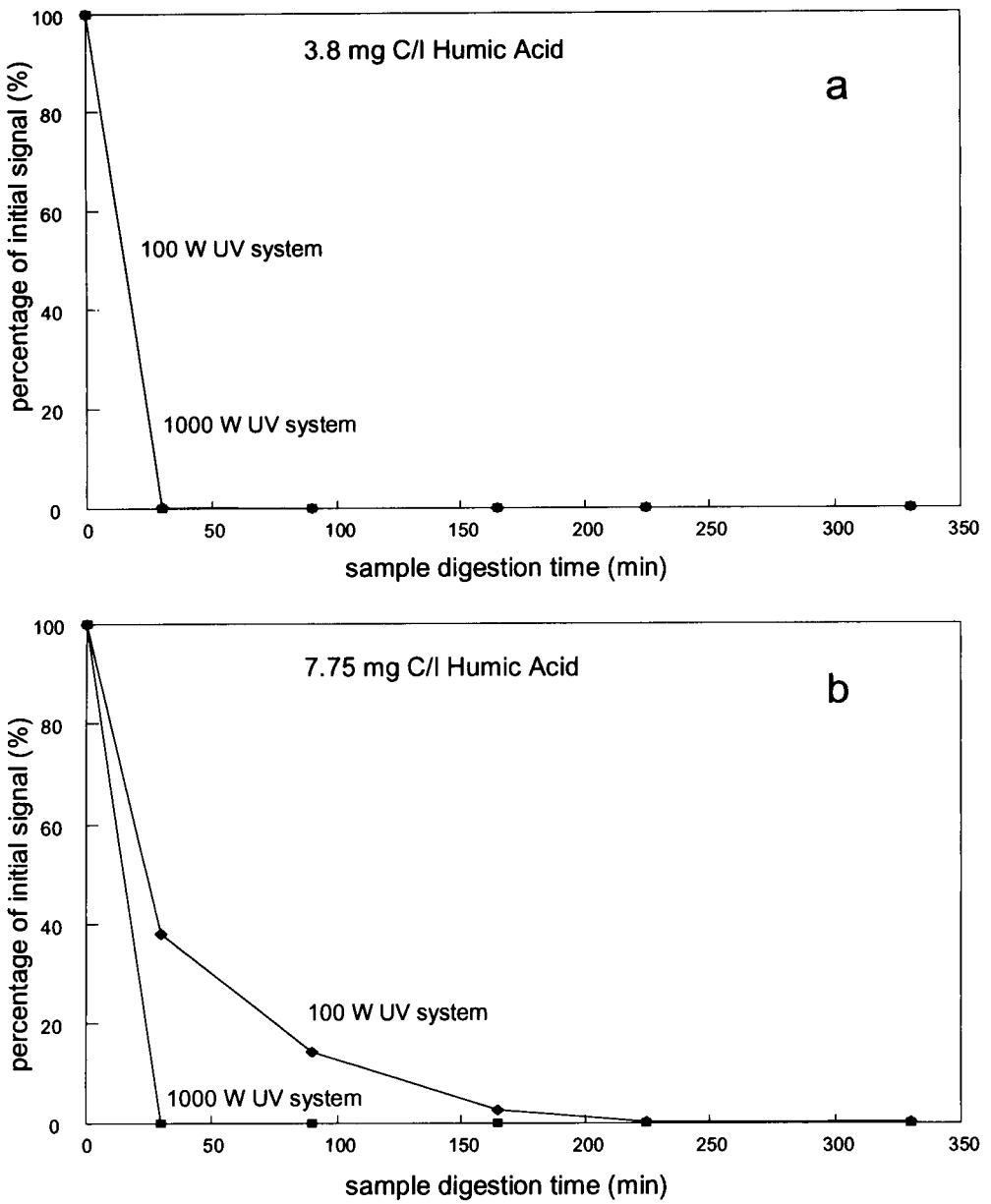

Fig. 2. Comparative batch-wise UV-digestion of humic acid in MQ using 100 W and 1000 W lamps. The humic acid concentrations were 3.8 (a), 7.75 (b) and 15.5 (c) mg C l^{-1}.

3. Results and discussion

3.1. Batch-wise UV-digestion of humic acid

The breakdown of humic acid solutions of 3.8, 7.75 and 15.5 mg C l^{-1} in MQ using conventional batch-wise UV-digestion is shown in Fig. 2a–c. The humic acid solutions had a strong yellow colour before irradiation. The colour vanished upon the UV-digestion. The residual fluorescence was used as an indication for survival of the humic acids. Using this method, it was possible to monitor the degradation of humic acids at carbon concentrations as low as 0.004 mg C l^{-1}, which is difficult to measure using a DOC analyser. Both the 100 W and the 1000 W UV-digestion systems completely broke down the humic acid solution containing 3.8 mg C l^{-1} within 0.5 h of irradiation (Fig. 2a). Resulting humic acid levels, based on fluorescence measurements, were below 0.004 mg C l^{-1}. Higher levels of humic acid (7.75 mg C l^{-1}) were fully broken down by the 1000 W system within 0.5 h (residual carbon concentration < 0.004 mg C l^{-1}), whereas the 100 W system needed 4 h to accomplish humic acid levels < 0.014 mg C l^{-1} (Fig. 2b). The destruction of the 15.5 mg C l^{-1} humic acid solution was more difficult for both systems: the 1000 W system needed at least 1.5 h to break the humic material down to 0.008 mg C l^{-1} (Fig. 2c), whereas no humic acid could be traced after 5.5 h (< 0.004 mg C l^{-1}). Using the 100 W system, an irradiation time of 5.5 h resulted in $> 99\%$ breakdown of a humic acid concentration of 15.5 mg C l^{-1} (residual carbon concentration < 0.05 mg C l^{-1}).

3.2. In-line UV-digestion of humic acid

The in-line UV-digestion was more efficient than the batch-wise method. A breakdown of $> 99\%$ was achieved for humic acid solutions of 3.8, 7.75 and 15.5 mg C l^{-1} in MQ, after a sample digestion time of only 100 s (Fig. 3a). Fig. 3a shows that complete destruction of a 3.8 mg C l^{-1} humic acid solution was achieved within 100 s (residual carbon concentration < 0.004 mg C l^{-1}). A 99.7% breakdown (residual carbon concentration 0.02 mg C l^{-1}) was achieved for an 7.75 mg

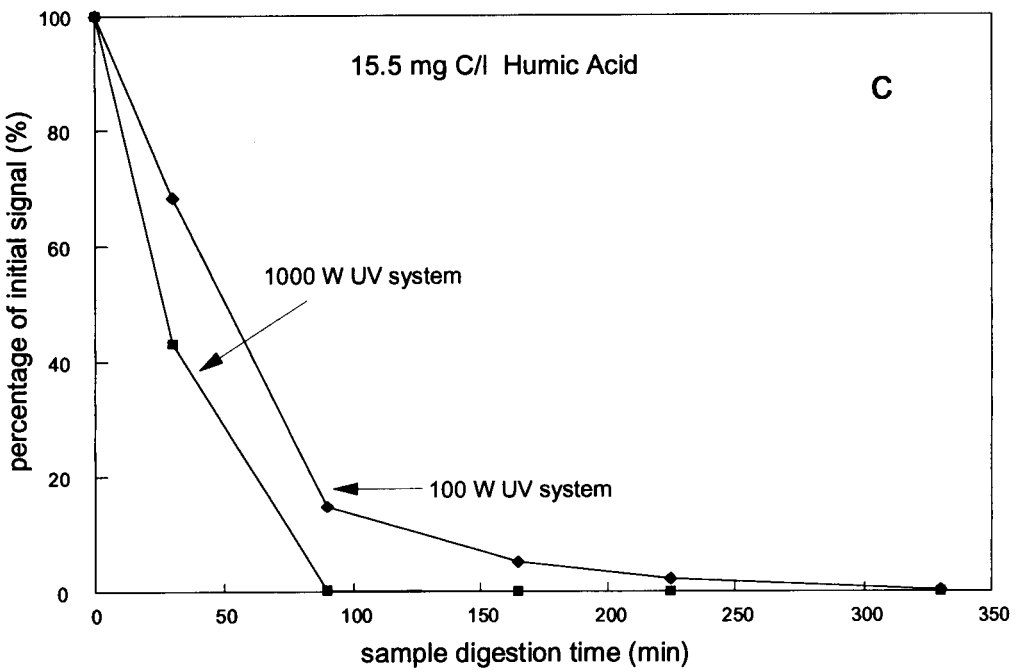

Fig. 2 (continued).

C l^{-1} solution after 275 s, and a 99.5% breakdown (residual carbon concentration 0.08 mg C l^{-1}) for an 15.5 mg C l^{-1} solution after 275 s.

Fig. 3b shows in-line UV-digestion of humic acid solutions in Mediterranean sea water. In sea water 98–99.5% breakdown was achieved for the three humic acid solutions, after a sample digestion time of 275 s, resulting in residual carbon levels of 0.03 mg C l^{-1} (3.8 mg C l^{-1} initial concentration), 0.1 mg C l^{-1} (7.75 mg C l^{-1} initial concentration) and 0.3 mg C l^{-1} (15.5 mg C l^{-1} initial concentration). The residual levels

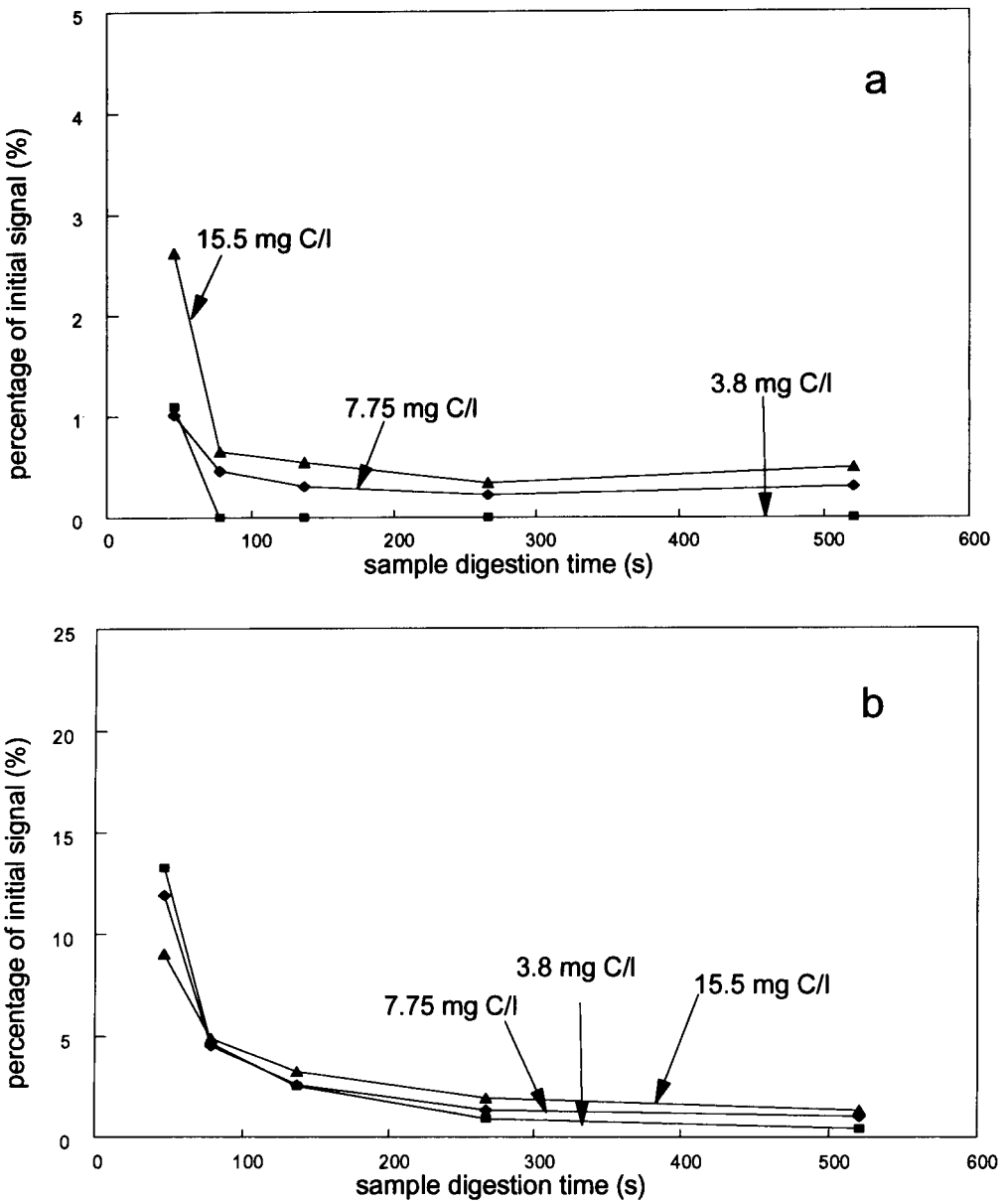

Fig. 3. In-line UV-digestion of humic acid (3.8, 7.75 and 15.5 mg C l^{-1}) in MQ (a) and sea water (b). The sample aliquots were oxygenated prior to treatment and hydrogen peroxide (9 mM) was added.

were higher in the sea water than in MQ, indicating that UV-digestion process is apparently less effective in a saline medium.

Dissolved oxygen and H_2O_2 were added to sample aliquots in order to improve the efficiency of in-line UV-digestion. Oxygen was bubbled (4 min) into the solution, immediately prior to the UV-digestion (no H_2O_2 was added during these experiments), and was compared with de-oxygenated samples, by bubbling nitrogen (4 min) into the solution. Comparative experiments were carried out using additions of 9 mM H_2O_2 to

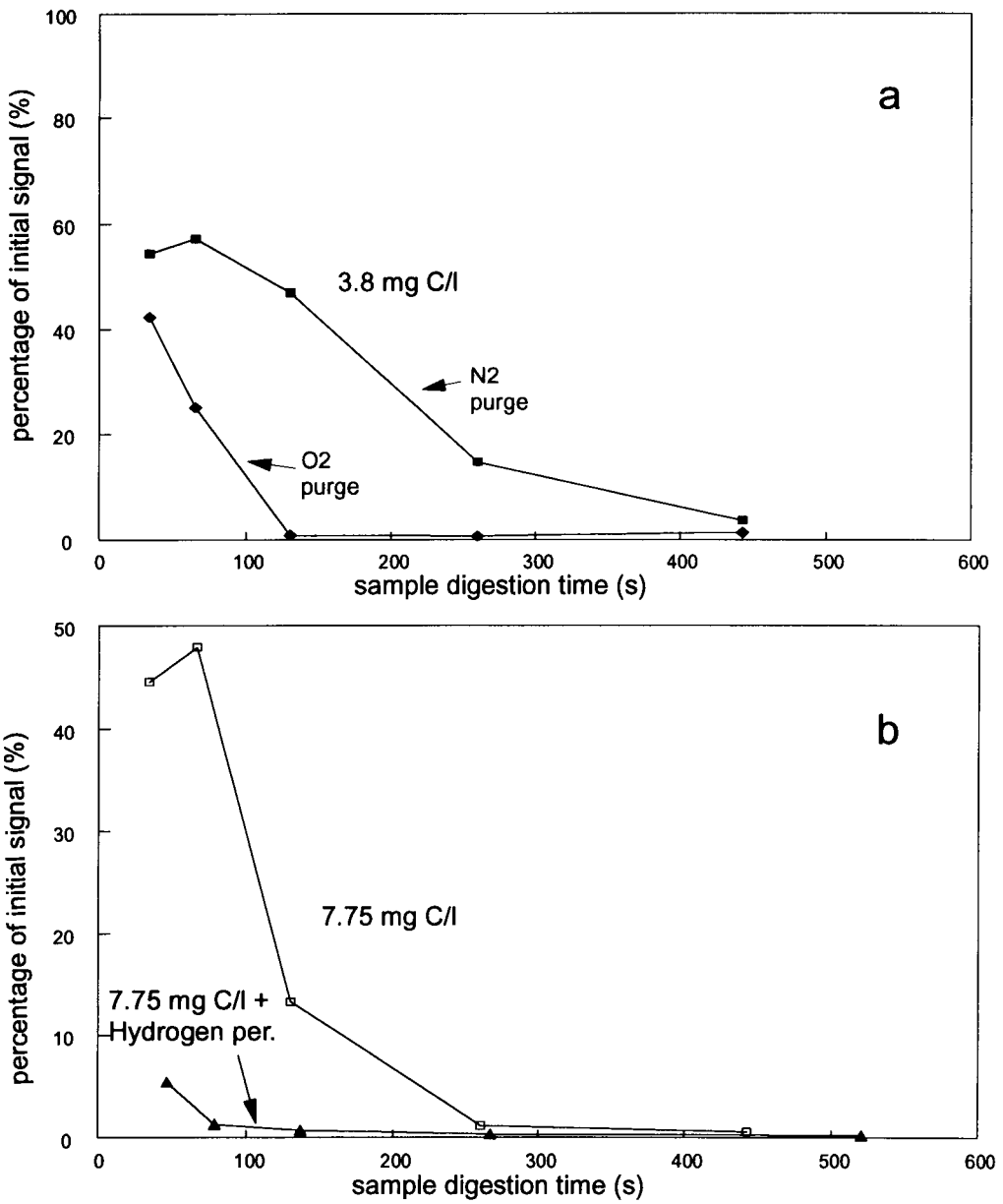

Fig. 4. The effect of oxygen (a), hydrogen peroxide (9 mM) (b) and temperature (c) on the in-line UV-digestion of humic acid in MQ.

humic acid solutions in MQ prior to UV-digestion. Effects of such additions are shown in Fig. 4a and b.

The efficiency of in-line UV-digestion was drastically improved by enhanced levels of oxygen (Fig. 4a). In the presence of oxygen, a sample digestion time of less than 125 s was required to reach 99% breakdown of a humic acid solution of 3.8 mg C l^{-1} in MQ (residual carbon concentration 0.04 mg C l^{-1}). Whereas with de-oxygenation, the residual humic acid fraction amounted to 46% (residual carbon concentration 1.75 mg C l^{-1}) after a similar digestion time.

Addition of 9 mM H_2O_2 to a humic acid solution in MQ of 7.75 mg C l^{-1} resulted in > 99% breakdown after a sample digestion time of 130 s (residual carbon concentration 0.06 mg C l^{-1}) (Fig. 4b). In the absence of a H_2O_2 addition, the residual humic acid fraction amounted to 13% (residual carbon concentration 1.0 mg C l^{-1}) after a similar digestion time. The addition of H_2O_2 therefore resulted in increased efficiency of in-line UV-digestion.

Variation of the sample temperature during in-line UV-digestion by partially closing the air inlet to the fan of the UV unit indicated that the effectiveness of in-line UV-digestion of humic material is enhanced by running the UV system at a high temperature (~ 70°C instead of ~ 25°C; sample aliquots were oxygenated prior to treatment) (Fig. 4c). At high temperature (70°C) a sample digestion time of 130 s resulted in 97.5% breakdown of a humic acid solution of 3.8 mg C l^{-1} in MQ (residual carbon concentration 0.10 mg C l^{-1}). Whereas, at the lower (25°C) temperature the residual humic acid fraction was 10% (residual carbon concentration 0.4 mg C l^{-1}), after a similar digestion time.

3.3. Formation of hypochlorite

Photochemical processes in sea water are still poorly understood. The formation of singlet oxygen and superoxide, and radical formation of organic molecules leading to their self-destruction have been reported [14]. Possible oxidation of chloride to chlorine has also been mentioned [14], but the role and fate of chloride in sea water

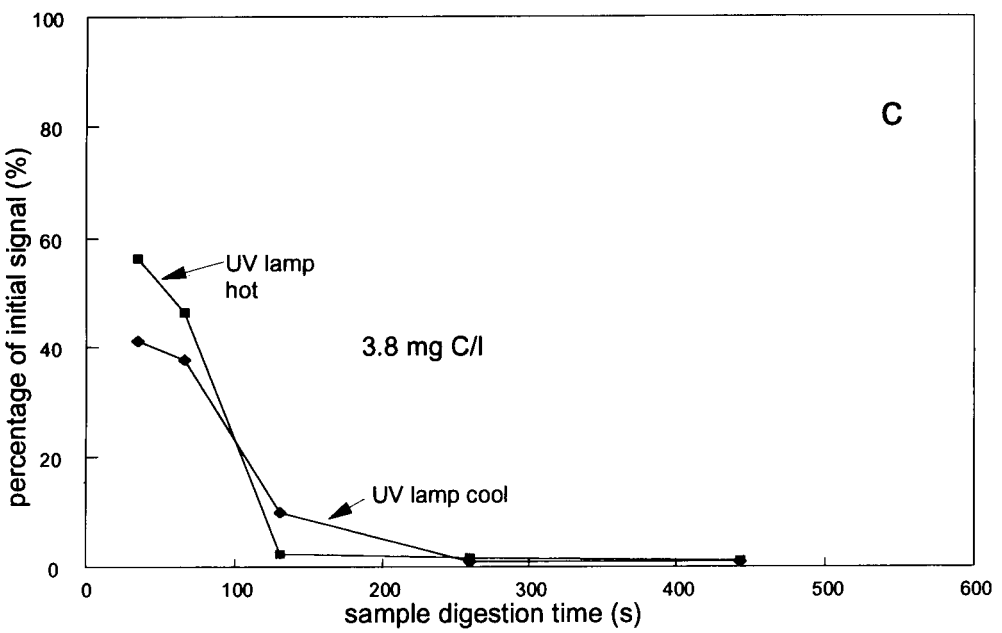

Fig. 4 (continued).

has not been examined in detail, despite its high reactivity, high concentration and strong nucleophilic character (stronger than water).

Preliminary experiments to evaluate the UV-digestion of organically complexed Cu indicated the formation of an unknown broad peak at −0.4

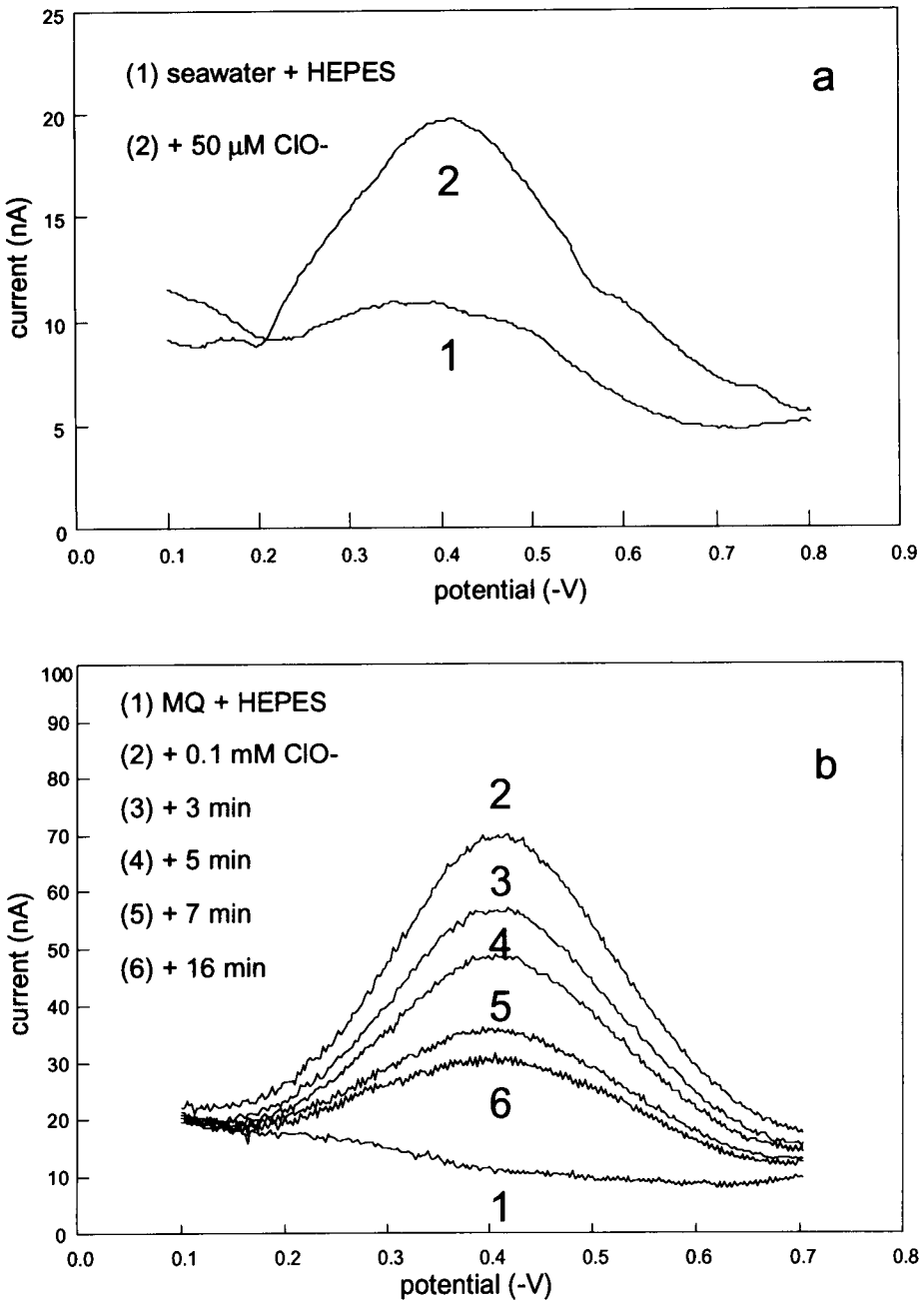

Fig. 5. Voltammetric scans of hypochlorite (a). Scan 1: hypochlorite produced during in-line UV-digestion of acidified Atlantic sea water (pH 2.2). Scan 2: sample plus standard addition of 50 μM hypochlorite. Scans made at pH 7.7 (HEPES buffer). (b) Time series of voltammetric scans showing reduction of 0.1 mM hypochlorite in MQ.

V, which seriously interfered with the cathodic stripping voltammetric (CSV) determination of Cu. Further tests indicated that this peak was due to hypochlorite which was apparently formed during the UV-digestion of acidified sea water (pH 2–2.5). Fig. 5a shows voltammetric scans of

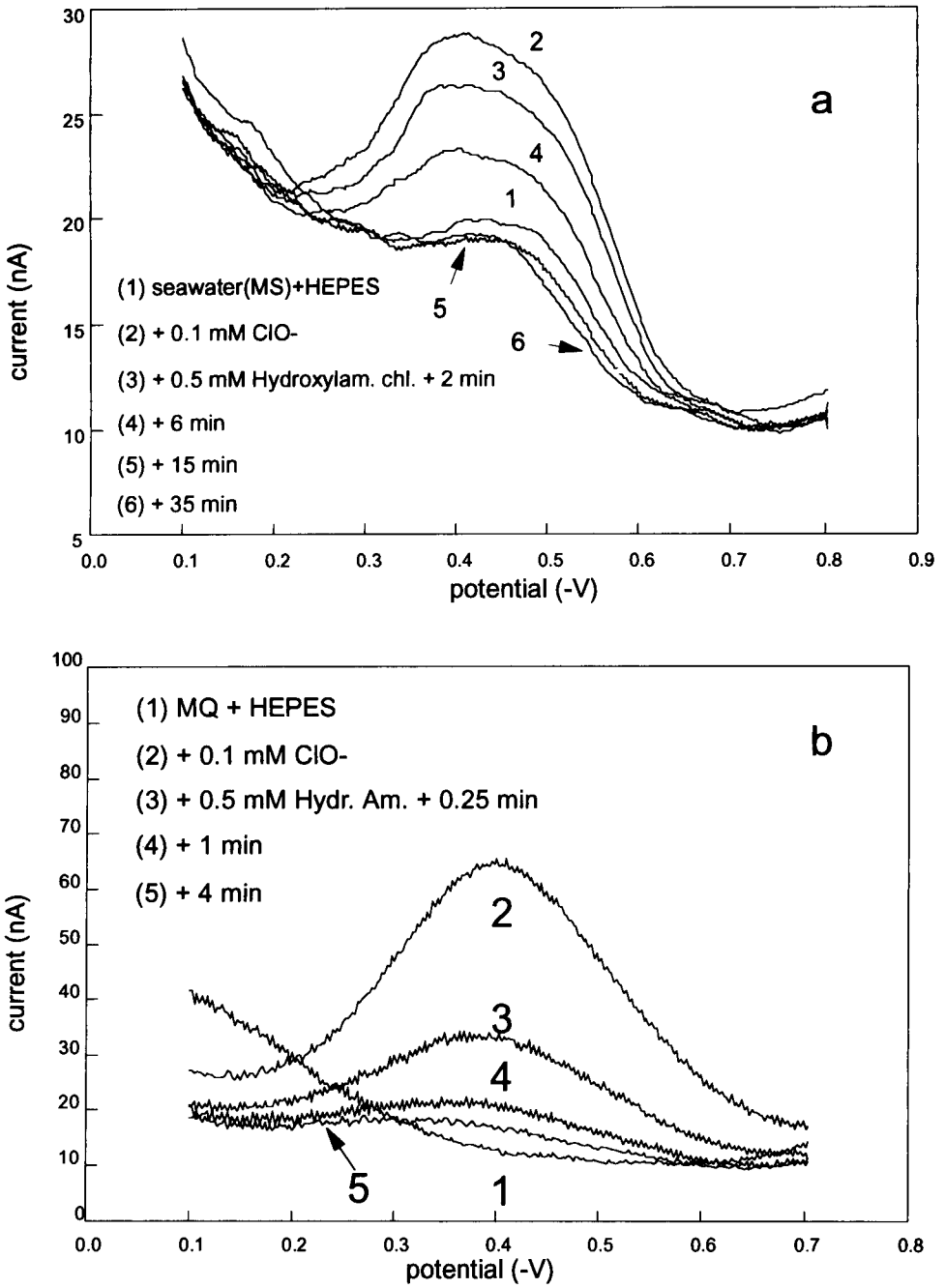

Fig. 6. Time series of voltammetric scans in sea water (a) and MQ (b) showing reduction of 0.1 mM hypochlorite by 0.5 mM hydroxylammonium chloride (pH 7.7).

hypochlorite produced in a sea water aliquot from the North Atlantic, irradiated at pH 2.2 using in-line UV-digestion. The hypochlorite peak was due to diffusion current as the peak was not enhanced by adsorption. The level of hypochlorite in the aliquot, immediately upon irradiation, was ca. 20 μM.

The hypochlorite appeared to be thermodynamically unstable as the reduction peak disappeared gradually. The reduction of 0.1 mM hypochlorite added to MQ, (pH 7.7 with HEPES buffer) is shown in Fig. 5b. Approximately 50% of the hypochlorite was reduced in MQ in a period of 6 min, whereas comparative experiments using pH 7.7 sea water indicated a 50% reduction requiring a period of several hours, suggesting that the reduction is much slower in sea water. Hypochlorite levels of 0.1 mM in sea water had decreased to $\sim$ 64 μM after 2 h, and the reduction was completed overnight (after 18 h).

Comparative experiments indicated that the formation of hypochlorite occurred during UV-digestion of acidified sea water in all types of UV systems (in-line, batch, 100 W and 1000 W). In the case of batch-wise irradiation, the hypochlorite production was more pronounced in the 1000 W system than in the 100 W system. This is probably due to the more intense irradiation of sample aliquots in the 1000 W UV system. In-line UV-digestion of acidified sea water samples caused the most pronounced production of hypochlorite. This may be explained by the intense irradiation of the sample in the silica coil and analysis of the sample aliquots immediately upon irradiation with little time for the reduction of the hypochlorite. UV-digestion of sea water at natural pH (pH $\sim$ 8) did not lead to the formation of hypochlorite, indicating that the pH is an important variable during the oxidation of chloride.

Hypochlorite formation during UV-digestion at low pH interfered with the determination of Cu, due to the similar peak potential (peak potential of Cu using CSV is ~ -0.3 to -0.4 V). The hypochlorite did not interfere with the Ni determination as the reduction peak for the Ni-DMG_2 complex appeared at ~ -1.0 V. The interference by hypochlorite was eliminated by addition of hydroxylammonium chloride to sea water. Fig. 6a and b shows the rapid disappearance of 0.1 mM hypochlorite after addition of 0.5 mM hydroxylammonium chloride to sea water and MQ, respectively. The hypochlorite disappeared from the sea water within ca. 15 min, whereas it disappeared within ca. 4 min from MQ (Fig. 6), after addition of hydroxylammonium chloride. Therefore, hydroxylammonium chloride (final concentration of 0.5 mM) was added to the UV digested sample flow along with the Cu reagent. The hypochlorite (formed during the in-line UV-digestion) was then reduced by the hydroxylammonium chloride during transport to and inside the voltammetric cell (total time $\sim$ 14 min) and during the purging of the sample with N_2 (4 min).

3.4. *In-line UV-digestion of oceanic and coastal waters*

Total dissolved Cu, Ni and Cr concentrations were determined in sea water samples in comparative experiments using batch-wise and in-line UV-digestion. A long period (4.5 h) of UV-treatment was used to ensure full batch-wise digestion with which the in-line digestion was compared.

Table 1
Total and labile trace metal concentrations in different oceanic and coastal waters (the total metal concentrations were determined after batch-wise UV-digestion (4.5 h) in a 100 W system. The labile levels were measured without prior sample treatment)

	total Ni (nM)	labile Ni (nM)	total Cu (trop.) (nM)	labile Cu (trop.) (nM)	total Cu (oxine) (nM)	labile Cu (oxine) (nM)	total Cr (nM)	labile Cr (nM)
Atlantic	3.76	3.34	1.85	0.69	ND	ND	ND	ND
Mediter.	3.28	2.08	2.08	0.78	2.03	1.66	1.91	1.64
Menai S.	9.00	5.80	16.3 [a]	4.49 [a]	10.7 [b]	5.4 [b]	1.81	1.47

[a,b] Sampled at different dates in the Menai Straits; ND = not determined.

The labile and total concentrations of Cu, Ni and Cr (before and after batch-wise UV-digestion) for water samples from the North Atlantic Ocean, Western Mediterranean Sea and Menai Straits are shown in Table 1. The difference between the total and labile trace metal fraction is the non-la-

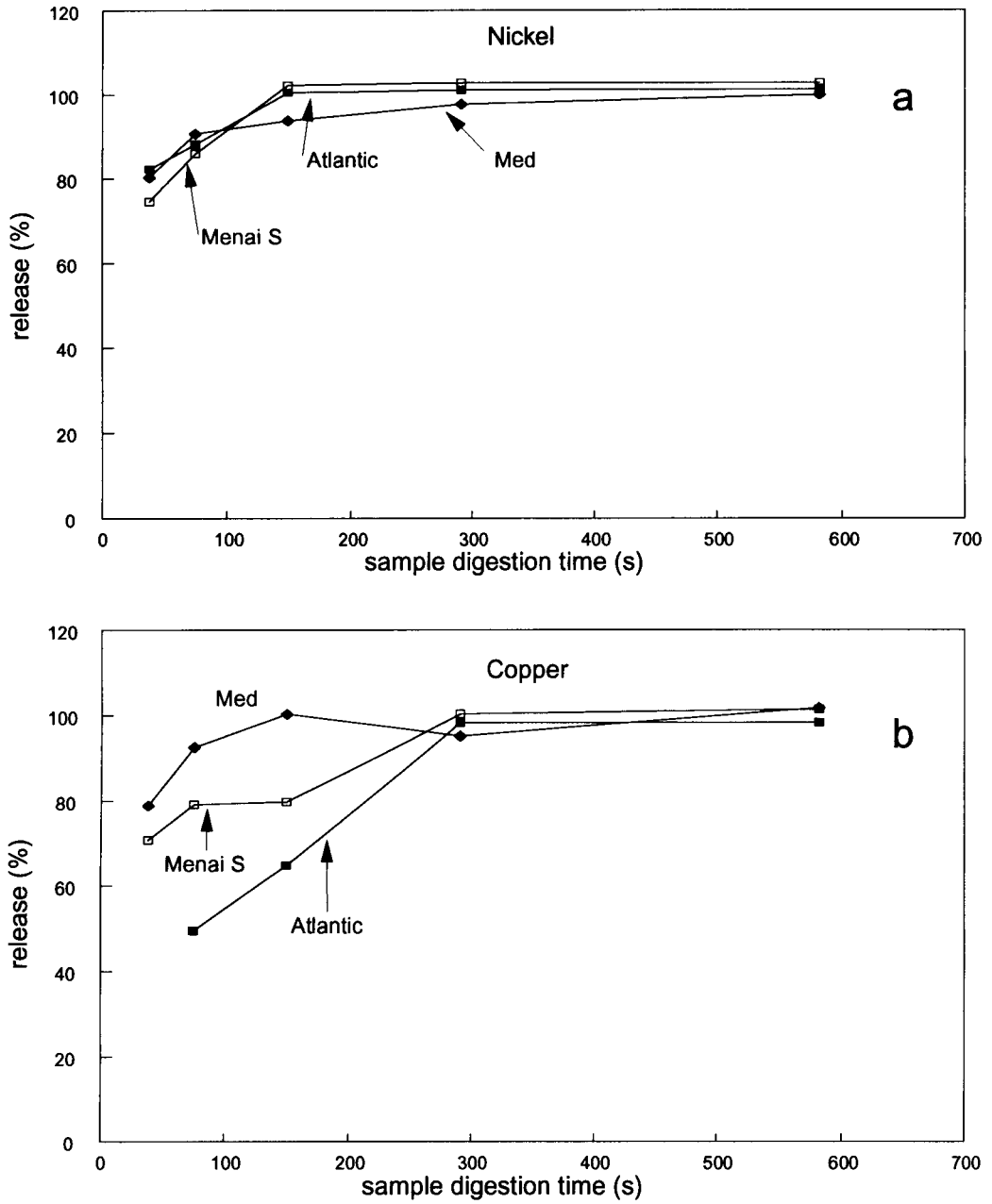

Fig. 7. Effect of in-line UV-digestion time on the release of Ni (a) and Cu (b and c) from organic complexes in coastal and oceanic sea waters. Cu was determined either using tropolone (b) as the added ligand after UV-digestion at natural pH (pH 8) or using oxine as the added ligand, in the presence of hydroxylammonium chloride (0.5 mM), after UV-digestion at pH 2.5 (c). (d) Effect of UV-digestion on the concentration of Cr in sea water as a function of time. The conversion of additions of 1.6 nM Cr(III) to Cr(VI) is also shown.

bile metal fraction, which is the fraction that is released by UV-digestion of sample aliquots. Previous work has shown that the non-labile fraction of Cu and Ni are complexed by natural organic matter [1,2], whereas that of Cr is Cr(III) [21,22].

The non-labile Ni fractions in the Atlantic, Mediterranean and Menai Straits samples amounted to 11%, 36% and 36% of the total Ni concentrations, respectively. Non-labile Cu fractions of 63%, 63% and 72% were obtained using tropolone in the Atlantic, Mediterranean and Menai Straits waters, respectively, whereas the non-labile fractions amounted to 18% and 50% in the Mediterranean and Menai Straits samples, respectively, when Cu was determined using oxine. The smaller non-labile Cu fractions obtained using oxine compared with tropolone are due to differences in complex stability: oxine forms more

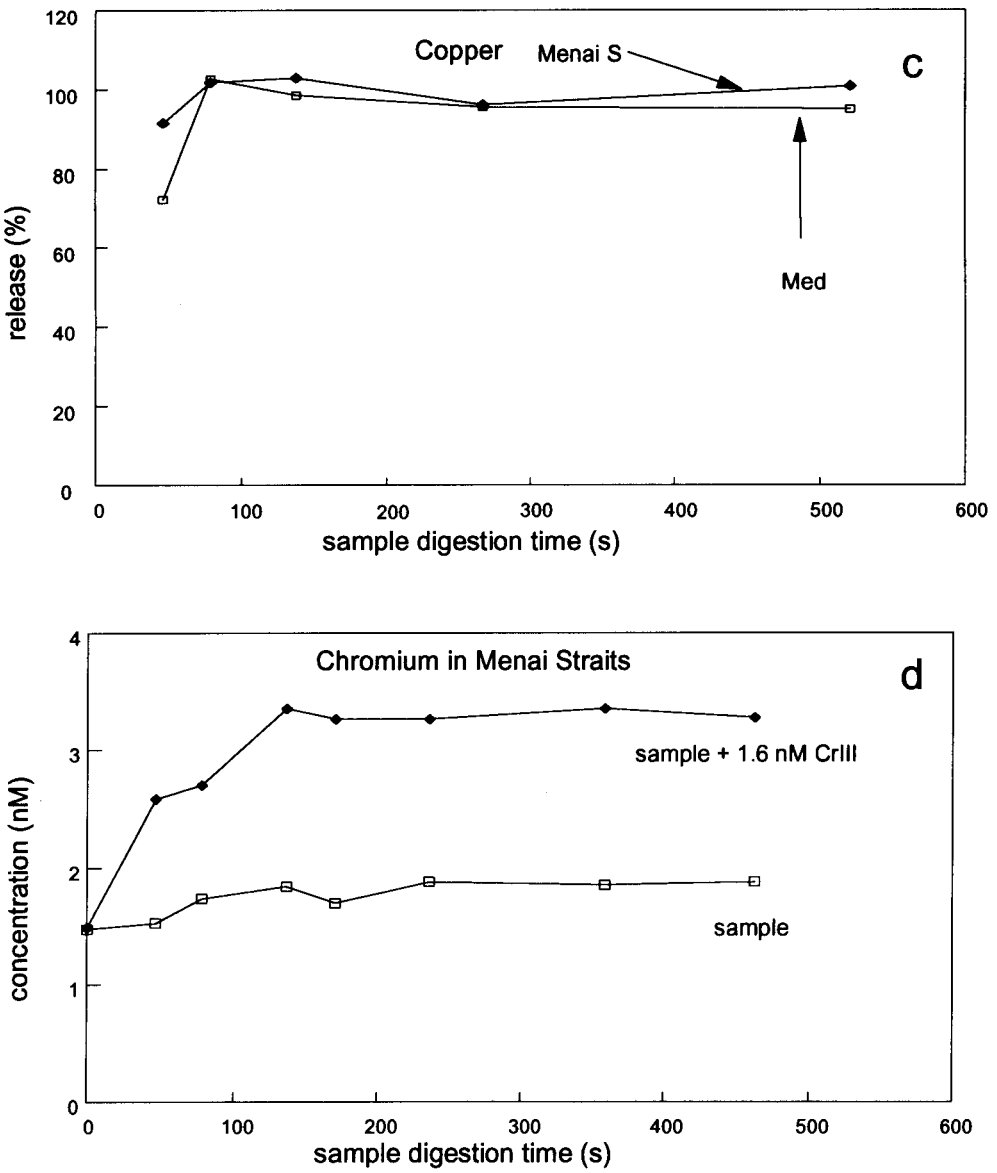

Fig. 7 (continued).

stable Cu complexes than tropolone ($\alpha_{Cu-Oxine} = \sim 4.2 \times 10^9$ and $\alpha_{Cu-Trop} = \sim 2000$). Non-labile Cr fractions of 14% and 19% were obtained in the Mediterranean and Menai Straits waters, respectively, indicating the presence of Cr(III).

The release of Ni and Cu from complexing organic material in sea water during in-line UV-digestion is presented in Fig. 7. A sample digestion time of 150 s was required for complete release of Ni from the organic complexes, whereas a sample digestion time of 275 s was required to release all Cu. A reason for this difference may be that the Ni-complexing organics are broken down more efficiently than Cu-complexing organics. However, a more plausible explanation may be the higher a coefficient for Ni–DMG ($\alpha_{Ni-DMG} = \sim 1.0 \times 10^9$) compared with Cu–tropolone ($\alpha_{Cu-Trop} = \sim 2000$), i.e. Ni–DMG complexes are more stable than the Cu–tropolone complexes.

The release of Cu from organic complexes by UV-digestion detected using oxine is shown in Fig. 7c. Complete release was achieved for both samples (Mediterranean and Menai Straits) at UV-digestion times < 100 s, indicating the efficiency of UV-digestion and the strength of the oxine in complexing Cu ($\alpha_{Cu-Oxine} = \sim 4.2 \times 10^9$).

3.5. Cr(III) / Cr(VI)

The efficiency of the in-line UV-conversion of Cr(III) to Cr(VI) was tested by comparison with batch-wise UV-conversion. The recovery of Cr(III) was examined using samples to which 1.6 nM of Cr(III) was added, at different sample digestion times. The UV apparatus was switched on at least 10 min before the start of the experiments in order to warm up (the water temperature during irradiation was ~ 70°C).

An in-line UV-digestion time of 150 s was sufficient to convert Cr(III) added to sea water to Cr(VI), and to convert the Cr originally in the sample to Cr(VI) (Fig. 7d), resulting in Cr concentrations comparable with those obtained using the batch-wise digestion. The data show that most of the added Cr(III) was converted to Cr(VI) at the shortest digestion times used (45 s), illustrating the effectiveness of the in-line UV-digestion

Table 2
Recovery of additions of Cr(III) and Cr(VI) to sea water and MQ upon UV-digestion in the presence and absence of humic acid and H_2O_2

	Cr(III) (nM)	Cr(VI) (nM)	Humic acid	H_2O_2	Recovery (%)
MQ (UV-batch)	20	–	+	+	96
Menai S. (UV-batch)	20	–	+	+	95
Menai S. (UV-batch)	–	20	+	+	92
MQ (UV in-line)	20	–	–	–	46
MQ (UV in-line)	20	–	+	–	77
MQ (UV in-line)	20	–	+	+	100
MQ (UV in-line)	–	20	–	–	81
MQ (UV in-line)	–	20	+	–	88
MQ (UV in-line)	–	20	+	+	100
Menai S. (UV-in-line)	20	–	–	–	80
Menai S. (UV-in-line)	20	–	+	–	35
Menai S. (UV-in-line)	20	–	–	+	90
Menai S. (UV-in-line)	20	–	+	+	93
Menai S. (UV-in-line)	–	20	–	–	91
Menai S. (UV-in-line)	–	20	+	–	42
Menai S. (UV-in-line)	–	20	–	+	92
Menai S. (UV-in-line)	–	20	+	+	95

for the determination of total Cr (Cr(III) + Cr(VI)) in natural water samples.

Further experiments were performed to investigate conversion of 20 nM levels of Cr(III) to Cr(VI) in MQ and sea water, using batch-wise and in-line UV-digestion in the presence and absence of humic acid (as a model compound for natural organic matter) and H_2O_2. Furthermore, Cr(VI) additions (20 nM) were made to MQ and sea water sample aliquots to obtain a measure of possible losses of Cr during batch and in-line UV-digestion. Silica particles (0.4 g l^{-1}) were added to UV digested sample aliquots to remove residual Cr(III) [21]. The determination of Cr as Cr(VI) was performed 20 min after the silica addition.

The batch-wise irradiation was performed using the 1000 W UV system for 2 h. The in-line UV-digestion was performed with the 100 W system and a sample digestion time of 275 s. The in-line system was run at high temperature (~ 70°C), and the sample aliquots were purged with oxygen (4 min) immediately prior to UV-digestion. The humic acid and H_2O_2 additions resulted in final concentrations of 4.0 mg C l^{-1} and 3.5 mM, respectively. The presence of Cr in the humic acid (4.0 mg C l^{-1} contained 7.1 nM Cr) and in Menai Straits water (1.6 nM) was taken into account for the calculation of the recovery.

The data show (Table 2) that addition of H_2O_2 to sample aliquots prior to the UV-digestion resulted in improved recovery rates for 20 nM spikes of Cr(III) and Cr(VI) in MQ and sea water. The efficiency to convert Cr(III) to Cr(VI) was similar for the in-line and batch-wise UV-digestion in the presence of H_2O_2. The Cr(III)/Cr(VI) conversion was not significantly affected by the humic acid (4.0 mg C l^{-1}) in the presence of the H_2O_2 (3.5 mM).

The recovery for Cr(III) in the presence of H_2O_2 was similar to that for Cr(VI), suggesting that complete conversion of Cr(III) to Cr(VI) occurred during the UV-digestion of sea water. However, recovery for Cr(III) and Cr(VI) were higher in MQ (100%) than in sea water (~ 95%), indicating that small losses of Cr occurred from sea water during UV-digestion.

3.6. Automated determination of Ni and Cu in certified sea water samples

The accuracy of the automated voltammetry with in-line UV-digestion was tested by determining the concentrations of Ni and Cu in certified sea water. BCR reference Southern North Sea water (CRM 403) was analysed for Ni automatically, as well as manually after batch-wise UV-digestion. Cu was determined fully automatically in NASS2 as well as BCR (CRM403) certified sea waters.

A total Ni concentration of 3.90 ± 0.03 nM ($n = 4$) was found in the certified sea water (BCR CRM 403) using the automatic voltammetric system, whereas a concentration of 4.03 ± 0.17 nM ($n = 2$) was found by the manual method. These concentrations compare well with the certified Ni concentration in the BCR sea water of 4.23 ± 0.34 nM [26].

A total Cu concentration of 3.52 ± 0.17 nM ($n = 3$) was found in BCR (CRM 403) certified sea water using the automatic voltammetric system with in-line UV-digestion. Measuring certified NASS2 sea water using the same procedures resulted in a total Cu concentration of 1.80 ± 0.05 nM ($n = 3$). These concentrations also compare well with the certified concentrations in these waters (BCR (CRM 403) Cu = 3.78 ± 0.35 nM, NASS2 Cu = 1.72 ± 0.2 nM).

3.7. Automated determination of oceanic depth profiles of total Ni and Cu

The automated voltammetric apparatus including in-line UV-digestion (Fig. 1) was used to determine concentrations of Ni in the Mediterranean and Cu in the North Atlantic Ocean. The Ni concentrations in the water column show depleted surface layer concentrations of ~ 3 nM, caused by a combination of inflow of trace metal depleted North Atlantic water into the Mediterranean and biological uptake of Ni in that water layer [27] (Fig. 8a). Higher Ni levels (~ 4.6 nM) were found in the deeper Mediterranean water layer (depth > 100 m).

The vertical profile for Cu in the North Atlantic (Fig. 8b) shows an increase with depth from low levels of 1.6–1.8 nM in the upper water

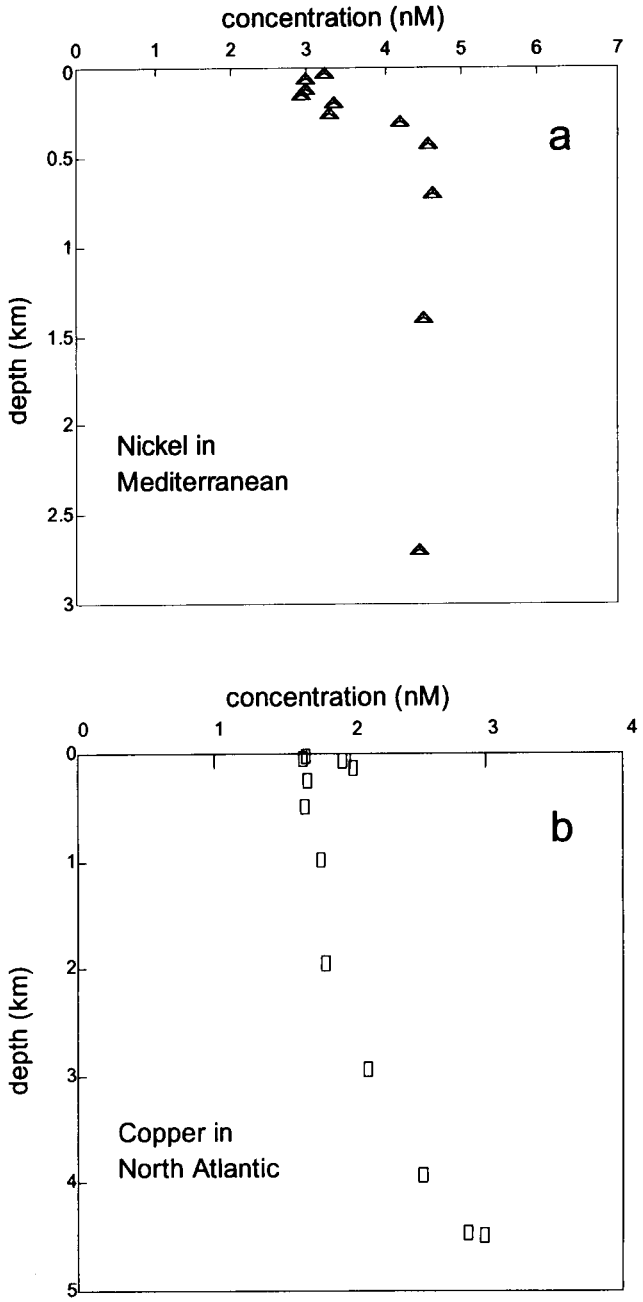

Fig. 8. Vertical distributions of Ni (a; Mediterranean Sea) and Cu (b; North Atlantic Ocean) determined using the automated voltammetric system with in-line UV-digestion.

column to ~3.0 nM in the deeper waters. The depleted surface concentrations in the North Atlantic are caused by a combination of biological uptake and particle scavenging of Cu in that water layer.

The total dissolved Ni and Cu concentrations in Fig. 8 are in agreement with levels previously observed in these waters [28,29].

4. Conclusions

Total metal concentrations in natural waters can be determined by cathodic stripping voltammetry after UV-digestion of complexing and interfering organic material. The in-line UV-digestion of coastal and oceanic sea water achieved complete release of metals (Cu and Ni) from organic complexes and destruction of interfering surfactants (in case of Cu, Ni and Cr determinations). The optimal in-line UV-digestion conditions prior to trace metal determination include the presence of oxygen, H_2O_2 (9 mM for Ni and Cu) and treatment at elevated temperatures (~ 70°C) at sample digestion times of 150–175 s.

The optimal in-line UV conditions for conversion of Cr(III) to Cr(VI) prior to the voltammetric determination of total Cr include addition of 3.5 mM H_2O_2 to sample aliquots, and treatment at elevated temperature at a sample digestion time of 150 s.

The in-line UV-digestion of humic acids was more efficient in MQ than in sea water. A breakdown of 99.5% and 98% was achieved for a 15.5 mg C l^{-1} solution in MQ and sea water, respectively, at UV-digestion times > 275 s. In view of the known refractory nature of the humic acid [19] it is likely that this digestion method also works with synthetic or other natural organic compounds.

This study shows that the in-line UV-digestion is more effective than the batch-wise UV-digestion. Using in-line UV-digestion, a breakdown of > 99% was achieved for humic acid solutions in MQ of 3.8, 7.75 and 15.5 mg C l^{-1}, at a digestion time of 100 s. To obtain similar results using the batch-wise treatment, digestion times have to be applied that are between 20 and 300 times longer. The higher efficiency of the in-line UV-digestion is most likely caused by a higher surface to volume ratio in the UV-coil compared

with the tubes used during batch-wise treatment, resulting in a more intense irradiation of a quantity of water per time unit.

Organic carbon concentrations in sea water are known to range between 0.1 and 4 mg C l^{-1} [30], between < 1 and 3 mg C l^{-1} in low productivity lakes and between 5 and 30 mg C l^{-1} in productive lakes [20]. The highest levels tested in this study (up to 15.5 mg C l^{-1}) were readily digested by the UV-digestion, indicating that this technique is suitable for all natural waters. The digestion efficiency for unusually high organic levels is increased by addition of H_2O_2.

The incorporation of in-line UV-digestion in an automatic voltammetric system resulted in a stand-alone metal monitor that is able to determine total trace metals at nanomolar levels in natural waters. The use of in-line UV-digestion minimises sample contamination because of reduced manual sample handling and minimised reagent additions.

Developments are under way for the application of in-line UV-digestion in a monitor for the automated determination of Cr speciation. In such a system the UV-digestion will be used for on-line conversion of Cr(III) to Cr(VI) for total Cr determination, whereas an on-line ion exchange column will be used for the removal of Cr(III) prior to the determination of Cr(VI).

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Determination of total phosphorus in waters and wastewaters by on-line microwave-induced digestion and flow-injection analysis

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Abstract

An on-line flow-injection system for the determination of total phosphorus in waters and wastewaters is described. Digestion was performed using a flow-through reactor in a modified domestic microwave oven, and the orthophosphate formed was detected as phosphomolybdenum blue after on-line filtration. The modifications and operation of the microwave complied with Australian Standards. Reagent and digestion conditions required to give optimal oxidation and hydrolysis of organic and condensed model species are reported. The flow-injection method gave complete recovery for almost all the model P compounds tested, with the exception of condensed phosphates. When the proposed method was applied to a series of wastewater samples, the results obtained were in close agreement with those from a conventional batch digestion method. The technique was rapid (7 samples h^{-1}, 4 replicates), with a linear range of interest for wastewaters 0–18 mg P l^{-1}, and a detection limit of 0.09 mg P l^{-1}.

Key words: Flow injection; Spectrophotometry; Digestion; Filtration; Phosphorus; Waters

Phosphorus is an essential but often limiting nutrient for algal and bacterial growth in natural waters [1]. The presence of elevated concentrations of phosphorus in natural waters frequently result in algal blooms with an accompanying deterioration in water quality. Total phosphorus (TP) concentration is commonly used as an indicator of water quality and as a control parameter in wastewater treatment processes; where TP consists of dissolved organic, condensed and orthophosphates, as well as particulate and colloidal-associated phosphates in both biotic and abiotic forms [2]. Currently, TP monitoring in many wastewater plants involves the use of occasional grab samples followed by batch TP analysis. Given the infrequency of the sampling, and the time lag for analysis, detection of any excur-

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sions from "within-limit" plant operating conditions would be unlikely.

Determination of TP necessitates the dissolution, oxidation or hydrolysis of particulate-associated phosphorus, and dissolved organic and condensed phosphates. Commonly-used digestion methods for TP include heating the sample with peroxydisulphate alone [3], or with acid peroxydisulphate [4]. However, these methods have been found to give incomplete phosphorus recoveries for some samples, and the use of mixtures of nitric-sulphuric [5] and nitric-sulphuric-perchloric [6,7] acids has been proposed. Other workers have used high temperature fusion of samples with magnesium sulphate or nitrate to give orthophosphate prior to detection [8,9]. However, all these methods are relatively slow and are poorly suited to determination of TP with the frequency necessary for process control or regulatory monitoring. Consequently automated methods for either continuous, on-line or frequent monitoring of phosphorus concentrations would be highly desirable.

A number of flow-injection (FI) methods have been reported for the determination of either total *dissolved* phosphorus (TDP) using thermal digestion [10–12] or dissolved organic phosphorus by photochemical oxidation [13]. With the exception of the photochemical method, all these use long reactor coils, and some involve the use of elevated pressures. More recently, Hinkamp and Schwedt [14] have described the determination of TDP by an FI system which utilizes a microwave oven for digestion and amperometric detection of the orthophosphate liberated. These methods have all the attendant advantages associated with flow-injection: increased sample throughput, minimal reagent use, and high precision, and all showed good recovery for model phosphorus compounds and TDP of real samples. To date, however, a method for total phosphorus (i.e., TDP plus particulate phosphorus) has not been reported.

This paper describes the development of a low cost and robust flow-injection method for TP in wastewaters. The proposed method uses a domestic microwave oven for on-line sample digestion, and a simple solid state detector of the type described by Worsfold et al. [15] for the photometric detection of phosphomolybdenum blue. Details of the microwave modifications, together with the optimal conditions for the digestion of model phosphates and the effectiveness of the microwave digestion for determination of TP in real wastewater samples are given.

1. Experimental

Reagents

All solutions were prepared in Milli-Q water and all reagents were of AnalaR (or equivalent) grade. The optimum concentrations for the three reagent streams were as follows: R1, 10 g l^{-1} ammonium molybdate (BDH) and 35 ml l^{-1} concentrated sulphuric acid (BDH); R2, 0.2 g l^{-1} tin (II) chloride (May & Baker), 2 g l^{-1} hydrazinium sulphate (Ajax) and 28 ml l^{-1} concentrated sulphuric acid; and O_X, 10 g l^{-1} potassium peroxydisulphate (Ajax) adjusted to pH 0.6 with concentrated sulphuric acid. The following inorganic and organic phosphorus standards, 100 mg P l^{-1} (Sigma unless otherwise stated) were prepared: Potassium dihydrogen orthophosphate (Ajax); phytic acid; phosphonoformic acid; 2-aminoethylphosphonic acid; D-glucose 6-phosphate; DL-α glycerophosphate; *O*-phosphorylethanolamine; adenosine 5′-monophosphate (Boehringer); adenosine 5′-diphosphate (Boehringer); adenosine 5′-triphosphate; ferric orthophosphate (Koch-Light); sodium pyrophosphate (Aldrich); sodium tripolyphosphate (Ajax); nitrophenylphosphate (Aldrich).

All the working standard solutions were prepared from the stock solutions immediately prior to the commencement of experimental work.

Reagents – digestion trials

During trials to select the most suitable digestion conditions, 5 mg P l^{-1} standard solutions were used unless otherwise specified. The following digestion mixtures were used.

(I) (i) Nitric acid (BDH) (1.5 M) and perchloric acid (BDH) (1.5 M). (ii) Nitric acid (2.0 M) and perchloric acid (2.0 M).

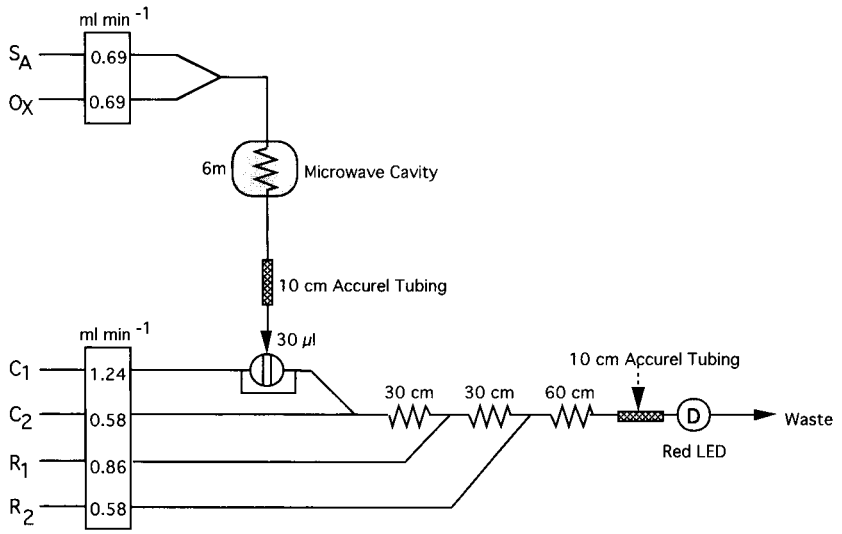

Fig. 1. FI manifold for the digestion and analysis of phosphorus containing material.

(II) Ceric ammonium sulphate (Sigma) (1 g l^{-1}) and perchloric acid (0.075 M).

(III) (i) Ammonium peroxydisulphate (BDH) (10 g l^{-1}) and perchloric acid (1.5 M). (ii) Ammonium peroxydisulphate (2–10 g l^{-1}) and perchloric acid (1.5 M). (iii) Ammonium peroxydisulphate (2–10 g l^{-1}).

(IV) (i) Sodium peroxydisulphate (BDH) (2–10 g l^{-1}) and perchloric acid (1.5 M). (ii) Sodium peroxydisulphate (2–10 g l^{-1}).

(V) (i) Potassium peroxydisulphate (2–22 g l^{-1}). (ii) Potassium peroxydisulphate (20 g l^{-1}) at pH 0.6–13.0 (adjusted with sulphuric acid or sodium hydroxide).

Instrumentation

Manual injection was used throughout this work; peristaltic pumps (Ismatec MS-CA 4 820 and MS-4 Reglo 8/100) were used to propel reagent, carrier, oxidant and sample streams

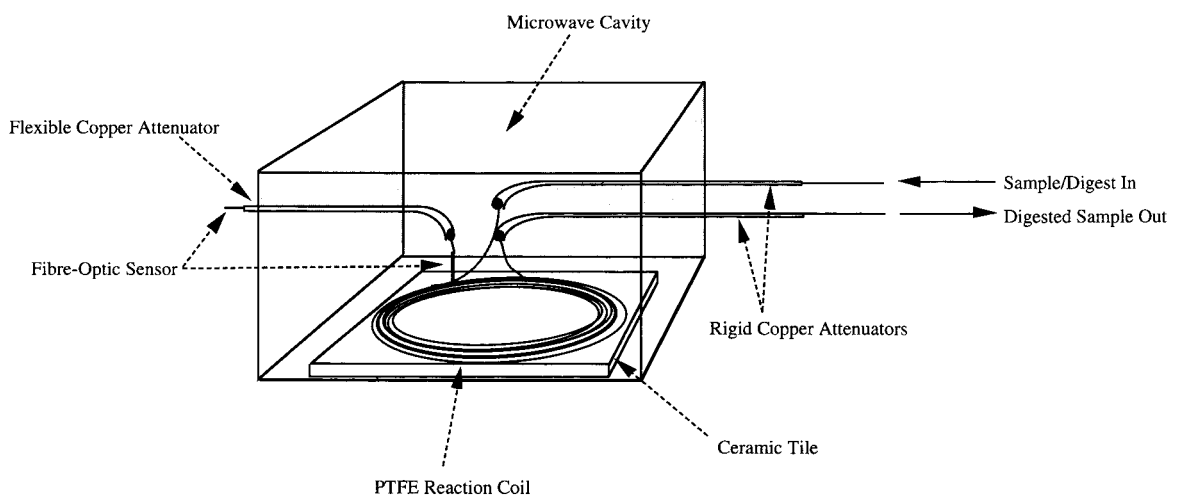

Fig. 2. Diagram illustrating modifications to domestic microwave oven.

through PTFE tubing (0.8 mm i.d.). Phosphorus standards or unfiltered effluent samples were continuously mixed on-line with a digestion reagent and this mixed solution was passed through a microwave reactor (further details given below), an aliquot (30 μl) of this was injected into a Milli-Q water carrier stream (C1) via a PTFE rotary valve (Rheodyne 5041), with an electronic actuator manufactured in-house, and sequentially merged with a further Milli-Q water carrier stream (C2) (to dilute the sample), the colorimetric reagent (R1) and the reducing reagent (R2). The transmittance was monitored using a solid-state photometer [16] incorporating red light-emitting diodes (635 nm) and the analogue signal obtained for transmission over a 0.8 mm optical pathlength was recorded on a chart recorder (Kipp & Zonen, BD111). The manifold used is shown in Fig. 1. This manifold included two debubblers, consisting of 10 cm lengths of microporous polypropylene tubing (Accurel S6/2, Enka), to ensure that any oxygen bubbles generated during the digestion procedure were removed prior to (i) injection of the sample, and (ii) measurement of the coloured complex. Flow-rates of 1.24 and 0.58 ml min^{-1} were used for C_1 and C_2 respectively, 0.86 ml min^{-1} for R_1, 0.58 ml min^{-1} for R_2 and 0.69 ml min^{-1} for O_X and S_A.

Microwave reactor modification. A domestic microwave oven, Sanyo EM-810F (Sanyo, Osaka), rated at 700 W, was modified (Fig. 2) such that a sample solution could be pumped through the microwave cavity in small bore PTFE tubing (1.0 mm i.d., 1.6 mm o.d.). The tubing passed into the cavity through two holes in the rear wall via attenuators designed to minimise the leakage of radiation. The attenuators were constructed from copper pipe (1 m × 2.45 mm i.d. × 4.75 mm o.d.) and fixed in the holes (ca. 10 mm in diameter) in the rear of the cavity by means of brass bulkhead unions. An additional attenuator, constructed from flexible copper braid, was later added to allow a fibre-optic cable to pass into the cavity. In addition to these modifications, the turntable was immobilised to prevent disturbance of the PTFE reactor coil during irradiation. The built-in stirrer fan was utilised to distribute the microwave radiation evenly throughout the cavity. In all experiments, unless otherwise stated, two ceramic tiles (220 × 247 × 10 mm, width × length × thickness) (Monier and/or Anchor tiles, 33–44% alumina, Cooinda Ceramics, Bayswater, Australia) were placed on the immobilised turntable to absorb excess microwave energy and hence reduce the amount of reflected energy.

The modifications to and operation of the microwave oven complied with Australian Standards [16] and routine testing for radiation leakage was conducted with a calibrated leakage detector (Model HI-1801, Holaday Industries, RFI Industries, Bayswater). At no time during operation, did the leakage exceed the maximum occupational exposure limit of 10 W m^{-2} [16], and because of the low-pressure nature of the sample digestion the risk of an explosion was minimised.

Procedures

Temperature and pressure measurement. Measurement of these physical parameters within the microwave cavity is difficult and the sensor used must overcome the restrictions associated with this operating environment. The primary requirements identified for the sensor were that it should be: (i) non-metallic and (ii) free of interference due to electromagnetic radiation. One suitable sensor that is commercially available and that was subsequently successfully applied to these measurements was a fibreoptic based multi-sensor (MetriCor Model 1400, MetriCor, Woodinville, USA).

Two probes were used in conjunction with the sensor unit: (i) temperature probe, T102-100A-01, −10 to +120°C; (ii) pressure probe, P100-100A-01, 0 to 6.9×10^5 Pa.

Installation of sensor probe. The sensor probe was introduced into the digestion solution at the end of the reaction coil by means of a PTFE "T" piece at a point just before the flow tubing exits from the microwave cavity. The exposed sensor within the cavity was shielded with a length of copper braid, securely grounded to the cavity wall, as a precautionary measure to prevent dam-

age as a result of long-term exposure to microwave radiation.

Digestion trials. In this series of experiments the microwave was operated on full power for the full digestion period and the first injection of sample made after a four minute stabilisation period. A dummy load of 1.4 l of cold water was placed in the oven prior to the commencement of each new digestion.

Effluent analysis. The operating procedure applied in the digestion trials was also used for the effluent analysis. The microwave oven was never operated without a dummy load in the cavity. The FI manifold illustrated in Fig. 1, was modified by the inclusion of two disposable syringe filters (5 μm and 0.45 μm, Lida) connected in series and inserted in the flow system at a point immediately before the injection valve in the "feed" line from the microwave cavity. To prevent contamination and blockage, these filters were replaced before the analysis of each new effluent sample.

Twenty-three samples including raw sewage, primary settled, secondary sedimentation, lagoon and final effluents were analysed for total phosphorus using the proposed FIA–microwave method. The effluents were collected in prewashed HDPE bottles and were stirred during introduction to the FI system to ensure that homogeneous sub-samples were taken. The total phosphorus in these samples was also determined by a manual batch procedure based on the nitric-sulphuric acid digestion of the sample [6], which was then treated with ammonium molybdate and antimonyl tartrate to form phosphomolybdic acid. Ascorbic acid was then used to reduce the phosphomolybdic acid to phosphomolybdenum blue and the transmittance of the complex recorded.

2. Results and discussion

Temperature and pressure measurement

With no microwave load other than the standard pyrex turntable, the temperature at the end of the reaction coil rose steadily to approximately 80°C after ca. 1 min (Fig. 3a). After this, the temperature increased following a regular "wave" pattern, with a final temperature of approximately 100°C being reached after 10 min. No difference was observed when the reactor coil was lifted off the turntable by a PTFE support (elevation ≈ 5 mm).

When either 1 or 2 ceramic tiles were placed in the cavity the temperature profile was considerably different, a steady rise in temperature to 100°C over 10 min was observed (Fig. 3b).

The pressure fluctuations observed with and without a load in the cavity were similar to those observed for temperature. Without a load, the pressure rapidly increased to a maximum value (ca. 8.6×10^4 Pa) and then decreased in an oscil-

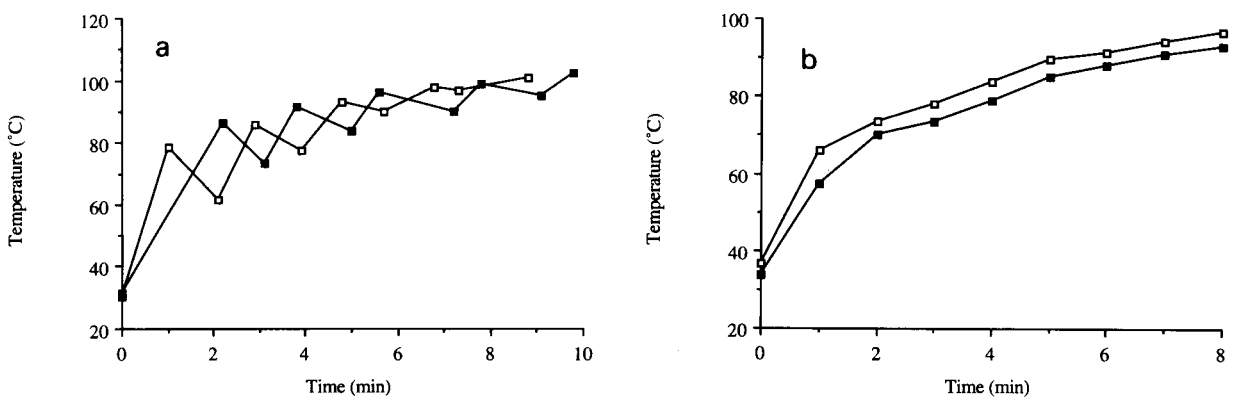

Fig. 3. (a) Temperature fluctuation of digestion mixture with no load in cavity. □, Run 1; ■, Run 2. (b) Temperature fluctuation of digestion mixture with ceramic load in cavity. □, 1 tile; ■, 2 tiles.

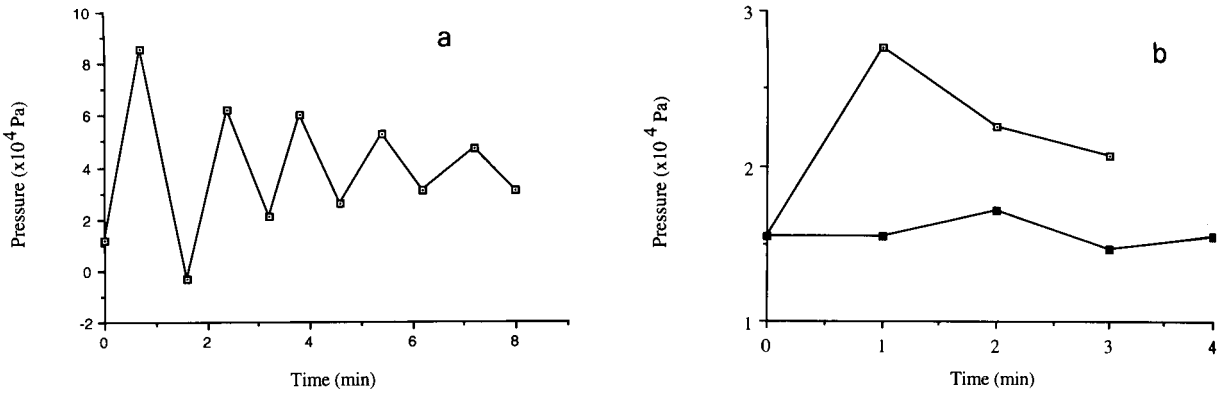

Fig. 4. (a) Pressure fluctuation in digestor reactor coil with no load in cavity. (b) Pressure fluctuation in digestor reactor coil with ceramic load in cavity. □, 1 tile; ■, 2 tiles.

lating manner (Fig. 4a). With a ceramic load of 2 tiles in the cavity, the pressure rose to a lower maximum value (ca. 1.6×10^4 Pa) at which it remained constant until the irradiation ceased (Fig. 4b).

The observed temperature and pressure oscillations suggest that the reflected energy caused the output of the magnetron to fluctuate and hence disrupt the smooth heating of the sample. However, when a load was inserted in the cavity, excess power was absorbed and the microwave heating was steady. This "smoothing" of the heating profile was achieved with either a ceramic load or a water load (1.4 l of water in a glass vessel). In all further experiments, a dummy load of 2 ceramic tiles and 1.4 l of water was used to ensure smooth temperature and pressure profiles and minimise damage to the magnetron.

Digestion trials

The suitability of the different digestion mixtures was examined and the results of these trials are summarised below. All percentage conversion values given were calculated with respect to the signal generated by a potassium orthophosphate standard at an equivalent phosphorus concentration.

Different reaction conditions are required for the successful conversion of simple organic phosphates (e.g., glucose phosphate) and condensed phosphates (e.g., sodium tripolyphosphate) to orthophosphate. Conversion of simple organic phosphates necessitates the use of an oxidation process, whereas condensed phosphates need to be hydrolysed. Digestion mixtures of either nitric acid and perchloric acid, Table 1 [I(i), I(ii)], or ceric sulphate, Table 1 [II(i)] were found to be effective for the conversion of condensed phosphates, but were used with no success for the conversion of organic phosphates. In order that the concentration of the oxidising agent, peroxydisulphate, could be increased in the presence of perchloric acid, the ammonium salt was selected in preference to the potassium salt because of a higher solubility in strong perchloric acid solutions. It was found that increasing the concentration of peroxydisulphate had little effect on the conversion of phytic acid (which remained constant at approximately 45%). However, this increase in peroxydisulphate concentration caused a significant decrease in the signals obtained for both phytic acid and orthophosphate (Fig. 5).

Table 1
Comparison of digestion reagents

Compound	Digestion solution/ conversion (%)			
	I(i)	I(ii)	II(i)	III(i)
Phytic acid	22	30	8	85
Glucose phosphate	22	29	– [a]	62
Adenosine triphosphate	75	77	91	40
Ferric orthophosphate	26	32	–	–
Sodium tripolyphosphate	102	97	74	37

[a] Not measured.

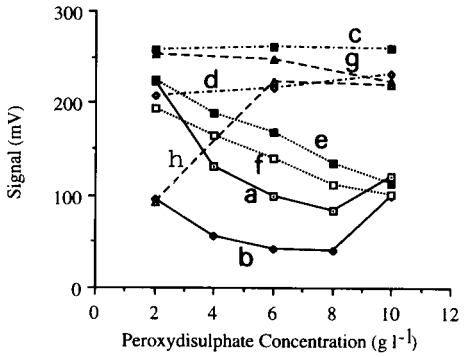

Fig. 5. Signal variation with digestion mixture composition.

Key	Digestion trial	Standard
a	III(ii)	Orthophosphate
b	III(ii)	Phytic acid
c	III(iii)	Orthophosphate
d	III(iii)	Phytic acid
e	IV(i)	Orthophosphate
f	IV(i)	Phytic acid
g	IV(ii)	Orthophosphate
h	IV(ii)	Phytic acid

This effect was also observed with sodium peroxydisulphate and perchloric acid mixtures (Fig. 5). It was concluded that perchloric acid when mixed with peroxydisulphate, suppressed both the signal and more importantly the oxidation process. Therefore these reagent mixtures were not used in any further trials.

The results of the trial with potassium peroxydisulphate were more successful, although it was found that this compound was not sufficiently soluble in perchloric acid to allow a high enough concentration of peroxydisulphate for adequate oxidation of the organic phosphates. Conversion efficiency for phytic acid remained unchanged with increasing peroxydisulphate concentration, regardless of whether the digestion mixture was acidified or not. Whereas, the oxidation of ATP increased with increasing peroxydisulphate concentration in the acidified mixture up to 10 g l^{-1}, after which no significant difference in conversion efficiency was observed. It was concluded that a 10 g l^{-1} acidified solution of potassium peroxydisulphate would offer the best all-round performance for the digestion of phosphorus material.

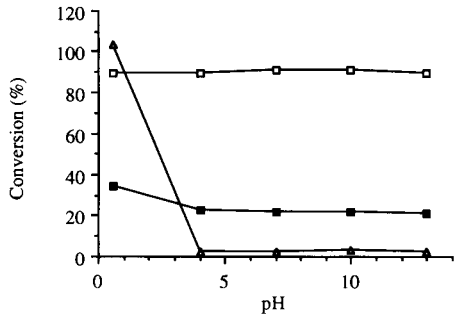

Fig. 6. Variation of oxidising efficiency with pH. □, Phytic acid; ■, adenosine triphosphate; △, nitrophenylphosphate.

The effect of varying the pH of the digestion solution on the oxidation efficiency for phytic acid, ATP and nitrophenylphosphate was examined. No significant change in signal size was observed for either orthophosphate or phytic acid, but a decrease was observed with increasing pH for both ATP and nitrophenylphosphate. This was matched with a decrease in oxidation efficiency (Fig. 6) and it was concluded that a low pH was most suitable. A practical pH of 0.6 was selected and was used in all further trials.

Oxidant trial – dissolved phosphorus. The optimum digestion reagents were used in a trial to examine the efficiency of the digestion procedure for 11 different phosphorus containing compounds. These compounds were selected on the basis of their structure and the variety in P bonds and consequently the different ease with which

Table 2
Digestion trial – dissolved phosphorus

P Compound	Conversion (%)	
	2 mg P l^{-1}	10 mg P l^{-1}
Phytic acid	88	94
Phosphonoformic acid	96	99
2-Aminoethylphosphonic acid	101	100
D-Glucose 6-phosphate	96	98
DL-α-Glycerophosphate	106	103
O-Phosphorylethanolamine	91	100
Adenosine 5′-monophosphate	102	98
Adenosine 5′-diphosphate	49	41
Adenosine 5′-triphosphate	47	47
Sodium pyrophosphate	32	29
Sodium tripolyphosphate	39	40

each will be converted to orthophosphate. The results of the trial (Table 2) show that these conditions are a compromise and conversion of condensed phosphates is low. Therefore special consideration would have to be given to samples known to contain large quantities of condensed material. It is envisaged that a two-step process where the conditions of the reaction mixture are adjusted for optimum hydrolysis and oxidation respectively could be used if real sample analysis highlighted large discrepancies in total phosphorus concentration.

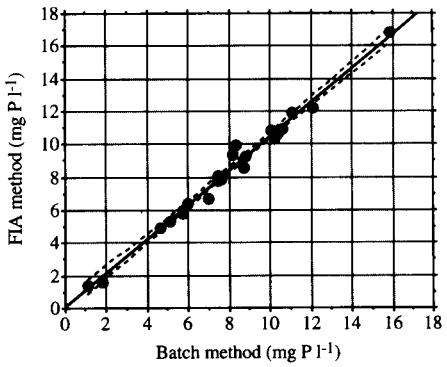

Fig. 7. Correlation between proposed FI–microwave method and standard batch procedure.

Effluent analysis

Typical calibration of FI method. Seven orthophosphate standards covering the range 0.5–18 mg P l^{-1} and a blank were analysed using the optimised manifold. The results were linear over this range ($r^2 = 0.9991$) and are described by the regression equation signal (mV) = 38.82 [P] (mg l^{-1}) + 18.66. The practical LOD, which was defined as the concentration corresponding to the y intercept + $3\sigma_{n-1}$ (the error in the calculated y values for the regression equation [17]), was 0.09

Table 3
Intermethod comparison – total phosphorus

Sample No.	Type [a]	Total P (mg l^{-1})		
		FI–microwave	$\sigma^{n=6}$	Batch [b]
1	R	6.7	0.2	7.0, 7.0
2	F	4.9	0.1	4.6, 4.5
3	R	7.9	0.3	7.6, 7.5
4	F	6.4	0.1	6.0, 6.0
5	R	5.8	0.0	5.7, 5.7
6	F	5.3	0.0	5.1, 5.1
7	R	12.2	0.3	12.1, 12.0
8	F	8.1	0.1	7.4, 7.6
9	L	9.3	0.1	8.0, 8.4
10	R	10.3	0.2	10.3, 10.1
11	PS	10.8	0.3	10.2, 10.1
12	F	9.2	0.1	8.8
13	F	1.6	0.0	1.8
14	F	8.6	0.1	8.7
15	R	16.8	0.5	15.9
16	R	10.9	0.2	10.6
17	PS	10.4	0.0	10.2
18	L	11.9	0.2	11.1
19	R	10.6	0.1	10.4
20	F	7.8	0.0	7.5
21	R	9.6	0.1	10.1
22	SS	1.4	0.0	1.1
23	R	9.9	0.2	8.3

[a] F = Final effluent, L = lagoon, PS = primary settled, R = raw sewage, SS = secondary sedimentation. [b] Individual analysis results.

mg P l^{-1}. Three phytic acid standards, 6, 8 and 10 mg P l^{-1}, were analysed following the same procedure and a 95% conversion was observed. Improvement in the detection limit could be achieved by the use of a digestion sample injection volume of > 30 μl, a volume which was chosen as being most appropriate for wastewaters. Further improvement could also be achieved by increasing the path length of the detector cell. Sample throughput was rapid at 7 samples h^{-1}, with 4 repeat injections per sample.

Comparison of FI and batch methods. The results from the proposed FI method and the batch method are given in Table 3. These illustrate the high reproducibility of the FI method and a plot of this data (Fig. 7) shows good correlation between the methods. The line of best fit ($r^2 = 0.986$) can be described by the regression equation [P] (total P by FI, mg P l^{-1}) = 1.04 [P] (total P by batch, mg P l^{-1}) + 5.05×10^{-2}. There is a small positive bias with the FI analysis, which is probably due to differences in the detection chemisty between the batch and FI methods. The agreement between the two methods is very good; most points lie within the 95% confidence limits. The data for raw and treated effluents can be separately correlated and described by the following regression equations (raw effluent includes samples classified as raw sewage or primary settled). Raw ($r^2 = 0.971$): [P] (total P by FI, mg P l^{-1}) = 1.05 [P] (total P by batch, mg P l^{-1}) − 9.86×10^{-2}. Treated ($r^2 = 0.990$): [P] (total P by FI, mg P l^{-1}) = 1.05 [P] (total P by batch, mg P l^{-1}) + 2.82×10^{-2}.

However, this separation does not highlight any particular differences in the data obtained using the two methods. It is clear that for the range of phosphorus concentrations typically encountered for wastewaters (2–30 mg P l^{-1}), that there is no significant difference between the values obtained using either method.

Although this method utilises disposable filters for the removal of particles from the digested stream prior to colorimetric determination, it is foreseeable that more suitable long-term filtration devices will have to be developed for routine environmental and process monitoring. Pedersen et al. [18] for example, have used tangential flow filtration in conjunction with flow-injection analysis for the determination of DRP in municipal wastewaters.

Conclusions

The proposed FI–microwave digestion and analysis procedure for total phosphorus has been shown to achieve rapid, effective and safe digestion of model phosphorus compounds. Furthermore, the method has been shown to be suitable for the analysis of sewage effluents without additional sample pretreatment.

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An integrated analytical strategy for liquid effluent management

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Abstract

As part of ZENECA's commitment to continually improve the environmental performance of its manufacturing sites, the design and construction of a liquid effluent management facility at Huddersfield Works has provided the hardware and infrastructure to allow a more timely and efficient response to abnormal occurrences resulting from the Works' manufacturing operations. The project comprised the installation of on-line analytical instrumentation designed to both detect and initiate a response to abnormal discharges to effluent drain, together with the construction of several containment tanks, associated ductwork and control mechanisms. Full commissioning of the £4 million facility took place during December 1992. The on-line analysers chosen, based on the determination of total carbon content and UV–visible spectrophotometry, were deemed to be the most appropriate for a manufacturing site handling in excess of 4000 different chemicals. An extensive development phase explored the response of the analysers to 'typical' and 'abnormal' loadings of selected chemicals and enabled realistic alarm levels to be set. The on-line installations have been complemented by the development of state of the art laboratory-based technologies, designed to characterise or confirm the precise nature of, and therefore the disposal options for, an abnormal discharge following its interception. Such techniques also have use in source attribution and subsequent enhancement of the environmental performance of individual plants and processes.

Key words: UV–Visible spectrophotometry; Environmental performance; Liquid effluent management; ZENECA

1. Introduction

A wide range of chemical compounds, together with their associated intermediates, are manufactured at Huddersfield Works in support of ZENECA's Agrochemical and Specialty Chemical businesses. The site occupies approximately 100 hectares, employs 1500 people and contains 30 manufacturing plants; many of these are multiproduct batch units and result in a portfolio in excess of 1000 distinct products.

Under normal flow conditions the Works' activities generate liquid effluent at a rate of 900–1100 m^3 per hour; this is collected via a complex drain network and consists of a combination of process effluent, floor washings, storage area and roof drainage. The diverse and constantly changing nature of the activities undertaken on the site mean that the composition of the effluent is a complex and variable, albeit dilute, mix of mainly

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organic chemicals, many of which are in aqueous solution but with some emulsification. Whilst some treatment at source is possible and indeed does takes place, there is a need for further downstream processing of the effluent and this entails several stages of treatment.

Within the Works boundary conventional primary treatment, i.e. pH adjustment and solids removal, is carried out before the effluent is biologically-treated by Yorkshire Water Services Ltd., utilising both aerobic activated sludge and trickling filter bed technologies. The quality of the effluent leaving the Works is subject to a comprehensive set of consent conditions by the Water Authority.

The treated effluent finally passes into the River Calder in combination with treated domestic and other industrial effluents, and is subject to a further stringent set of consent conditions by the National Rivers Authority.

Since both Yorkshire Water's biological treatment units and the river ecosystem beyond could potentially be adversely affected by the discharge of toxic materials, historical practice has been to divert 'abnormal' effluent, resulting from accidental discharges occurring on the Works, to containment lagoons provided by the Water Authority. Following analysis and an evaluation of environmental impact, a disposal strategy would then be determined, possibly involving chemical/physical treatment prior to controlled discharge.

The Water Authority took a decision in the late 1980's to undertake major site redevelopment in the area of the containment lagoons and therefore Huddersfield Works initiated a comprehensive effluent management project, encompassing the installation of monitoring, diversion and containment facilities within the site boundary.

The scope of the project included the identifi-

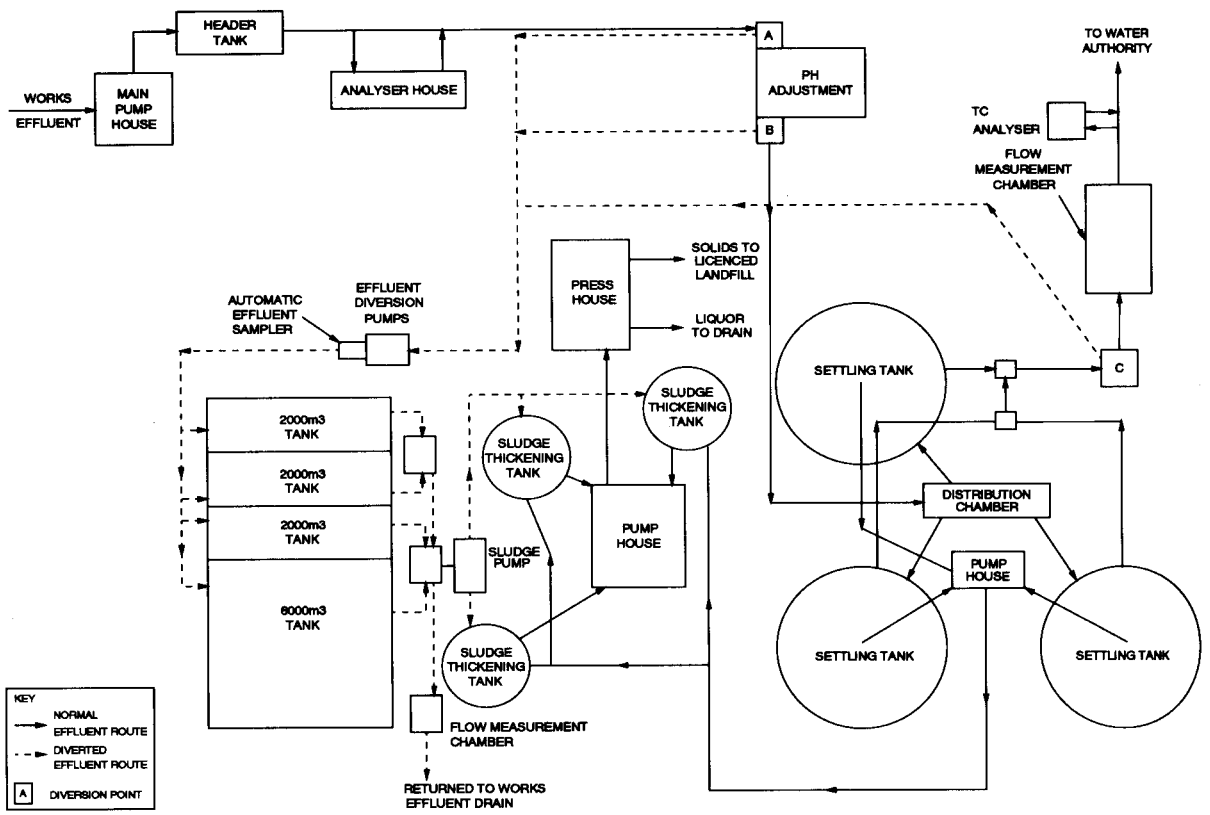

Fig. 1. Schematic diagram of effluent management plant.

cation and installation of appropriate on-line analytical instrumentation, and the construction of diversion and containment facilities capable of handling envisaged abnormal occurrences. The latter entailed the statistical analysis of both historical and possible future scenarios and involved a consideration of factors such as process effluent flows, rainfall data and abnormal occurrence frequency and duration. From this information the most appropriate nature, size and location for the facilities was determined. Fig. 1 schematically depicts the layout of the final plant.

This paper covers the analytical aspects of the project, from initial choice of technology to final operating philosophy.

2. Choice of on-line analytical technology

It is probably true to say that analytical chemistry has now reached the stage where, if time taken to perform the analysis and complexity of installation were not constraining factors, there are very few analytes that cannot be determined on-line. However, if the on-line installation is to be used for the early detection and subsequent management of abnormal occurrences, as it is in this case, whilst additionally being capable of detecting a very wide range of (mainly) organic chemicals against a constantly varying background, such an approach is not valid. The key requirements of any chosen technique are listed in Table 1. In consideration of these criteria, and in the light of the nature of abnormal occurrences which have taken place historically, the choice of technology was seen at the time as being limited to those techniques listed in Table 2.

Table 1
Key requirements of on-line technique(s) useful for this application

Robust	Low maintenance
Reliable	Safe
Relatively simple	Well-proven
Response to wide range of chemicals	Rapid response
Reasonable capital cost	Suitable operating
Reasonable operating cost	range

Table 2
Technology shortlist

Total carbon	Toxicity measurement
Total organic carbon	Flammability measurement
Total oxygen demand	Wet chemical techniques
UV–visible spectrophotometry	Electrochemical detection
IR spectrophotometry	Volatilisation/
Chemical oxygen demand	photoionisation
Biochemical oxygen demand	detection
Adsorbable organic halogen determination	

Following an evaluation of both the attributes and limitations of each of the shortlisted techniques, it was concluded that a dual analytical configuration utilising both total carbon (TC) and UV–visible spectrophotometry was most appropriate for this application.

TC analysis has the advantage of being well-proven in on-line applications and will respond to a wide range of compounds. It was chosen in preference to its close relative total organic carbon (TOC) since it offers an enhanced response time (2.5 vs. 5 min), no requirement for sample sparging, thereby maintaining the integrity of effluents containing volatile components, and simpler instrumentation. Earlier studies on Works effluent had shown relatively low levels of inorganic carbon to be present and therefore meant that TC values would closely reflect TOC. An analysis of those substances on the Works' chemical inventory list likely to cause problems if inadvertently discharged to drain showed most to have a carbon content $> 50\%$ by weight, thus rendering them suitable analytes for TC analysis.

Many of the chemicals likely to be present in Works effluent possess a UV–visible chromophore, indeed many of the company's dyestuff ranges are manufactured at Huddersfield, and therefore it was considered that the use of an on-line UV–visible spectrophotometric technique would facilitate rapid detection of abnormalities.

Whilst both total carbon and UV–visible spectrophotometric technologies in an on-line configuration were felt to closely meet the analytical requirements listed earlier, there remained the problem of differentiating an 'abnormal' occurrence from 'normal' day to day Works operations.

3. Laboratory trials

Extensive laboratory trials were carried out in order to explore the degree of variability of composition of effluent with respect to the chosen analytical parameters, leading to the proposal of realistic alarm levels to be applied on initial commissioning of the on-line instrumentation. The trials also helped define the various instrumental variables, thus maximising the value of the analytical data generated.

4. Experimental

4.1. Analytical instrumentation

Sartec DC-190 TC / TOC analyser

The DC-190 is a multi-mode instrument, capable of measuring total carbon, total organic carbon, inorganic and purgeable organic carbon. Any carbon present in the sample is subjected to high temperature catalytic oxidation to carbon dioxide, which is then determined spectroscopically thereby enabling the original carbon content to be calculated.

Cecil 6600 series UV–visible spectrophotometer

The Cecil 6600 Series is a conventional multi-mode UV–visible double beam spectrophotometer.

4.2. Sampling

A microprocessor-controlled portable sampling unit (Sigma Streamline 800SL) was used to collect representative samples from the Works effluent treatment plant. A range of programmes were used to collect samples reflecting effluent variability over a range of different timescales, from a few hours to several weeks.

All samples were stored at 4°C in amber glass bottles until required for analysis.

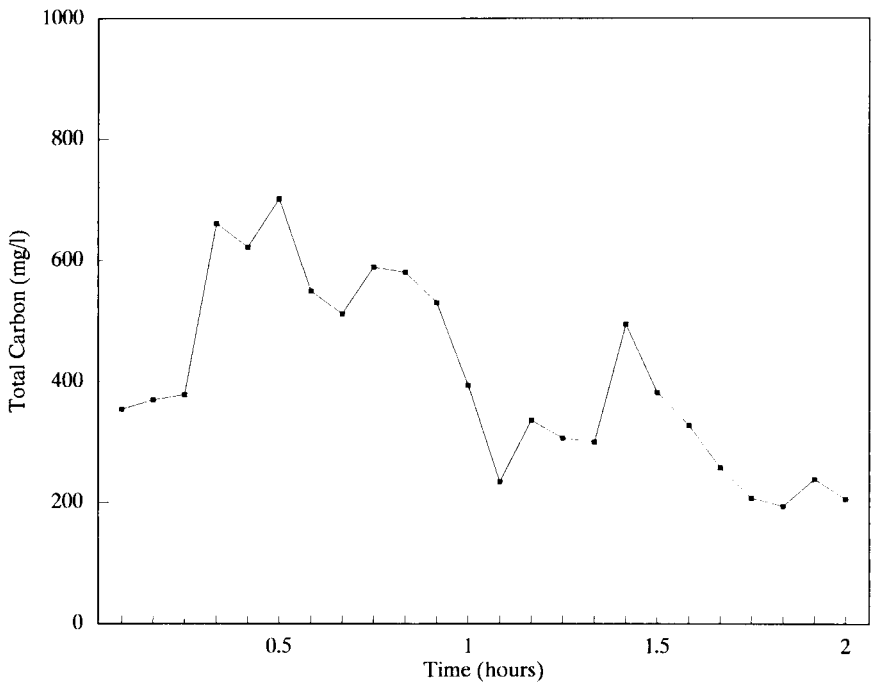

Fig. 2. Typical variation in effluent TC content over a 2 h period (grab samples).

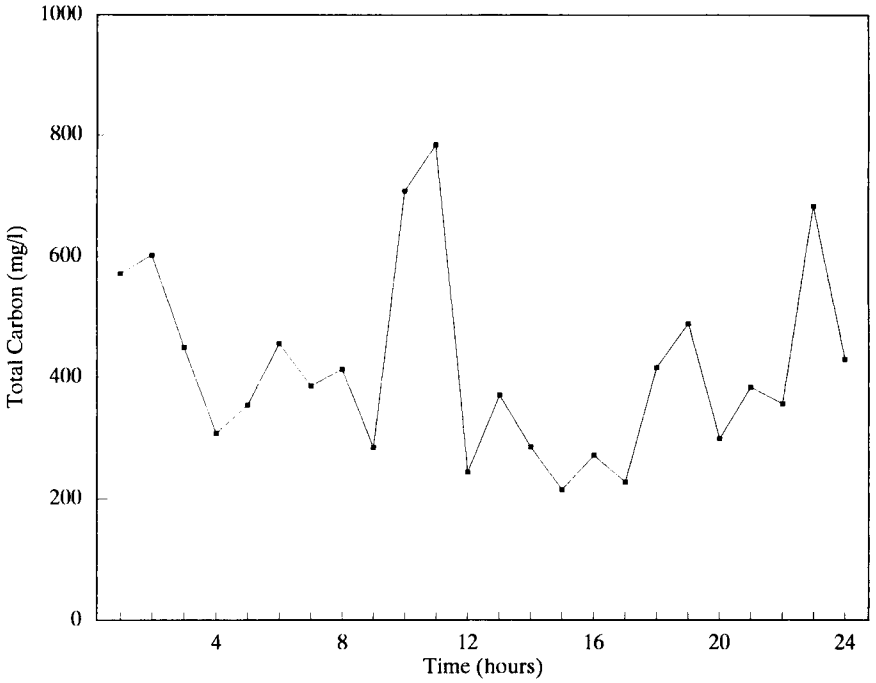

Fig. 3. Typical variation in effluent TC content over a 24 h period (hourly composite samples).

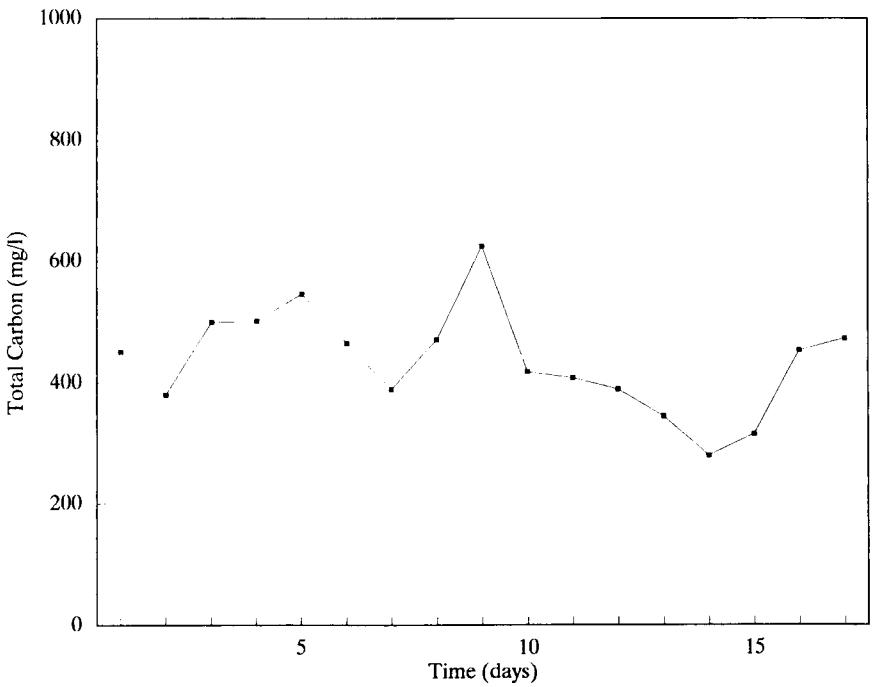

Fig. 4. Typical variation in effluent TC content over several days (daily composite samples).

4.3. Analysis

TC / TOC

Samples were analysed for TC, TOC, IC and POC in order to investigate the normal variability in composition of the effluent, and to confirm the choice of TC as the most appropriate technology to use on-line. Studies were also carried out on the oxidation efficiency versus oxidation temperature, in order to establish the optimum oxidation temperature for the on-line analysers. A series of spiked samples were also analysed, in an attempt to mimic abnormal occurrences.

UV–visible spectrophotometry

Full sample spectra (220–800 nm) were obtained, again to determine what constituted normal variability. In addition, selected wavelength analysis, selected bandwidth integrals and 1st and 2nd derivative spectra were measured in order to define the optimal analysis mode. A series of spiked samples were examined to establish likely analyser response to abnormal occurrences.

5. Results and discussion

5.1. TC / TOC / IC / POC analysis

Works effluent was found to have a typical TC value of between 200 ppm (mg l^{-1}) and 600 ppm, of which less than 10% was IC, confirming earlier work. A small amount (< 50 ppm) of POC was also typical. This endorsed the use of TC as the chosen mode of operation for the carbon analyser.

Figs. 2, 3 and 4 show the typical variation in TC value over different timescales. It can been seen that although levels are generally in the 200–600 ppm range, excursions above this range are quite common.

For all samples the TC result increased as the oxidation temperature was increased from 680 to 900°C, the operating range of the instrument. Whilst it is likely that operating at even higher temperatures would further increase oxidation efficiency, by degrading even the most refractory compounds, the choice of temperature becomes a compromise between combustion tube/catalyst life and result accuracy. For this reason most instruments will only allow combustion temperatures up to 900°C. Since absolute TC values are not required for this application this was not seen as a problem.

Having investigated the normal range and variability of TC in Works effluent, samples were spiked with potentially deleterious compounds at concentrations likely to occur in the event of an abnormal occurrence, typically 1000 ppm. From this, information was gained on the range of compounds readily detectable by this technique.

5.2. UV–visible spectrophotometry

Full range wavelength scans from 220–800 nm were carried out on all samples to define the normal absorbance spectrum of Works effluent. Using 1 mm cells, readings typically varied between 1 and 2 absorbance units at 220 nm, decreasing in a characteristically-shaped curve to close to zero at 400–500 nm. There is typically very little absorbance in the visible region of the spectrum.

Absorbances were integrated between selected wavelengths in both the UV and visible regions of the spectrum, leading to the establishment of a fairly stable 'normal' level of absorbance for each spectral window, thereby increasing the potential for an abnormal occurrence to be identified.

Selected wavelength analysis and derivative spectra were performed on several samples. The former was deemed unsuitable due to the wide local absorbance fluctuations characteristic of 'normal' Works effluent whilst the latter, although potentially very sensitive to small changes in the spectrum, would require more complex on-line instrumentation than that sought for this application.

Samples were spiked with potentially deleterious compounds at concentrations likely to occur in the event of an abnormal occurrence. Analysis of the selected bands in the UV and visible regions of the spectrum gave a high enough signal-to-noise ratio to detect many such compounds at 500 ppm, rendering this technique superior to

Table 3
Preferred technique for abnormal occurrence detection

Total carbon	UV–visible spectrophotometry
n-Butanol	Aniline
Cyclohexylamine	4-Butylaniline
Piperidine	*N,N*-dimethylaniline
Butylethoxol	*N,N*-diethylaniline
Methanol	Bipyridyls
Dimethylformamide	3-Cresol
Methylethylketone	Nitrobenzene
Methylisobutylketone	*N*-phenylglycine
Acetone	Procion dyes
Acrylonitrile	Pyridine
Ethanol	Certain pesticides
Acetic acid	Sodium dispersol
Chloroacetaldehyde	*tert.*-Butylbenzaldehyde
tert.-Butanol	

TC for those chemicals with strong chromophores.

The laboratory trials clearly showed the potential for the two chosen technologies to be used on-line for the detection of abnormal occurrences in Huddersfield Works effluent. Furthermore, whilst many compounds with the potential for accidental release would result in a sufficiently large signal-to-noise ratio using either technology for abnormality detection, for several others the techniques are complementary (see Table 3).

5.3. On-line analysis

Having established the technologies most appropriate for this application, and the optimum configuration of laboratory-based instrumentation used for the trial work, it was then necessary to specify and identify suitable on-line instrumentation. It should be noted that the translation of an effective laboratory-based analytical technique into an effective on-line monitor is non-trivial, new problems have to be addressed and in particular the complex issue of effectively sampling the effluent stream to be measured needs resolving. The problems associated with taking a representative sample and transporting it to the analyser are beyond the scope of this paper but it is sufficient to note that this is an area that demands equal, if not greater, attention than the 'analytical' part of the on-line system if the installation is to be a success.

Choice of TC analyser

Most TC analysers fall into one of two basic types, differing chiefly in the mode of oxidation of the sample. A high temperature catalytic oxidation instrument was used for the laboratory trials; the main alternative instruments utilise low temperature UV/sodium persulphate oxidation. It was felt that the latter technology would suffer from several disadvantages when used in this particular application, summarised as follows:

(a) Instrument manufacturers' literature suggests that the low temperature oxidation method performs inefficiently when presented with the more refractory compounds, in particular those of long carbon chain length or containing several heteroatoms.

(b) Because oxidation is dependent on free radical formation, utilising a UV source for energy input, the presence of particulate material can render oxidation incomplete. Sampling system considerations, and the integrity of the measurement itself, dictated that the tolerance to particulate material needed to be as great as possible consistent with effective oxidation of the sample, therefore the high temperature method was preferred.

(c) Any fouling of the reaction chamber leads to less efficient oxidation due to a reduction in the amount of UV radiation reaching the sample solution; Works effluent can contain high levels of sub-μm particles even after filtration, with a consequent risk of fouling. In addition, as a dyestuff manufacturer the effluent can sometimes assume a distinct colouration, thus reducing the ability of the UV source to fully penetrate the sample line.

(d) Instruments utilising high temperature oxidation have a slightly shorter combustion cycle time, an important consideration in this application.

Following consideration of several instruments from different manufacturers, each utilising the high temperature catalytic combustion method, the Ionics 6800 unit was chosen based largely on

its successful use elsewhere in the company, albeit not for this precise application.

Choice of UV–visible analyser

The Procal Pulsi 650 was chosen on the grounds of it being an instrument well-proven in industrial process applications, and capable of meeting the criteria demanded of an on-line installation. Utilising simple spectrophotometric filters the instrument can be configured to record up to 5 separate absorbance channels and hence provide the sort of basic spectral integration required to reproduce the laboratory trial work.

It should be noted that the on-line application of technically more complex laboratory-based instrumentation, incorporating for example diode array technology, was at this time neither proven nor commercially-available and in any case would not have afforded significant advantages over the simpler approach adopted in this instance.

Trial work

Extensive on-line trials were undertaken following installation of the selected instrumentation in order to determine the efficacy of the chosen systems, fully appraise the sampling system and confirm the operating range and alarm levels established during the laboratory trials. In addition, it was possible to take the Procal UV–visible analyser off-line in order to determine its response to effluent samples spiked with a range of potentially-deleterious compounds.

As indicated earlier, the majority of the problems experienced during the trial phase centred around the sampling system, and for example included blocked filters, blocked lines and ineffective pumps. The need to develop a system effective for each particular application cannot be over stressed. In general the analysers themselves performed well, although it soon became apparent that the UV–visible spectrophotometer required a finer mesh filter in order to prevent frequent fouling of the sample cell windows.

Analyser location and configuration

The final location of the analysers was largely determined by considering the concentration profile of an abnormal occurrence as it passes through the effluent primary treatment plant. Historical data, together with mathematical modelling techniques, show that the majority of abnormal occurrences exhibit plug flow characteristics as they move through the site liquid effluent drainage system, but are subject to significant dispersion during primary treatment. During the dispersion process the concentration of the various analytes is effectively reduced, rendering abnormal occurrence detection more difficult. In the light of these observations, and taking into consideration the response time needed prior to initiating a diversion of contaminated effluent into the containment tanks, the primary analyser house was located approximately 7 min upstream of the treatment plant, conveniently at the point where the effluent is pumped above ground in order to give it the potential energy to move through the system under gravity (Fig. 1).

In order to allow for instrument downtime, for example to enable maintenance to be carried out, it was necessary to build some redundancy into the analytical capabilities of the system. For this reason two examples of each instrument type were procured, with, in the case of TC only, the addition of a third instrument to decrease the response time in the event of an abnormal occurrence. Whilst the UV–visible analysers provide a real-time output, ignoring sampling time delay, the TC systems have an analytical cycle time of approximately 2.5 min. By utilising 3 instruments in parallel, and by synchronising their cycle times such that a fresh reading is obtained every 50 s, the delay prior to abnormal occurrence notification is minimised.

A fourth TC analyser was located at the final Works outfall as an indicator of the quality of the effluent reaching Yorkshire Water Services, several hundred metres downstream.

Alarm settings

During the on-line trials a considerable amount of data was generated enabling a statistical analysis of the effect of applying various alarm levels to the two measurement criteria to take place. The following important considerations needed to be borne in mind when assessing the results of the analysis:

(a) The acceptable frequency and volume of diversion, noting that the total containment tank capacity is 12000 m^3, equating to approximately 12 h flow under normal conditions. Following every diversion time is needed to treat and/or bleed back into the effluent system, thus reducing total tank capacity for the management of subsequent abnormal occurrences.

(b) Many of the diversions would require further laboratory analysis, since their origins may be unknown or non-quantifiable. The resource implications could therefore be significant.

In consideration of the above and the data obtained from the trials an initial TC alarm level was set, together with 5 UV–visible spectrophotometric band alarm levels. It is intended that all alarm levels will be reduced in due course as the environmental performance of the Works improves.

Instrument maintenance

A common cause of on-line instrument malfunction is simply lack of an appropriate level of maintenance, resulting from ill-defined ownership of the hardware. Both during the trial work, and particularly later on for the commissioning and full utilisation phases, the wisdom of having dedicated maintenance personnel was clearly demonstrated. Primary responsibility for routine maintenance now lies with the plant process operators, with more complex problems being handled by a central technical support team, who were set up to troubleshoot all such on-line installations on the Works.

5.4. Operating philosophy

Detection of abnormal occurrences

It is important to realise that detection of an abnormality by on-line analytical instrumentation is complementary to, and not a replacement for, other notification mechanisms. For example, a manufacturing plant may be aware of an accidental release of a potentially toxic compound and would immediately telephone the Works' emergency response team so that appropriate measures could be taken. Indeed, for this scenario a PC-based effluent flow modelling technique is utilised as a means of calculating arrival time at the effluent primary treatment plant, which can be anything from 5 to 40 min following release, and therefore the required diversion start time and duration. In such instances the on-line instrumentation is a valuable confirmatory tool for management of the release.

Where an abnormal occurrence is not prenotified, but has been detected instrumentally by one or more of the installed on-line analytical technologies, diversion is initiated either automatically or via a manual override capability. This would occur following successive high readings from two of the TC units or sustained high readings from one or more of the spectral bands monitored by the UV–visible spectrophotometers. The diversion is ended manually via close observation of the analyser outputs, which are relayed to VDU's in two strategic locations.

Although the on-line analysers were chosen on the basis of their response to a wide range of compounds, it may be that an abnormal occurrence is undetectable on the basis of the quantity of material released, or its physical/chemical properties. Whilst in many instances this would not be a problem, resulting for example from a normal routine operation within a plant, for highly toxic compounds the outcome could be serious in terms of the deleterious effect on the biological treatment processes further downstream, or even the ecosystem beyond. For this reason, it is company policy to build in safeguards at individual manufacturing plants, for example impervious bundling of storage vessels, so that scenarios like this cannot occur.

Diversion options

Automatic, instrument-initiated diversion occurs at a point prior to the effluent primary treatment plant, allowing interception of an abnormal occurrence at its point of maximum concentration, and hence least volume. This has obvious advantages in terms of management of the problem, by occupying less of the containment facilities and rendering subsequent processing easier to undertake.

For manually-initiated diversions the point of interception is ideally also prior to the effluent

primary treatment plant, but late notification may mean that this is not always possible, some or all of the release having passed into the plant. In this instance there are two further diversion possibilities, one part way through the plant following pH adjustment and the other at the final exit from

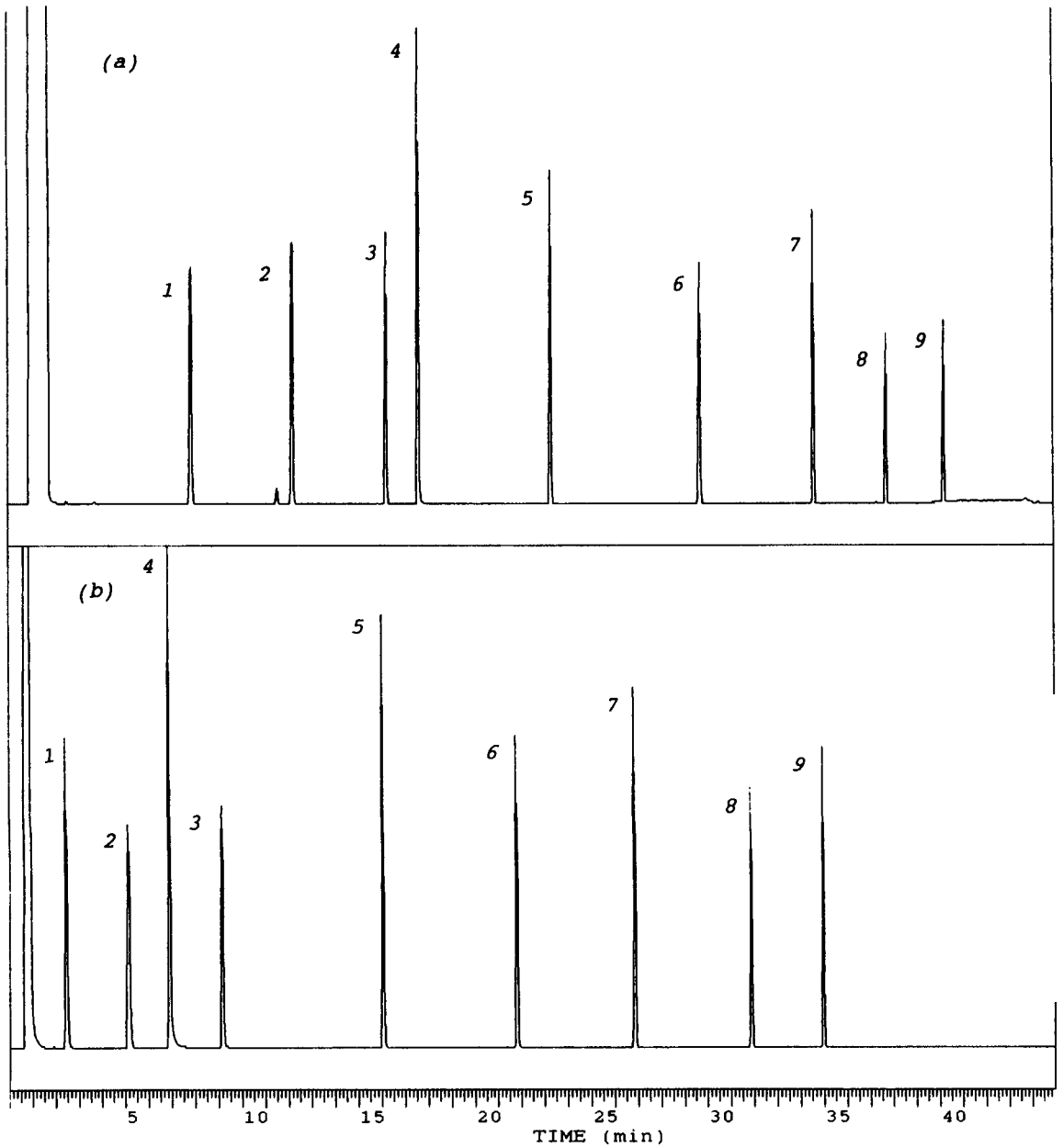

Fig. 5. Chromatograms obtained from liquid effluent spiked with 500 ppm aniline; column type (a) Chrompack CP Sil 13CB, (b) Chrompack CP Sil 5CB, both 25 m × 0.25 mm i.d., 0.25 μm film thickness; column temp. 40°C for 5 min, then to 150°C at 5°C/min, held for 2 min, then to 260°C at 10°C/min, held for 3 min.; inj. 30:1 split at 220°C; det. FID at 250°C; sample vol. 3 μl; carrier gas He at 40 cm/s; peak identities: 1 = octane, 2 = nonane, 3 = decane, 4 = ANILINE, 5 = dodecane, 6 = tetradecane, 7 = hexadecane, 8 = octadecane, 9 = eicosane.

the Works, the last point at which interception can occur. In spite of the disadvantages of larger effluent volume and more complex handling procedures these alternative points of interception are seen as important to the efficacy of the system (Fig. 1).

From whichever point effluent is intercepted and sent to the containment facilities, immediately prior to tank entry a flow-proportional composite sample is automatically taken. This sample is totally representative of the tank contents, obviates the need for direct sampling from the containment vessel, and provides sufficient effluent for subsequent laboratory-based analysis to take place.

Analytical response

The analytical strategy adopted where the nature of the diverted effluent is known, for example following manual notification of an accident on plant, is quite different from the approach used for an unknown abnormality, for example where an automatic diversion has been initiated by the on-line instrumentation following a signal exceeding the pre-set alarm levels.

In the former case, for the vast majority of chemicals held on the Works there are fully documented analytical methods available, most of these being based on either gas or liquid chromatography. A permanently-manned laboratory means that confirmation of identity and subsequent quantification is accomplished in a timely fashion.

In order to address the problem of characterisation of an unknown abnormality, a technique was needed that was both analytically powerful and relatively straightforward to use. It was not deemed appropriate to train technician-level personnel in advanced analytical techniques, for example gas chromatography–mass spectrometry (GC–MS).

Purchase of the Nordion Micromat GC system with Micman 5 software provided an elegant and effective answer to the problem. The system basically comprises a capillary GC fitted with two columns of differing polarity, two flame ionisation detectors and a data reduction software package. A sample of effluent requiring characterisation is combined with a standard series of alkanes (octane, nonane, decane, dodecane, tetradecane, hexadecane, octadecane and eicosane) and is injected on to both columns simultaneously via a common injection port. Following chromatography a pattern recognition algorithm is applied to the standard series, with any significant unknown peaks being assigned retention index values via measurement of their retention times relative to those of the standard set. A comparison can then be made between the observed retention indices and those held in the system library. Providing a good fit is obtained, ideally on both columns, the identification of the contaminant(s) can be established with a high degree of confidence (Fig. 5). There are currently approximately 75 compounds, used on the Works in quantities sufficient to cause a problem if released to drain, held in the library.

Following tentative characterisation using the Nordion system, existing well-documented methodology is used to confirm contaminant identity and provides a quantified analysis. If the Nordion system is unable to characterise the contaminant, for example because the chemical involved is not held in the library or a poor pattern fit is obtained, alternative analytical techniques need to be used. This would usually require an input from specialist analytical personnel, and could encompass techniques such as gas, liquid and ion chromatography with a variety of sample inlet and detector options, the former including mass spectrometric detection. It may also be necessary to utilise the services of other groups, based at the company's corporate research centres, who are able to offer advanced characterisation techniques including LC–MS, MS–MS, and GC–MS–FTIR.

Disposal

Having utilised some, if not all, of the capacity of the containment tanks, and knowing the nature and concentration of the contaminant(s), the disposal options then need to be considered. Central to the decision-making process is a knowledge of the degree of hazard presented by the material, with a judgement being made as to whether controlled release back into the effluent

primary treatment plant is appropriate, or further more complex physical/chemical treatment is required.

The company's Environmental Laboratory at Brixham, Devon, is an invaluable source of expertise when assessing the options. The Laboratory is usually able to recommend a 'safe' concentration target below which the diverted effluent can be released back into the primary treatment plant, based on a consideration of the toxicological properties of the compound(s) involved. This is normally a factor of 10 below the level known to cause any adverse ecological effects, which when coupled with the dilution and biodegradation/adsorption that occurs at Yorkshire Water creates a wide margin of safety. In certain cases it may prove necessary to further treat the diverted effluent prior to release or transfer to licenced disposal contractor. Oxidation utilising hydrogen peroxide and/or ozone, or adsorption onto, for example, activated carbon, are the options most likely to be applicable. The containment tanks have been designed with a view to facilitating these operations.

6. Conclusions

The design, construction and commissioning of a major liquid effluent management facility at Zeneca Ltd's Huddersfield Works, to complement the existing primary treatment system, has resulted in a significant improvement in the Works' ability to detect and manage effluent abnormal occurrences.

Careful choice of the analytical technology incorporated into on-line instrumentation, involving extensive laboratory and field trials, has meant that the widest possible range of chemicals with a potential for accidental release can be detected in time for interception and treatment.

It is envisaged that as a more comprehensive knowledge of the nature of abnormal occurrences on the Works is gained, source attribution will be possible leading to operational improvements and a subsequent enhancement of environmental performance. This is also likely to include further development of analyser systems closer to individual plants, nearer the source of the problem.

Finally, the installation of on-line TC and UV–visible spectrophotometric analysers as part of this project is only seen as being an important first stage in the detection and management of abnormal occurrences. In particular the development of an on-line toxicity monitoring capability is viewed as a further essential activity, as the Works' manufacturing portfolio tends more towards lower volume, highly active agrochemical and specialty chemical products.

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Fibre optic reflectance sensor for the determination of aluminium(III) in aqueous environment

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Abstract

An optical Al(III) sensor based on the use of Eriochrome cyanine R (ECR) immobilised on XAD-2 (styrene–divinylbenzene cross-linked copolymer) and diffuse reflectance spectrophotometry has been developed. A kinetic approach was used to quantify sensor response to Al(III) concentration in which the reflectance signal is measured at a fixed time interval of 3 min. Reproducible measurement of Al(III) was possible using the same probe (R.S.D. = 1.8%). Linear response was obtained for Al(III) concentration 1.3×10^{-5}–4.0×10^{-4} M with limit of detection of 1.0×10^{-5} M of the metal ion. The sensor was also used for the determination of Al(III) in aqueous samples and the results obtained were comparable to those obtained by graphite furnace atomic absorption spectrometry.

Key words: Sensors; Fibre optic reflectance sensor; Aluminium

1. Introduction

Al(III) is widely known to create strong threats to the water ecosystem [1] and has been long associated with some diseases such as Alzheimer's and bone softening [2]. Since much of the Al(III) intake is caused by drinking water, a selective Al(III) sensor which can operate remotely and continuously monitor the water quality, would be immensely useful.

The development of optical fibre chemical sensors is a subject of growing interest since they offer several advantages over the conventional sensors. Optical sensors are electronically passive and are not subject to electrical and electromagnetic interference [3]. They can be corrosion resistant and are capable of real time monitoring of sample. Furthermore, they are also flexible, easily miniaturised, inexpensive and of rugged construction.

ECR has been used previously for Al(III) determination [4]. In the presence of long chain cationic surfactants, its sensitivity towards Al(III) was found to be significantly increased [5,6].

This paper describes the use of ECR immobilised on XAD-2 as the reagent phase in the development of an optical sensor for determination of Al(III) in aqueous environments.

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2. Experimental

2.1. Instrumentation

The apparatus used to measure reflectance is shown in Fig. 1. It consists of a quartz-halogen lamp (12 V, 100 W) whose optical radiation was modulated by an optical chopper (Bentham 218) and focused onto one end of a bifurcated optical fibre. This light is guided to the probe, which contained the reagent phase, and the light reflected back to the fibre is guided by the second branch of the bifurcated fibre to a monochromator (ISA Jobin-Yvon H1061). The light was then detected by a photomultiplier (Hamamatsu R446), enhanced by a current amplifier (Bentham 286) and a lock-in amplifier (Bentham 223) before finally being recorded on a chart recorder (DBC SE120).

Atomic absorption spectroscopy of the samples were measured using a Perkin-Elmer 3100 graphite furnace atomic absorption spectrometer (GF-AAS).

2.2. Reagents and buffer solution

Buffer solution pH 6.0 of ammonia–acetic acid was prepared by adding 50 ml of glacial acetic acid (AR) to approximately 500 ml distilled deionised water. Sufficient ammonia solution (s.g. 0.88, AR) was added to adjust its pH to 6.0 (approximately about 50 ml required), and the mixture was diluted to 1 l with distilled deionised water.

Standard Al(III) (4.0×10^{-4} M) and cetylpyridinium chloride (2.0×10^{-4} M) solutions were prepared by dissolving the required amount of

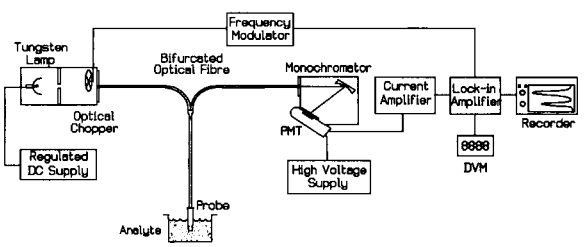

Fig. 1. The instrument used for reflectance measurement.

Table 1
Determination of Al(III) in aqueous sample by using both GF-AAS and optical fibre Al(III) sensor

Concentration of Al(III) (M)	GF-AAS	Reflectance (M)
3.0×10^{-4}	$3.0 (\pm 0.2) \times 10^{-4}$	$3.1 (\pm 0.4) \times 10^{-4}$
8.0×10^{-5}	$8.0 (\pm 0.2) \times 10^{-5}$	$8.4 (\pm 0.4) \times 10^{-5}$
2.0×10^{-5}	$2.0 (\pm 0.1) \times 10^{-5}$	$1.8 (\pm 0.3) \times 10^{-5}$

aluminium potassium sulphate (Aldrich) and cetylpyridinium chloride (BDH), respectively in the buffer solution. Saturated fluoride solution was prepared by dissolving excess amounts of sodium fluoride (Aldrich) in buffer solution. Solution of ECR (Fisons) was prepared daily in the buffer solution.

2.3. GF-AAS method

For determination with GF-AAS, three different samples of known concentration (see Table 1) were prepared in the laboratory. These sample were diluted 100 times and 20 μl of the diluted sample was injected into the graphite furnace. The reading (peak height) was recorded at a wavelength of 309.3 nm. The calibration graph was prepared in the similar way using standard Al(III) solution in the concentration range 1.3×10^{-5} M–8.0×10^{-4} M.

2.4. Immobilisation procedure

ECR was immobilised onto XAD-2 as its Al(III) complex in the presence of cetylpyridinium chloride, in order to prevent the functional groups involved in complexation from interaction with the XAD-2 polymer. To about 2 g of washed XAD-2, 50 ml of ECR solution (2.0×10^{-4} M), 50 ml of cetylpyridinium solution and 15 ml of Al(III) solution (2.0×10^{-4} M) were added and the mixture was stirred for about 5 h. The immobilised reagent was removed from the supernatant liquid, washed with deionised-distilled water and allowed to dry in a desiccator. The dried immobilised ECR was soaked in the fluoride solution to remove the Al(III) ions before use.

2.5. Probe design

Two probe designs were investigated here.

The first design was based on a common type where the immobilised reagent was encapsulated at the end of the optical fibre using PTFE membrane which in turn was held to the fibre by heat shrink tubing. The second probe design was studied after noting poor performance of the first probe design (see Results and Discussion).

2.6. Reflectance measurement

The analysis of reflectance measurements was carried out by the use of a kinetic approach where the signal is measured after 3 min of the insertion of the probe into the Al(III) solution. The measurements taken were expressed as a relative reflectance (in arbitrary units, a.u.), which is defined as the difference between the reflectance of the Al(III) complex and that of the immobilised ECR alone, both recorded at the measurement wavelength (643 nm).

2.7. Interference studies

Interference from foreign ions was studied by introducing amounts (moles) of interfering ion comparable to that of Al(III), to the sensing probe. The degree of interference of these ions were evaluated with reflectance measurement, recorded in the presence and absence of the interfering ion for the determination of a given concentration of Al(III).

3. Results and discussion

3.1. Probe design

Fig. 2a shows the first design of the Al(III) sensing probe which was based on the conventional design of the optical sensor, e.g. pH sensor [7]. No response was observed with this probe when PTFE membrane was used. This may be due to sluggish diffusion characteristics of the membrane. Another limitation of this design includes a small surface area of the fibre which makes it difficult to apply immobilised reagent on its surface. Again, since this design is of a permanent type, it had to be cut and repolished again before replacing the reagent for re-use.

This design was improved by replacing PTFE membrane with a nylon mesh (40 μm) and increasing the surface area of the probe, and having a disposable type of design at the sensor end rather than that in the permanent type. The surface area of the probe was increased by adding two rubber tubes outside the fibre with the second tube (PTFE with 5 mm diameter) protruding slightly outward to aid holding the reagent phase in place (Fig. 2b). The probe had the disposable

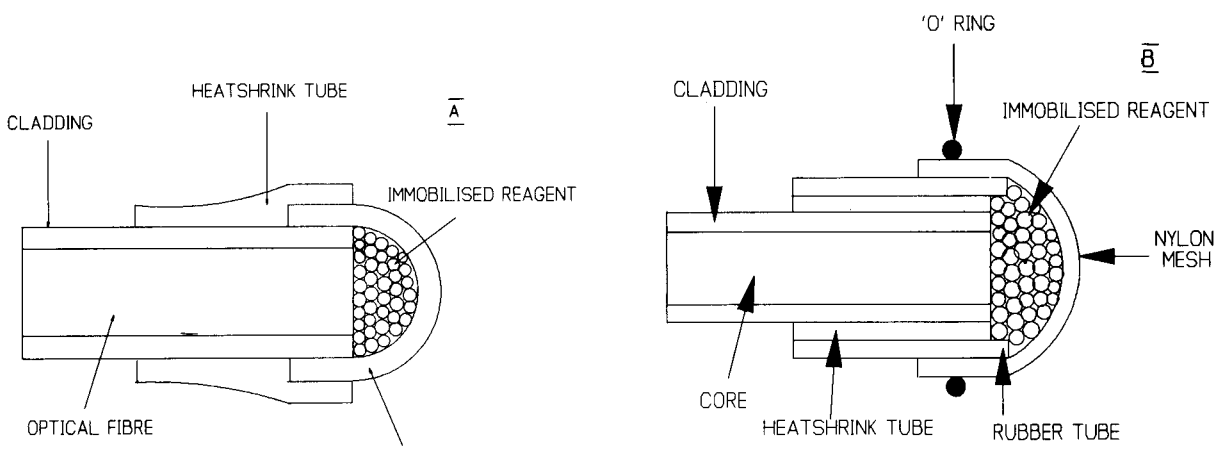

Fig. 2. The design of the probe: (A) based on the well established design of optical sensor for pH, (B) improved design.

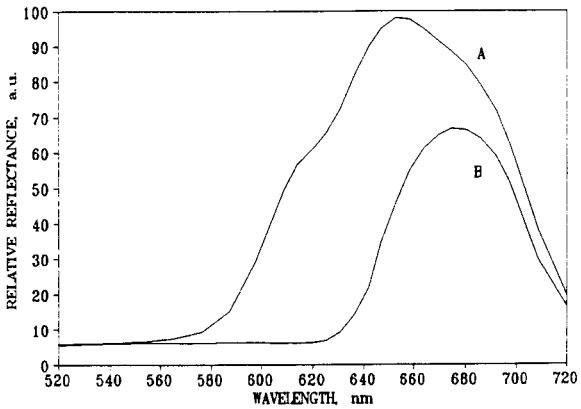

Fig. 3. The diffuse reflectance spectra (520–720 nm) of the immobilised ECR alone (A) and after reaction with Al(III) (B).

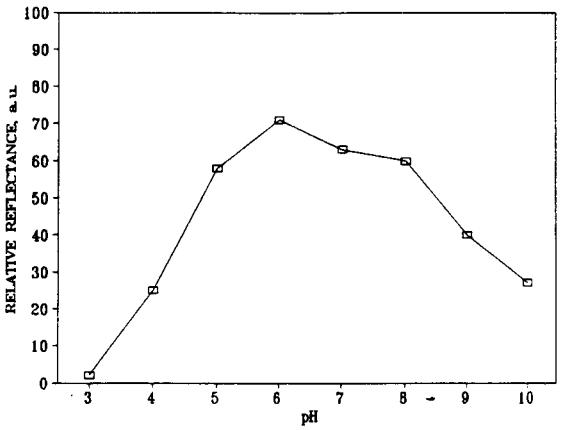

Fig. 4. The effect of pH to the probe response towards Al(III).

type of reagent tip which was constructed by using an O-ring to hold the nylon mesh against the fibre.

3.2. Response to Al(III)

Fig. 3 shows the diffuse reflectance spectra of the immobilised ECR before (A) and after (B) reaction with Al(III). It can be noted that reaction with Al(III) causes a large decrease in reflectance, which is due to a sharp change in colour of the reagent phase (red to dark blue). The maximum reflectance difference was found to be at the wavelength of 643 nm and this wavelength was therefore used for all the analytical measurement. The sensor probe was observed to produce the same steady state response and thus was the reason why a kinetic approach was used in this study to quantify the Al(III) concentration.

3.3. Parameter affecting the probe response

The response of the probe towards Al(III) was governed by the pH of the solution (Fig. 4). At lower pH, the probe showed low reflectance signal because of the tendency of the reagent to leach from the XAD-2. At higher pH, the reflectance signal decreased due to the formation of a solid $Al(OH)_3$, which effectively removes the Al(III) from the system. In this work, the measurement was carried out at a pH of 6.0.

Stirring the Al(III) solution had a very significant effect on the probe response. As shown in Fig. 5, the steady state signal increased by the factor of more than 10 with stirring. This is due to the fact that stirring could facilitate the diffusion of more Al(III) ions across the nylon mesh to the reagent phase and therefore increase the rate of reaction between the reagent and the Al(III) ions. Without stirring, however, the diffusion depended solely on the concentration differences set across the membrane.

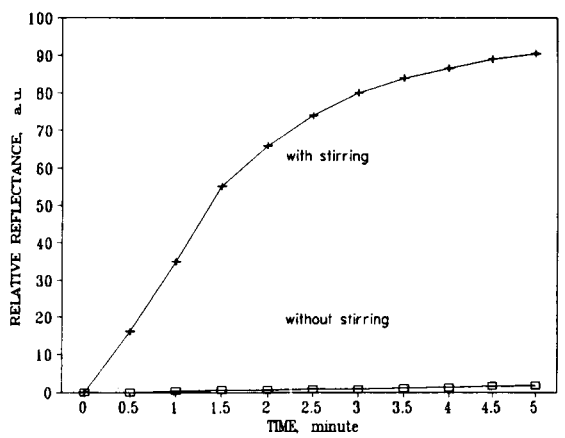

Fig. 5. The effect of stirring to the steady state response of the probe.

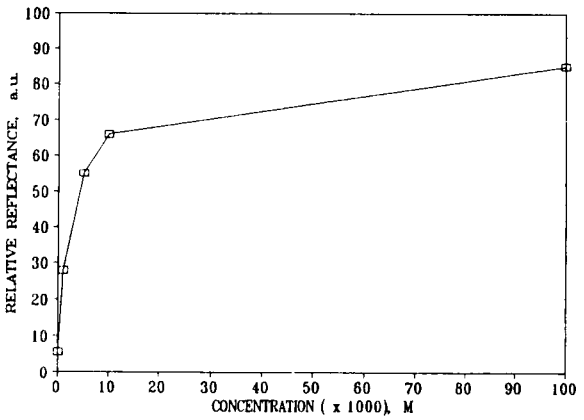

Fig. 6. The effect of initial ECR concentration to the response of the probe.

The probe response was also noticed to be dependent on the initial ECR concentration used for immobilisation. Fig. 6 indicates that the higher the ECR concentration used for immobilisation, the higher the reflectance signal obtained for the same concentration of Al(III).

3.4. Regeneration of the probe

It was found in this study that ethylenediaminetetraacetic acid (EDTA) gave the highest degree of interference in Al(III) determination when compared to many other ions. This can be explained by the fact that it has six binding sites which are capable of binding the Al(III) ion. The use of this anion and other anions such as tartrate, fluoride, oxalate and phosphate as regenerating agents was also studied. The result (Fig. 7) shows that EDTA, produced long probe regeneration times (~ 1 h) while fluoride ion gave the shortest time (2 min). This can be explained by the fact that EDTA is more bulky than fluoride ion and therefore produces greater steric effect on approaching the Al(III) complex in the probe. The F^- ion, on the other hand, is more accessible to the probe and the Al(III) ions can therefore be readily removed from the probe. Regeneration time is defined in this study as time taken for the probe to reach it base line signal soon after it has been dipped in the regenerating solution.

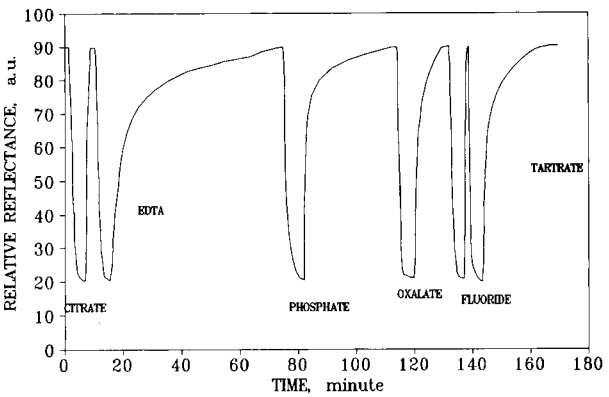

Fig. 7. Response obtained by the use of different types of anions for the regeneration of the probe.

3.5. Reproducibility

The reproducibility of the probe was studied by measuring the response of the probe to the Al(III) solution of the same concentration (2.0×10^{-4} M). Reflectance measurement obtained by using the same probe gave a very good reproducible result (R.S.D. = 1.8%). The variation between probes, however, was found to be slightly high (R.S.D. = 5.0%).

3.6. Determination of Al(III)

A linear relationship was obtained between the relative reflectance and the logarithm of

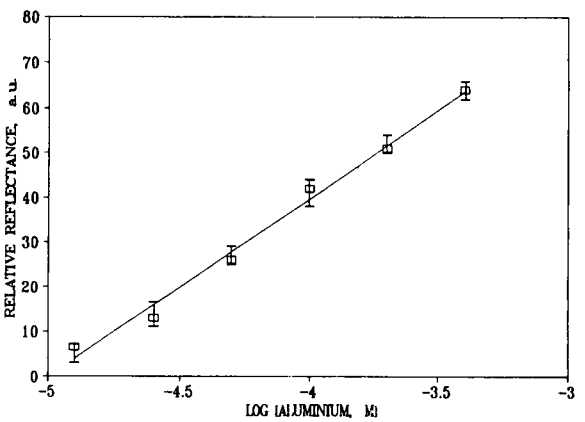

Fig. 8. Linear response produced by the sensor in the range of 1.3×10^{-5} to 4.0×10^{-4} M Al(III) (slope = 39.79 ± 1.97; intercept = 198.86 ± 2.47 and regression coefficient = 0.9903).

Al(III) concentration in the range of 1.3×10^{-5} M to 4.0×10^{-4} M (Fig. 8). An attempt was also made to study the reliability of the results obtained using this probe by comparison with the well established method such as atomic absorption spectrometry. The results obtained are shown in Table 1. Although the standard deviation of the reflectance method is found to be slightly greater than that of the graphite furnace-AAS, the results obtained show very good agreement with the Al(III) concentration determined in the sample using the optical sensor.

The limit of detection (LOD) as defined as the concentration of sample that yield a detector response equal to three times the detector noise was found in this study to be 1.0×10^{-5} M.

3.7. Interference

The degree of interference measured from some foreign ions at an ion to Al(III) ratio of 1 : 1 is summarised in Table 2. As expected, EDTA, citrate and phosphate gave a degree of interferences higher than that obtained from fluoride because each of these ions possess more than two sites that are capable of binding Al(III). An attempt to use the solution of these ions to regenerate the reagent however indicated that a longer recovery time was needed than when fluoride solution was used. This could be due to the steric effect caused by the bulkiness of these ions as explained earlier. The main cation interference was obtained from Be^{2+} and this would be expected because ECR also forms complexes with Be^{2+} and has been used for the Be^{2+} analysis [8]. Other ions such as Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, CO_3^{2-}, OH^-, CH_3COO^-, In^{3+}, Ni^{2+}, Ca^{2+}, CO^{2+}, Cr^{3+}, Pb^{2+}, Sn^{4+}, Sn^{2+}, Zn^{2+}, Mg^{2+}, Ti^{3+}, Ti^{4+}, Ce^{3+} and Zr^{3+}, showed no interference in Al(III) determination even if present at the molar ratio of up to 1 : 100 (Al(III) to ion).

Table 2
The degree of interference measured from some foreign ions at an ion to Al(III) ratio of 1 : 1

Anion	Degree of interference (%)	Cation	Degree of interference (%)
F^-	−15.5	Be^{2+}	+13.4
EDTA	−75.3	Ca^{2+}	+7.0
PO_4^{3-}	−50.6	Fe^{3+}	+10.4
$C_2O_4^{2-}$	−2.9	Ga^{3+}	+5.4
$C_6H_5O_7^{2-}$	−71.4	Fe^{2+}	+5.1
$C_4O_6H_4^{2-}$	−10.2		

4. Conclusion

Immobilised ECR on XAD-2 provides a good reagent phase for the development of a sensitive, regenerable and reproducible optical Al(III) sensor. This sensor is comparable to the well established method, graphite furnace-AAS when used for Al(III) determination.

Acknowledgement

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Selective extraction of organochlorine and organophosphorus pesticides using a combined solid phase extraction–supercritical fluid extraction approach

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Abstract

Supercritical fluid extraction (SFE) of pesticides has been investigated for the possibility of selective extraction between organochlorine (OCPs) and organophosphorus (OPPs) compounds. Three OCPs and OPPs were trapped onto a C_{18} Empore™ extraction disk prior to SFE with CO_2 only and methanol-modified CO_2 at various pressures and constant temperature. The results indicate that OCPs can be quantitatively extracted using CO_2 only whereas OPPs require a modifier for extraction.

Key words: Gas chromatography–mass spectrometry; Pesticides; Selective extraction; Supercritical fluid extraction

1. Introduction

There has been increasing concern in recent years about the long-term effects of organochlorine pesticides (OCPs) in the environment. Their known toxicity has led to their control and an overall move to replace them with less toxic compounds. Organophosphorus pesticides (OPPs), which exhibit lower mammalian toxicity, have been used as a replacement and have overtaken OCPs as the most-used insecticides [1].

Traditional extraction methods used to remove pesticides from environmental samples usually consist of liquid–liquid extraction (i.e. Soxhlet) followed by a preconcentration step. These techniques are often time-consuming, require large volumes of organic solvents and are non-selective for the types of compounds which are extracted. In comparison, supercritical fluid extraction (SFE) allows rapid extraction of analytes due to the unique properties of the extraction fluid which include high diffusivity and low viscosity [2]. Carbon dioxide is the most widely used extraction fluid and is an excellent solvent for non-polar analytes. The addition of an organic entrainer, either from a second pump or added directly into the extraction cell, allows extraction of more polar analytes. In addition, solvent strength is directly related to supercritical fluid density which can be easily altered by changing the pressure and temperature of the extraction fluid. This leads to the possibility of performing selective

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extractions. Class-selective extractions between alkanes and polyaromatic hydrocarbons (PAHs) have been demonstrated by Hawthorne and Miller for the extraction of diesel exhaust [3].

There have been few publications where SFE has been used to extract analytes from an aqueous matrix directly mainly because of the inherent problems associated with this technique [4,5]. Water has a moderate solubility in supercritical CO_2 (0.3%) [6]. This can lead to restrictor plugging due to ice formation during extraction and also carry over of water into the collection vessel which can interfere with any subsequent detection. One way to overcome these problems is to use solid phase extraction (SPE) disks (Empore™) to trap analytes prior to elution with a supercritical fluid [7–10]. These disks [11] offer an alternative to the conventional SPE cartridges as they can be used with a traditional filtration apparatus arrangement which allows high sample flow-rates. Once the analytes have been adsorbed onto the C_{18} packing, contained in the disks' PTFE membrane, the disks can be simply rolled and placed in an extraction cell. Supercritical carbon dioxide can then be used to elute the analytes.

2. Experimental

2.1. Apparatus

Supercritical extractions were undertaken on a Carlo Erba SFE 30 system (Fisons Instruments, Crawley, UK) comprising a 150-ml syringe pump (SFC 300). Fig. 1 illustrates the main components of the system. Extractions were carried out in off-line mode with the pressure being maintained by a heated (150°C) stainless steel restrictor. The flow-rate through the restrictor was approximately 2 ml min^{-1} as it was found that lower flow-rates resulted in less quantitative extraction. The analytes solubilized in supercritical CO_2 were depressurized into 5 ml of hexane contained in a septum-capped 25-ml vial. Loss of analytes by aerosol formation was minimized by passing the eluted CO_2 through a syringe needle to which a C_{18} SPE cartridge is attached. Any escaping analytes are then trapped onto the cartridge and can be subsequently back-flushed with a small amount of hexane. The collection vessel was not cooled as the expansion of the depressurized CO_2 caused sufficient cooling (Joule–Thompson) to effectively trap the analytes. This may account for the increased recovery found at higher flow-rates as the higher the flow-rate the cooler the vessel becomes. The extraction temperature was held at 50°C throughout the experiments.

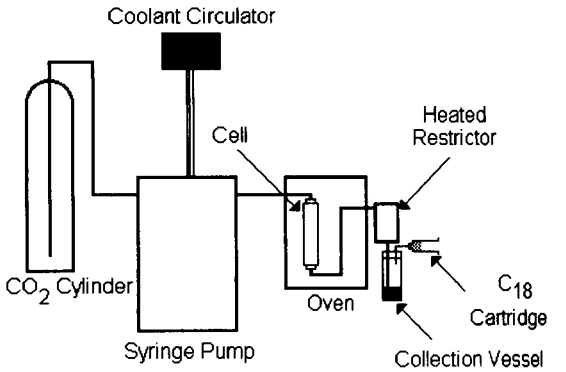

Fig. 1. The main components of the Carlo Erba SFE system.

The extraction pressure was set using the syringe pump and varied between 7.5 MPa and 40 MPa. The 10-ml extraction cells used were supplied by Jasco (Mettler–Toledo, Halstead). Methanol modifier (used to successfully extract OPPs) was introduced directly onto the disk in the cell.

Analysis of the extracted analytes was performed by gas chromatography with mass-selective detection (GC–MSD) using a Hewlett-Packard GC (Model 5890) with MSD (Model 5971A) (Avondale, PA). A 0.5 μl volume was injected onto a 25 m × 0.20 mm i.d. HP-1 fused silica capillary column through a split/splitless injection system maintained at 250°C. The transfer line was kept at 280°C. Helium was used as a carrier gas with a head pressure of 9 p.s.i. The GC temperature program was as follows: initial oven temperature, 85°C; held in split mode for 0.75 min. A linear ramp was then used at 16°C min^{-1} to a final temperature of 285°C which was held for a further 2 min. The MSD was operated

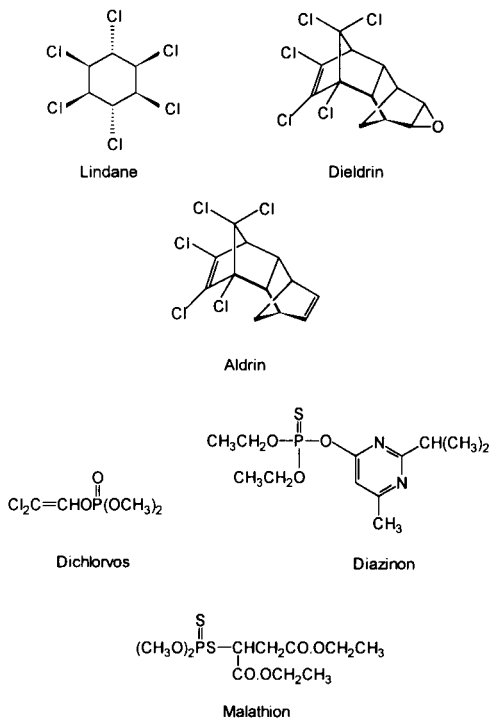

Fig. 2. The six organochlorine and organophosphorus pesticides studied.

in the selective ion monitoring mode with data acquisition started after 5 min.

2.2. Reagents

Supercritical fluid grade carbon dioxide (purity 99.995%) was supplied by Air Products (Sunderland, UK). All of the pesticides used (Fig. 2) were obtained from Promochem (St. Albans). Hexane, methanol and acetone (AnalaR) were obtained from various sources. Empore™ solid phase extraction disks were obtained from Jones Chromatography (Glamorgan, UK). C_{18} Sep-Pak (Millipore) cartridges were supplied by Fisons (Loughbrough).

2.3. Procedure

All six of the pesticides studied were initially spiked (in acetone) into a 200-ml distilled water sample at a 200 μg concentration level. The sample was then pretreated before Empore™ filtration by adding 5 ml of methanol and adjusting the pH to less than 2 with concentrated hydrochloric acid [12]. The disks were then wetted with 10 ml methanol, to activate the C_{18} ligands, for 3 min. Air was then pulled through under vacuum for 1 min followed by a further 10 ml of methanol. Finally 10 ml of distilled water were added immediately prior to sample filtration to remove excess methanol. The sample was then filtered in approximately 5 min and the disk partially dried by drawing air through for 10 min. A further oven drying stage was found to be necessary (45°C for 30 min) to remove all residual water which caused restrictor plugging during extraction and hence a severely reduced flow-rate. The disks were then rolled and placed in the 10-ml extraction cell.

At the beginning of each extraction a 10-min period of static extraction was used, where no actual flow of fluid occurred, to allow the modifier to be effective in a dynamic extraction mode. This is necessary to allow modifier–analyte interaction. Then 30 ml of CO_2 were dynamically passed through the cell which took approximately 15 min. The extractions were undertaken at nine different pressures ranging from 7.5 MPa to 40 MPa with the extracts being made up to a 10-ml volume with hexane and an internal standard (Demeton-*S*-methyl) added. The disks were then removed from the cell and retained for further extraction. The same disks were then extracted with the addition of 300 or 400 μl of methanol, spiked directly onto the disk, as a modifier.

3. Results and discussion

The six pesticides shown in Fig. 2 were chosen as a selection of common organochlorine and organophosphorus pesticides. The properties of the two groups are quite different and their marked difference in polarity being used to obtain selectivity in extraction. The differences in polarity are shown by their octanol–water partition coefficients (log P) [13] determined by a shake flask method. The log P values for each pesticide are listed in Table 1. Supercritical car-

Table 1
Octanol-water partition coefficients (log P) for organochlorine and organophosphorous pecticides

Pesticide	log P (shake flask method)
Lindane	3.72
Aldrin	6.50
Dieldrin	4.32
Dichlorvos	1.47
Diazinon	3.14
Malathion	2.84

bon dioxide is directly suitable for extraction of non-polar analytes and therefore is capable of extracting OCPs at relatively low density (low solvent strength). However, because of their higher polarity, indicated by lower log P values in Table 2, the OPPs require the addition of a polar modifier and an increase in fluid density to extract from an Empore™ disk. It is this difference which can be utilized to selectively extract OCPs and OPPs.

An advantage in selectively extracting OCPs from OPPs is in their subsequent GC detection. Both classes of pesticide are detected with greatest sensitivity using electron capture (ECD) and nitrogen–phosphorus (NPD) detectors, respectively. However, NPDs do not favour the presence of any chlorinated compounds and therefore the removal of OCPs from a mixture of both pesticide classes will be favourable prior to OPP detection.

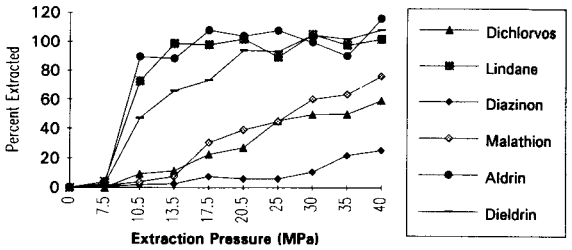

Fig. 3. Percentage extracted versus extraction pressure. OCPs versus OPPs (carbon dioxide only).

The results obtained for the initial extraction with carbon dioxide only and carbon dioxide plus modifier are shown in Figs. 3 and 4. It can be seen from Fig. 3 that there is a marked difference in the recovery of OPPs compared to that of OCPs when extracted with pure CO_2 only. Recoveries close to 100% are possible for two of the three OCPs even at pressures as low as 13.5 MPa

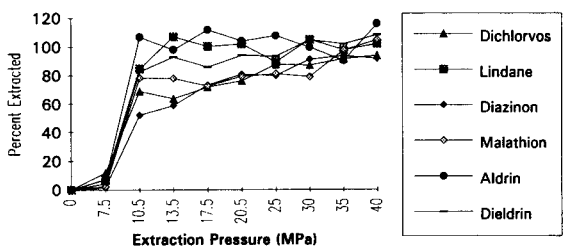

Fig. 4. Percentage extracted versus extraction pressure. OCPs (CO_2 only) and OPPs (CO_2 + MeOH).

Table 2
Recoveries of organochlorine and organophosphorus pesticides after solid phase extraction – supercritical fluid extraction – gas chromatography with mass selective detection (SPE-SFE-GC-MSD)

Extraction with CO_2 only at 13.5 MPa			
Compound	Aldrin	Dieldrin	Lindane
Mean percentage	98.1	72	91.4
Recovery (individual recovery)	(103.8, 107.7, 94.7, 89.6, 94.7)	(68.8, 70.2, 68.2, 76.3, 76.5)	(86.1, 96.5, 98.2, 82.3, 94.1)
Standard deviation	7.42	4.08	6.89
% RSD	7.6	5.7	7.5
Extraction with CO_2 and modifier (400 μl) at 35 MPa			
Compound	Dichlorvos	Diazinon	Malathion
Mean percentage	95.6	93	84.6
Recovery (individual recovery)	(92.8, 91.6, 88.7, 100.4, 92.5)	(89.7, 93.6, 91.2, 102.5, 87.9)	(82.2, 85.5, 83.3, 86.1, 85.9)
Standard deviation	6.28	5.72	1.75
% RSD	6.6	6.1	2.1

(corresponding to a density of 0.66 g ml^{-1}) whereas recoveries of approximately 10% or less are observed for all three OPPs. As the pressure and hence the density of the fluid is increased an increase in the overall amount of all pesticides extracted is seen. However, it is observed that although 100% recovery of all OCPs is rapidly reached the maximum recovery of an OPP (malathion), even at high extraction pressure, does not exceed 80%.

After the disks were extracted with CO_2 only a second extraction was carried out on the same disks using identical extraction conditions with 300 μl of methanol modifier added to the disk prior to extraction. The results for this second extraction (OPP only) are combined with the recoveries of OCPs from the CO_2 only extractions and are shown in Fig. 4. Here it is observed that recoveries of almost 100% can be achieved for all three OPPs with the addition of an organic modifier using high extraction pressure. From analysis of the data it was deduced that the optimum conditions required to selectively extract OCPs from OPPs would be to initially extract the disk with pure CO_2 at 13.5 MPa (density 0.66 g ml^{-1}) and then to extract the disk again with the addition of methanol at a pressure of 35 MPa (density 0.93 g ml^{-1}). This extraction procedure was repeated five times to obtain a standard deviation for the extraction. The mean recoveries and relative standard deviations (R.S.D.s) are shown in Table 2.

Typical chromatograms from GC–MS injections are shown in Figs. 5 and 6. Fig. 5 shows the single-ion monitoring trace for OCPs when extracted at 13.5 MPa with CO_2 only (Fig. 5a) while Fig. 5b shows the low extraction efficiency of the more polar OPPs obtained at this pressure. Con-

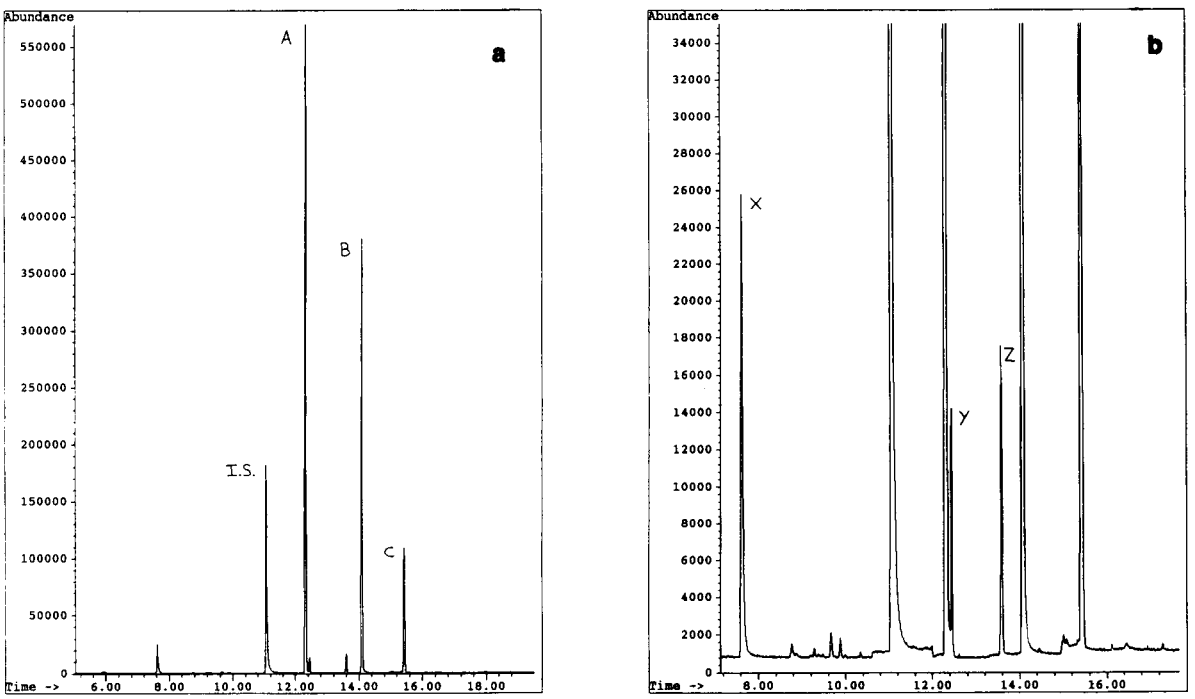

Fig. 5. (a) Single-ion monitoring of OCPs after extraction (13.5 MPa) with CO_2 only. (IS = internal standard; A = Lindane; B = Aldrin and C = Dieldrin). (b) Single-ion monitoring of OPPs after extraction (13.5 MPa) with CO_2 only. (X = Dichlorvos; Y = Diazinon and Z = Malathion).

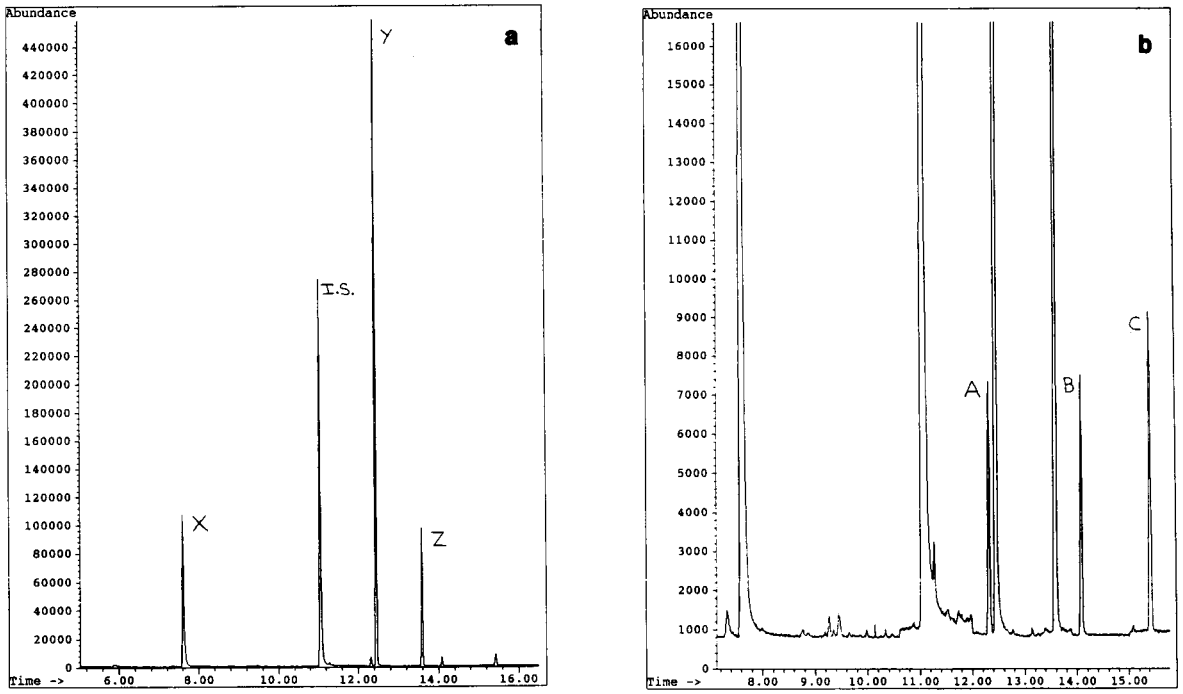

Fig. 6. (a) Single-ion monitoring of OPPs after extraction (35 MPa) with CO_2 + MeOH. (IS = internal standard; X = dichlorvos; Y = Diazinon and Z = Malathion). (b) Single-ion monitoring of OCPs after exhaustive extraction (35 MPa) with CO_2 + MeOH. (A = Lindane; B = Aldrin and C = Dieldrin).

versely, Fig. 6a shows the single-ion monitoring trace for OPPs when extracted at 35 MPa with CO_2 and MeOH and the low residual recovery of OCPs (Fig. 6b). This clearly demonstrates the capability of SFE to selectively extract OCPs without any significant extraction of OPPs at moderate density (0.66 g ml^{-1}). In contrast, the addition of MeOH as a modifier allows the extraction of the more polar OPPs at higher density (0.93 g ml^{-1}) with only minimal residual extraction of the OCPs which had already been removed with CO_2 only.

4. Conclusions

The possibility of selectively extracting OCPs from OPPs has been investigated using SPE–SFE. While compromise extraction conditions are required to achieve the selectivity reported the effectiveness of SFE has been clearly demonstrated. The relatively small differences in polarity, indicated by log P values, indicate the viability of selective extraction using CO_2 only followed by modified CO_2.

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ANALYTICA CHIMICA ACTA

Solid phase extraction of metal ions using immobilised chelating calixarene tetrahydroxamates

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Abstract

The synthetic macrocycles, known as calixarenes, have been shown to possess high ionophoric selectivity and form inclusion complexes with many ions. A tetrameric calixarene bearing hydroxamic acid functional groups was synthesised and supported on octadecylsilica and XAD-4 resin. When loaded with Fe(III), V(V) and Cu(II), the materials display the characteristic visible absorption spectra associated with hydroxamate coordination. The chemical bonding of the calixarene tetrahydroxamic acid to silica particles was also carried out using the triethoxysilane derivative of the *p*-allylcalix[4]arene hydroxamic acid. When packed into small cartridges, these materials are used for the solid phase extraction of transition metal ions from aqueous solution. Metal uptake profiles as a function of pH are presented for Fe(III), Co(II), Pb(II), Cd(II), Mn(II), Ni(II), Zn(II) and Cu(II). The quantitative trace enrichment of Cu(II), Zn(II) and Mn(II) from water samples prior to ion chromatographic analysis is demonstrated.

Key words: Calixarene; Hydroxamic acid; Solid phase extraction; Metal complexation

1. Introduction

The separation and sensitive determination of trace metal ions very often necessitates the use of preconcentration or trace enrichment [1,2]. The solid-phase extraction approach has gained rapid acceptance because of its amenability to automation and the availability of a wide variety of sorbent phases.

Chelating solid phases can be tailor-made for selective trace metal analysis, with applications in both solid-phase extraction and liquid chromatography. Appropriate chelating reagents can be chemically bonded to or otherwise immobilised onto support matrices, thus providing complexing or chelating solid phases. For example, silica immobilised 2-[(2-(triethoxysilyl)ethyl)thio]aniline has been prepared and used as a selective sorbent for the preconcentration of palladium [3]. Polymeric resins incorporating a chelating ligand have been extensively used, in particular poly-

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styrene–divinylbenzene matrices. One such resin containing iminodiacetic acid has been used for the preconcentration of heavy metals from natural waters [4]. More recently, a polystyrene resin containing azobenzylphosphonic acid has been utilised for the separation of Pb(II), U(VI), Cu(II), Mn(II), Zn(II) and Fe(III) [5].

Hydroxamic acids are well known as effective chelating agents for a broad range of transition metal ions [6–8]. Much attention has centred on the effect of N-substitution on metal chelate stability and selectivity, and on the immobilisation of the chelating agents to the chosen solid supports, e.g., as in the coupling of hydroxamic groups to epoxy activated Sepharose [9]. Other solid support materials used to produce polymeric hydroxamic acids include cross-linked poly(acrylonitrile) [10,11], polyacrylic acid resin [12], XAD-4 [13] and also silica bonded hydroxamate phases as used in the solid phase extraction of many transition metals [14].

Recently a new class of ionophoric compound, calixarenes [15], which are the macrocylic products of phenol–formaldehyde condensations, have been shown to act as efficient extractants of alkali metals when modified at the phenolic oxygen atom [16–18]. Some chemically modified calix[4] arenes have been reported to be effective ionophores in ion selective electrodes for the analysis of sodium in blood plasma [19]. Chromatographic selectivity for sodium ions on a calix[4]arene silica-bonded phase has also been demonstrated [20]. Hexameric and tetrameric calixarenes containing hydroxamate functional groups have recently been reported by Nagasaki and Shinkai [21] to selectively solvent extract Fe(III), Cu(II) and Pd(II) from aqueous solution. The alternative approach is to prepare a chelating sorbent incorporating the calixarene hydroxamate for the solid phase extraction of trace metal ions. In this work, both the covalent and non-covalent immobilisation of chelating calixarene hydroxamates onto silica particles and hydrophobic supports is described. Metal uptake profiles by solid phase extraction, using cartridges packed with these materials, were determined and their use in trace metal preconcentration prior to ion chromatographic analysis is demonstrated.

2. Experimental

2.1. Synthesis of calix[4]arene hydroxamic acids

The calixarene hydroxamic acid (shown in Fig. 1a), 25,26,27,28-tetrakis(*N*-hydroxycarbamoylmethoxy)-*p-tert.*-butylcalix[4]arene was synthesised [22] as follows. To a cooled solution of 1.8 g (0.0018 mol) of the tetraethylacetate of *p-tert.*-butylcalix[4]arene previously prepared [17] and 2.02 g (0.029 mol) hydroxylamine hydrochloride in a mixture of 80 ml methanol and 40 ml THF, was added a solution of 2.04 g (0.036 mol) KOH in a mixture of 24 ml methanol and 12 ml THF dropwise at −5°C following a method of Liguori [23]. After stirring for 5 h at −5°C and then 5 days at room temperature, all volatiles were removed under vacuum and dilute acetic acid was added to the residue to give 1.5 g product, which was recrystallised from dichloromethane to give colourless crystalline shiny platelets: m.p. 198–200°C; ν_{max}(KBr)/cm^{-1} 3230 (NH) and 1655 (C=O). Found: C, 64.59; H, 7.57; O, 20.35. Calc. for $C_{52}H_{68}O_{12}N_4$: C, 66.36; H, 7.28; O, 20.40. The ^{1}H NMR spectrum of the product in DMSO-d_6 shows a broad band at 10.8 ppm attributed to the NHOH protons of the hydroxamic acid functional groups.

The *p*-allylcalix[4]arene tetraacetate was prepared from the *p*-allyl derivative of calix[4]arene by treatment with ethylbromoacetate and was converted to its hydroxamic acid derivative by treatment with hydroxylamine hydrochloride in

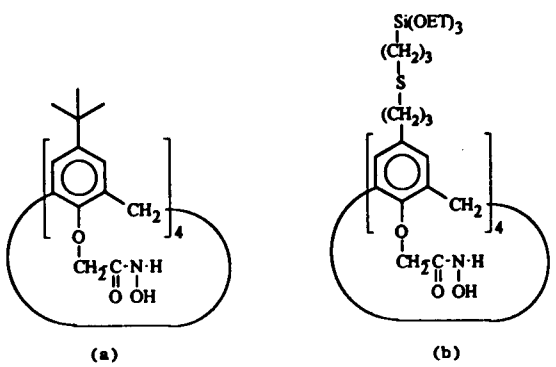

Fig. 1. Structure of (a) *p-tert.*-butylcalix[4]arene tetrahydroxamic acid and (b) the triethoxysilane derivative.

the presence of KOH in THF–methanol as previously described for the *p-tert.*-butyl derivative [17,22].

Treatment of this product with mercaptopropyltriethoxysilane at 70°C for 1 h in the presence of cumene hydroperoxide (free radical source for thiolene addition) gave the triethoxy derivative shown in Fig. 1b.

For the chemical immobilization of calix[4]arene tetrahydroxamate on silica, 0.15 g triethoxysilane derivative of calix[4]arene tetrahydroxamate (Fig. 1b) was refluxed with activated silica (1.35 g), i.e., in a 1:9 (w/w) ratio in 15 ml chlorobenzene for 24 h in an inert atmosphere of N_2(g). The chelating material was filtered and washed with chlorobenzene and suction dried. On testing with Pb(II), a breakthrough capacity of 0.3 μmol g^{-1} was determined. For the activation of silica, 5 μm silica (Nucleosil) was refluxed with 1:1 conc. HCl–H_2O for 8 h. The silica was filtered and washed with H_2O until pH 4 was reached and then dried in a vacuum oven at 110°C for 8 h. Elemental analysis provided evidence for carbon coverage on the silica particles: C = 4.14%, while activated silica alone gave value of C = 0.69%.

2.2. Materials

Unless otherwise stated, all chemicals were analytical reagent grade quality. Ethylenediaminetetraacetic acid disodium salt (EDTA) was purchased from BDH (Poole), LiChroprep RP-18 (25–40 μm) was obtained from Merck (Darmstadt) while Amberlite XAD-4 and Tris[hydroxymethyl]aminomethane (Tris) were obtained from Sigma (Poole).

Metal ion stock solutions of 100 parts per million (ppm), were made up from their nitrate salts (Spectrosol or Merck 1000 ppm standards). Nitric acid (conc.), potassium hydroxide (5 M) and a 35% ammonia solution were used for pH adjustment. Deionised water from an Elgastat spectrum water purification unit, with a resistivity of 15 MΩ cm or better was used for this work. All glassware was washed by soaking overnight in concentrated nitric acid.

2.3. Instrumentation

A cartridge (plastic container, 1.25 × 0.9 cm i.d.) containing the appropriate sorbent was used in association with a masterflex peristaltic pump equipped with two channel head pumps (Cole-Parmer Instruments, Chicago, IL). An Orion Research Model 231 pH meter was employed for the pH measurement of all metal ion solutions. Ultraviolet and visible absorption spectra were recorded on a Shimadzu UV 260 spectrophotometer. Atomic absorption analyses were carried out using either a Pye Unicam SP 9 spectrophotometer or a Perkin-Elmer 2380 spectrophotometer. Ion chromatographic analysis was carried out using a Dionex 4500i Ion chromatographic system with a CS5 cation separator column. The mobile phase consisted of 0.05 M oxalic acid and 0.095 M lithium hydroxide with a flow rate of 1 ml min^{-1} at a pH of 4.8. The injection volume was 25 μl with post column derivatisation involving the use of 0.3 mmol 4-(2-pyridylazo)resorcinol in 1.7 M acetic acid–7.4 M ammonium hydroxide (60:40).

2.4. Chelating solid phase extraction cartridges containing calixarene hydroxamates

In total three solid phase extraction cartridges of equal dimensions were constructed using either the chemically bonded calix[4]arenetetrahydroxamate silica, ODS or XAD particles. The *p-tert.*-butylcalix[4]arene tetrahydroxamic acid was supported on the latter two materials as described below.

A set amount of LiChroprep RP-18 (25–40 μm) (i.e., 0.35 g) was added to a plastic cartridge (1.25 × 0.9 cm i.d.) fitted with a frit at its base and methanol was pumped through to wet the octadecylsilica. Thereafter, a suspension consisting of 0.094 g (0.1 mmol) calixarene hydroxamate in 10 ml methanol was slowly passed through the octadecylsilica using a pressurised glass syringe. Another frit was placed on top of this silica bed and pressure was applied to ensure that the bed was tightly packed.

To immobilise the calixarene hydroxamate onto XAD-4 resin, the calixarene (0.094 g, 0.1 mmol)

and 1,4-dioxan (50 ml) were stirred together in a covered 100 ml beaker until the reagent had dissolved. XAD-4 (0.2613 g) was introduced into the solution and the solution was stirred for two days, allowed to settle and the top 25 ml of the supernatant solution was removed by pipette and replaced with 15 ml water. The solution was stirred for 30 min and the former step repeated three more times. The resultant solution was added as a slurry to the plastic cartridge fitted with a frit at its base. A frit was placed on top of the XAD material. The cartridges were connected to a peristaltic pump and water was pumped through at a rate of 1 ml min^{-1}.

2.5. Visible absorption spectrophotometry

On passage of selected metal ions through the chelating cartridges, all three solid phases gave colours that are indicative of hydroxamate binding, red-brown for Fe(III), brown for V(V) and green for Cu(II).

In order to further characterise complex formation by the immobilised calixarene hydroxamates, solutions of Fe(III) (25 ppm, pH 2.5, 50 ml), V(V) (25 ppm, pH 4.0, 50 ml) and Cu(II) (25 ppm, pH 6.0, 100 ml) were passed separately through cartridges prepared as described above. Quantities of the chelating solid phases (0.1 g) were finely ground and dispersed in nujol and subsequent spectrophotometric analysis using 1 mm cells gave the visible absorption spectra of the complexes formed.

2.6. pH dependency of transition metal uptake

Metal solutions (5 ppm, 25 ml) were adjusted to the required pH and pumped at a rate of 1 ml min^{-1} through the cartridge. This was followed by 25 ml of water to wash off any remaining uncomplexed metal ions. Thereafter the metal was eluted with 25 ml of 0.01 M EDTA or acidified water (pH 2.0). Subsequent atomic absorption analysis gave the % uptake of the various metal ions and % recovery of the eluted metals. With the silica material, the pH range studied was 2.0–7.5 and the pH was adjusted using 5 M KOH. The pH range studied for the XAD-4 resin was extended to 8.5 and this required the addition of 2 mmol Tris as a buffer to the metal solutions to prevent precipitation.

The dependency of the uptake of transition metals on the flow rate was also investigated for Cu(II), Co(II), Pb(II), Fe(III), Mn(II), Ni(II), Zn(II) and Cd(II). With the pH of the aqueous metal ion solutions chosen for maximum complexation, the solution flow rate was varied from 0.5–12 ml min^{-1}.

2.7. Preconcentration and analysis of trace metal ions in water samples using ion chromatography and atomic absorption spectrometry

The use of the chelating cartridges for the preconcentration and analysis of transition metal ions present in laboratory tap water was examined. A 250-ml sample of fresh tap water was acidified to pH 2.0, boiled for ten minutes [24], allowed to cool and then filtered through a 0.45-μm filter (Tris, 0.0606 g, 2 mmol was added as a buffer after cooling, for applications involving the XAD-4 immobilised calixarene). After adjusting the pH to 7.5 for the silica based materials and to 8.5 for the XAD based resin using a 35% ammonia solution, the sample was pumped through the cartridge at a flow rate of 1 ml min^{-1}. Thereafter, 50 ml of deionised water was washed through and any complexed metals were eluted off with 10 ml of acidified water (pH 2.0). Ion chromatographic and atomic absorption analyses of the initial sample, filtrate and eluate, were used to determine the concentration, %uptake and recovery for the metals present in the sample.

3. Results and discussion

The hydroxamic acid functional group when present in microbial siderophores plays an important part in the sequestration of Fe(III) from aqueous solution into microbial cells. The very high stability constants for complexation of Fe(III) can be attributed to the nature of the oxygen donor groups, N-substitution on the hydroxamate and in some cases, conformational stability. The

calix[4]arene tetrahydroxamic acid, under study here provides a structurally rigid organic backbone and with its anchored chelating groups is essentially a synthetic siderophore. Unlike most naturally occurring siderophores, the calix[4]arene tetrahydroxamic acid (Fig. 1a) is water insoluble. This property is useful in solid phase extraction applications, as the physically immobilised calixarene would be less easily leached from a suitably chosen support material. On the down side however, could be the high hydrophobicity, which might not allow satisfactory water–receptor contact for metal complexation to occur.

A characteristic feature of Fe(III) complexation by hydroxamic acids in aqueous solution and as solid complexes is the presence of an absorption band in the 420–480 nm region. In order to verify that hydroxamate complexation of metal ions, can occur, when the calix[4]arene tetrahydroxamic acid is immobilised onto ODS or XAD, molecular absorption spectra were recorded for the materials dispersed in nujol following exposure to aqueous solution of metal ions. The calix[4]arene tetrahydroxamic acid supported on ODS displayed broad bands with λ_{max} values at ~ 430 nm for Fe(III) and V(V) while Cu(II) gave a broad absorption at ~ 740 nm (Fig. 2). These bands are characteristic of hydroxamate complexation and represent significant wavelength shifts and increases in absorption values from those obtained in the spectra of the uncomplexed metal ions in aqueous solutions. The results also indicate that sufficient water–receptor contact exists when the calixarene receptor is supported on ODS or XAD resin. Similar metal binding prop-

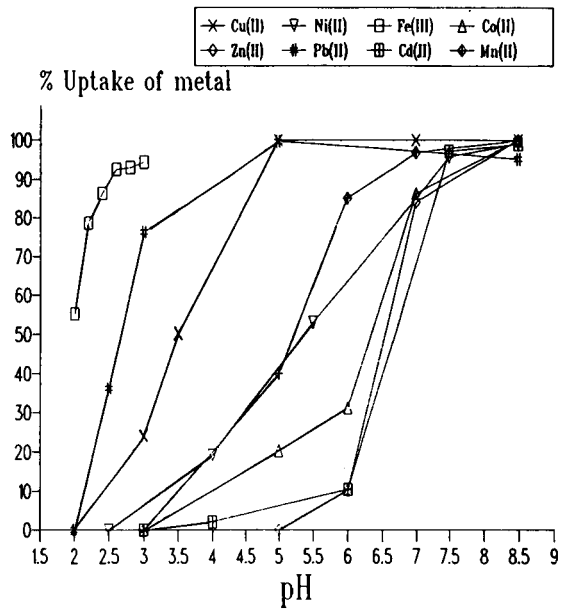

Fig. 3. Effect of pH on percentage metal extraction of metal ions by calixarene tetrahydroxamic acid supported on XAD-4 resin.

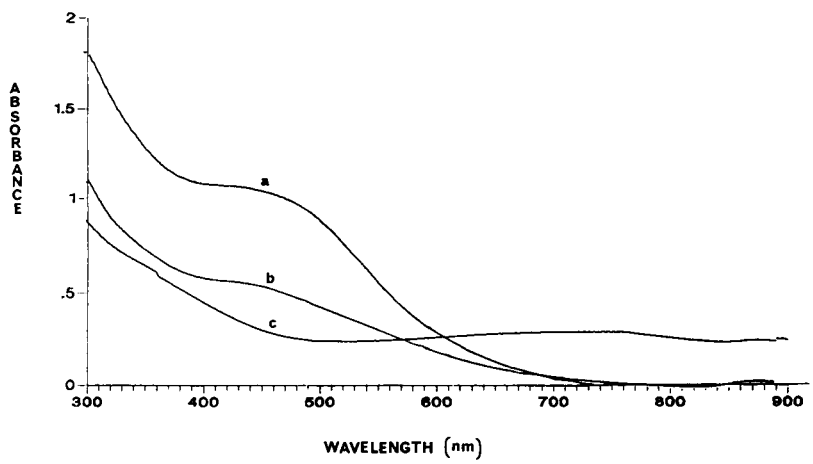

Fig. 2. Absorption spectra of metal calixarene tetrahydroxamate complexes supported on ODS: (a) Fe(III), (b) V(V) and (c) Cu(II).

erties were exhibited by the chemically bonded calix[4]arene tetrahydroxamate silica phase.

Using flame atomic absorption spectrometry, the percentages of metal uptake were determined for Fe(III), Co(II), Pb(II), Cd(II), Mn(II), Ni(II), Zn(II) and Cu(II) as a function of pH. The uptake profile obtained for the cartridge containing calixarene supported on XAD is given in Fig. 3. A similar pattern of metal uptake was obtained using the calixarene supported on ODS. Fe(III), Pb(II) and Cu(II) are quantitatively extracted below pH 5.0 while the quantitative extraction of the other metals requires a pH as high as 8.0. With the exception of Pb(II), this behaviour is similar to the extraction behaviour of alkyl-substituted hydroxamic acids such as the *N*-phenylacylhydroxamic acids reported by Hojjatie et al. [25]. Under these conditions, however, the calix[4]arene tetrahydroxamic acid displays a different selectivity for Pb(II), with complexation occurring in the range pH 2–5 rather than pH 4–8.

The metal uptake profile for the chemically bonded calixarene tetrahydroxamate silica is shown as a function of pH in Fig. 4. The pH dependency of metal uptake for Pb(II), Cu(II) and the other metal ions studied is similar to the supported calixarene tetrahydroxamic acid phases. The most noticeable difference is that for Ni(II), Zn(II), Co(II) and Mn(II), the uptake curves are shifted to a lower pH and overlap to a greater extent. This could be due to improved water–receptor contact on the silica phase or to additional complexation, possibly through the sulphur containing anchoring side chain.

For all three chelating sorbents, elution of the complexed metal ions was readily achieved using 0.01 M EDTA or acidified water (pH 2.0). In addition the chelating phases were reusable and stable to metal loading and washing cycles with minimum reduction in metal complexation capacity on repetitive usage.

3.1. Applications

The chelating calixarene cartridges are useful for the off-line and on-line preconcentration of trace metal ions from aqueous samples, in con-

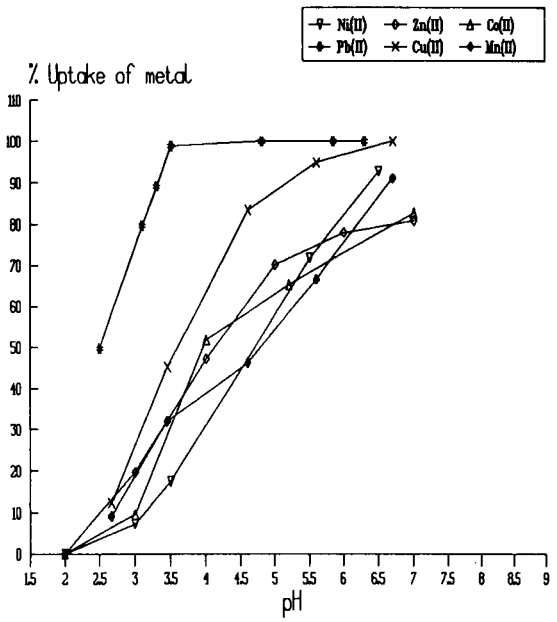

Fig. 4. Effect of pH on percentage metal extraction of metal ions by chemically bonded calixarene tetrahydroxamic acid on silica.

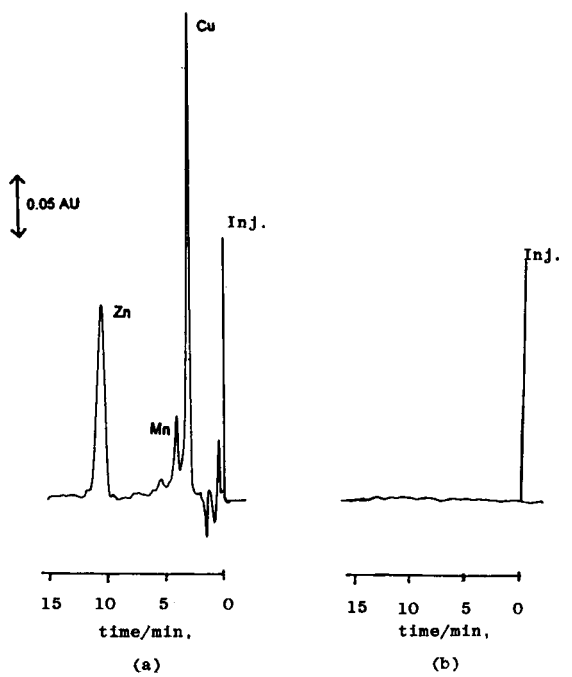

Fig. 5. Ion chromatographs of tap water sample (25 μl injections), (a) original sample, (b) after passing through chelating cartridge.

junction with atomic absorption spectrometry or ion chromatography. To demonstrate this, the preconcentration of trace Cu(II), Mn(II) and Zn(II) ions from a laboratory tap water sample was carried out using a cartridge containing the calixarene tetrahydroxamate on XAD resin. The pH was adjusted to 8.5 to ensure quantitative uptake and metals were removed using acid elution. A 25-fold preconcentration factor was used and the metal content of the acid wash was determined by FAAS, yielding the following concentrations (ppm) for the original water sample: Cu(II) (0.41), Mn(II) (0.04) and Zn(II) (0.29). Direct analysis of the original water sample by ion chromatography using post column derivatisation with PAR yielded metal ion levels (ppm) of Cu(II) (0.41), Mn(II) (0.05) and Zn(II) (0.30). The effectiveness of the chelating cartridge is illustrated in Fig. 5 where the ion chromatographs for the water samples are given for, before and after cartridge treatment. Further studies of the applications of these chelating calixarene cartridges in environmental trace metal analysis, are in progress.

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Evaluation of a sequential extraction procedure for the speciation of heavy metals in sediments

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Abstract

The three-stage sequential extraction procedure for speciation of heavy metals, proposed by the Commission of the European Communities Bureau of Reference (BCR), has been applied to a freshwater sediment collected from the River Clyde, Lanarkshire, UK. Initial studies were carried out using flame atomic absorption spectrometry (FAAS). Iron, manganese and zinc could be determined in the sediment extracts by FAAS, but the technique proved insufficiently sensitive for the determination of chromium, copper, nickel and lead. Detection methods based on electrothermal AAS were therefore developed and applied. Furnace conditions were optimised for the determination of the four analytes mentioned, plus molybdenum and vanadium, in acetic acid, hydroxylammonium chloride and ammonium acetate matrices. Interferences by components in the sediment extracts necessitated analysis by the method of standard additions in most cases. The sequential extraction procedure was found to be both repeatable and reproducible. The amounts of analytes released by the sequential extraction procedure plus aqua regia digestion of the residue remaining after extraction were similar to those released by pseudo-total digestion of the sediment (using aqua regia).

Key words: Atomic absorption spectrometry; Sequential extraction; Speciation; Freshwater sediment; Electrothermal atomic absorption spectrometry; Waters

1. Introduction

It is now widely accepted that the role of aquatic sediments as a sink for pollutants or a source of pollutants cannot fully be assessed by measurement of total metal concentrations. In addition, determination of total elements does not give an accurate estimate of the likely environmental impact when, for example, aquatic sediments are removed from their depositional environment by dredging and used in landfill [1]. Instead, it is desirable to have information on the potential availability of metals (whether toxic or essential) to biota under various environmental conditions. Since availability critically depends upon the chemical form in which a metal is present in the sediment, considerable interest exists in trace element speciation.

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Chemical speciation can be defined as the process of identifying and quantifying the different species, forms or phases present in a material, or the description of these. The species can be defined (a) functionally e.g. 'plant-available species', (b) operationally, according to the reagents or procedures used in their isolation or, most specifically, (c) as particular compounds or oxidation states of an element [2,3]. Operationally defined speciation often involves the use of single or sequential extractants to release species associated with particular sediment phases. In sequential extraction, the sample is treated with a succession of reagents intended to specifically dissolve different sediment phases. A large number of sequential extraction methods have been reported, many of which are variants on the Tessier procedure [4] in which the exchangeable metals and those nominally associated with carbonate, Fe–Mn oxides, organic material and silicate residues were extracted with magnesium chloride, sodium acetate–acetic acid, hydroxylammonium chloride, hydrogen peroxide and hydrofluoric–perchloric acid, respectively. Although the reagents used in sequential extraction procedures may be insufficiently specific to dissolve exclusively the 'target' phases, and results obtained can vary widely when different extraction schemes [5] and experimental conditions [6,7] are used, useful information has been gained from such studies [8].

The Commission of the European Communities Bureau of Reference (BCR) has proposed the production of a sediment reference material certified for its extractable, as distinct from its total, heavy metal contents. An interlaboratory comparison, using a protocol [9] based on the sequential extraction scheme of Salomons and Forstner [10], was conducted to assess the feasibility of preparing such a material. The results obtained were promising, but indicated that more sensitive analytical techniques were required for use both in feasibility studies and in reference material certification.

Even when polluted sediments are studied, flame atomic absorption spectrometry (FAAS) may be insufficiently sensitive for the determination of some important heavy metals in sediment extracts [11]. Electrothermal atomic absorption spectrometry (ETAAS) has recently been applied in the determination of cadmium, chromium, copper, nickel, lead and zinc in ammonium acetate extracts of soils [12]. This paper describes an extension of the study to the analysis of solutions of acetic acid, hydroxylammonium chloride and ammonium acetate obtained by sequential extraction of a freshwater sediment. Zinc, iron and manganese were determined by FAAS, and chromium, copper, molybdenum, nickel, lead and vanadium by ETAAS. Matrix interference effects were encountered in ETAAS, but could be overcome by the use of matrix modification or by analysing the sediment extracts by the method of standard additions. The methods developed were used to compare the amounts of metals liberated by sequential extraction with those obtained by aqua regia digestion of the sediment samples.

2. Experimental

2.1. Apparatus

A Unicam PU 9100 flame atomic absorption spectrometer with deuterium background correction was used for the analysis of the sediment digests and extracts. The instrument was controlled by an IBM ps2 personal computer. A fuel-lean air acetylene flame was used for determination of iron, manganese and zinc. Chromium, copper and lead were determined by electrothermal atomic absorption spectrometry with a Unicam PU 9400 spectrometer, GF 90 atomiser and PU 9390 power supply, a PU 9380 autosampler and pyrolytic graphite-coated, part-ridged electrographite tubes. Deuterium background correction was used for copper and lead, but not for chromium because the intensity of the D_2 lamp was too low at 357.9 nm. Initially, nickel was also determined using this system. However, high blank signals were caused by contamination of the autosampler capillary tube during the wash stage of the injection cycle. Molybdenum, nickel and vanadium were determined by ETAAS with a Perkin-Elmer PE 3030 atomic absorption spectrometer equipped with deuterium background

correction, an HGA 500 furnace programmer, AS40 autosampler and pyrolytic graphite-coated tubes. The GF 90 and HGA 500 temperature programmes, optimised for the determination of the analytes in the reagents used for sediment extraction, are listed in Table 1. For all elements except lead, the same furnace programme could be used for analysis of the acid digests and the three extractants.

The method of standard additions was used for the determination of chromium, copper, nickel and lead in sediment extracts. Molybdenum and vanadium were determined by standard additions in sediment digests and by direct calibration in extracts. Palladium matrix modification ($Pd(NO_3)_2$, 200 mg l^{-1}) was used for the determination of molybdenum in extracts, and $NH_4H_2PO_4$ (5000 mg l^{-1}) for the determination of vanadium in extracts and digests.

Analytes were determined at the following wavelengths (nm): Cr 357.9, Cu 324.8, Fe 248.3, Mn 279.5, Mo 313.3, Ni 232.0, Pb 217.0, V 318.4, and Zn 213.9.

2.2. Reagents

Ammonium acetate (analytical reagent grade) was obtained from Fisons, Loughborough. All other reagents were obtained from Merck (formerly BDH), Poole. Nitric acid, hydrochloric acid, acetic acid and 30% w/v hydrogen peroxide (8.8 mol l^{-1}) were Aristar grade and hydroxylammonium chloride was AnalaR grade. Calibrant solutions were prepared from 1000 μg ml^{-1} Spectrosol standard solutions in aqua regia diluted (4:25) with distilled water, acetic acid, hydroxylammonium chloride or ammonium acetate,

2.3. Procedures

Sample collection and pretreatment

Surface sediment was collected from the River Clyde near Motherwell, Lanarkshire, UK, and

Table 1
Furnace programmes for ETAAS

	Chromium			Copper		
	Temp. (°C)	Ramp (°C s^{-1})	Hold (s)	Temp. (°C)	Ramp (°C s^{-1})	Hold (s)
Dry	90	10	30	90	10	30
Char	1400	50	20	1000	50	20
Atomise	2500	> 2000	3	2100	> 2000	3
Clean	2650	> 2000	2	2650	> 2000	2
	Molybdenum			Nickel		
	Temp. (°C)	Ramp (°C s^{-1})	Hold (s)	Temp. (°C)	Ramp (°C s^{-1})	Hold (s)
Dry	100	10	20	100	10	20
Char	1750	100	20	1000	100	20
Atomise	2600	> 2000	5	2500	> 2000	3
Clean	2700	100	2	2650	150	2
	Lead			Vanadium		
	Temp. (°C)	Ramp (°C s^{-1})	Hold (s)	Temp. (°C)	Ramp (°C s^{-1})	Hold (s)
Dry	90	10	40	100	10	20
Char	[a]	50	20	1200	100	20
Atomise	1900	> 2000	2	2600	> 2000	5
Clean	2650	> 2000	2	2700	100	2

Purge gas (Ar) flow was stopped during atomisation.
[a] 600°C for extractants and acid digests, 400°C for acetic acid extracts, 850°C for hydroxylammonium chloride and ammonium acetate extracts.

was air-dried by spreading it thinly on a plastic sheet at room temperature. It is possible that air-drying may have altered the chemical forms of the metals present since speciation can critically depend on the sampling and pretreatment protocols applied [13]. However, there are also advantages in terms of ease of handling if the sample is dried. After drying, the sediment was separated into size fractions with a set of sieves of known mesh size (250, 125 and 63 μm.) The < 63 μm fraction, which constituted 3% w/w of the dried sediment, was collected and stored in plastic bottles at room temperature. Analysis of this fraction is recommended in sediment studies because clay and silt particles generally contain the highest concentrations of pollutants, and are most readily transported in suspension in natural waters [14]. The homogeneity of the sediment was checked by total digestion ($HF/HCl/HNO_3$) of 0.2 g sediment subsamples followed by determination of nickel. The R.S.D. value obtained for three replicate digestions was 3.3%. The organic matter content of the sediment (loss on ignition) was 6.1% and the carbonate content, as determined by back-titration with NaOH, was negligible. The average pH of the riverwater at the site during the month in which the samples were taken was 7.8 [15].

Microwave digestion

A 0.2 g subsample of the dry sediment (< 63 μm fraction) was weighed into a PTFE bomb, and dampened with 0.5 ml water. To this, 3 ml concentrated HCl and 1 ml concentrated HNO_3 were added. After any effervescence had subsided, the bomb was sealed, heated in a domestic microwave oven (850 W) for 50 s, and allowed to cool to room temperature. The heating–cooling cycle was repeated four times. The digest was filtered (Whatman No. 541 filter paper) into a standard flask and the solution made up to 25 ml with distilled water. Sediment residues from the sequential extraction procedure were digested in a similar way.

Sequential extraction

Step one. A 40 ml volume of acetic acid (0.11 mol l^{-1}) was added to 1 g of dry sediment (< 63 μm fraction) in a 100 ml polypropylene centrifuge tube. The tube was then shaken for 16 h (overnight) at ambient temperature (approx. 20°C) on an end-over-end mechanical shaker at a speed of 40 rpm The extract was separated from the solid residue by centrifugation at 4000 rpm, 2333 *g* (MSE Mistral 1000 benchtop centrifuge). The liquid was decanted into a clean polyethylene container and stored at 4°C for analysis. The residue was washed with 20 ml distilled water by shaking for 15 min, centrifuged, and the washings discarded.

Step two. A 40 ml volume of hydroxylammonium chloride (0.1 mol l^{-1}, adjusted to pH 2 with nitric acid) was added to the residue from step one. The extraction procedure was repeated as described above, i.e. the sample was shaken overnight, the extract separated by centrifugation, and the residue washed with distilled water.

Step three. A 10 ml volume of hydrogen peroxide (8.8 mol l^{-1}) was carefully added, in small aliquots, to the residue from step two. The centrifuge tube was covered with a watch glass and the contents digested at room temperature for one hour with occasional manual shaking. Digestion was continued by heating the tube to 85°C in a water bath for one hour. The watch glass was

Table 2
Extractants used in sequential extraction and nominal sediment phases dissolved

Extraction step	Reagent	Phase(s)
One	CH_3COOH (0.11 mol l^{-1})	Exchangeable, water and acid soluble
Two	$NH_2OH \cdot HCl$ (0.1 mol l^{-1}) at pH 2	Reducible (e.g. iron/manganese oxides)
Three	H_2O_2 (8.8 mol l^{-1}); followed by CH_3COONH_4 (1 mol l^{-1}) at pH 2	Oxidizable (e.g. organic matter and sulfides)

then removed and the tube contents reduced to a small volume (1–2 ml). A second 10 ml aliquot of H_2O_2 was added and the tube was again covered and heated to 85°C for one hour. The cover was removed and the volume reduced as before. A 50 ml volume of ammonium acetate (1 mol l^{-1}, adjusted to pH 2 with nitric acid) was added to the cool, moist residue. The sample was then shaken, centrifuged and the extract separated as described in step one. The solid residue was retained for microwave digestion. The nominal sediment phases dissolved by each of the extractant reagents are given in Table 2.

3. Results and discussion

Reproducibility of the sequential extraction procedure

To assess the reproducibility of the BCR protocol, three sediment subsamples (Set A) were taken simultaneously through the extraction procedure, along with a 'blank' centrifuge tube containing reagents but no sediment (Table 3). This was repeated twice (Sets B and C), at approximately weekly intervals. Extracts were analysed by FAAS to determine the concentrations of iron, manganese and zinc. Attempts to determine copper, nickel and lead in the extracts were unsuccessful because these elements were present at too low concentrations.

Even for elements far from their detection limits, significant 'in-set' and 'between-set' variation occurred. The 'between-set' variance was generally larger than the 'in-set' variance. Acceptable precision (R.S.D. values < 15%) could be obtained for iron and zinc in acetic acid and hydroxylammonium chloride and for manganese in acetic acid and ammonium acetate. The Set A results obtained for iron in ammonium acetate, although self-consistent, were disparate from those of the other sets of extractions, leading to a degradation in the overall precision. This is also apparent in the results for manganese in hydroxylammonium chloride. Poor precision was obtained in each set of measurements for zinc in ammonium acetate. Similar variability between replicate sequential extractions was observed in the BCR interlaboratory trial [3]. Several factors may contribute to the lack of precision, including variability in sampling and in the efficiency and selectivity of the extraction procedure. Although the precision obtained for some of the elements and extractants was poorer than would normally be considered acceptable, where high concentrations in a particular phase are found, the method is reproducible enough to obtain useful information on heavy metal speciation.

Table 3
Reproducibility of the sequential extraction procedure ($\mu g\ g^{-1}$ sediment dry weight)

Element	Extraction step	Set A		Set B		Set C		Grand mean ($n = 9$)	R.S.D. (%)
		mean ($n = 3$)	R.S.D. (%)	mean ($n = 3$)	R.S.D. (%)	mean ($n = 3$)	R.S.D. (%)		
Fe	1	27.6	8.2	29.7	13	26.2	8.8	27.8	9.0
	2	1890	1.4	2270	1.8	1910	2.0	2020	15
	3	1010	3.8	1490	3.0	1440	12	1320	28
	Sum	2930		3790		3380		3370	18
Mn	1	263	1.7	284	12	271	1.0	273	5.5
	2	607	1.3	329	11	380	5.0	439	48
	3	56.4	1.4	67.7	0.5	60.0	0.8	61.4	13
	Sum	926		677		709		771	25
Zn	1	23.9	1.4	26.9	1.2	23.1	0.3	24.6	12
	2	17.9	10	16.2	1.0	16.0	1.8	16.7	8.8
	3	25.6	42	15.2	17	30.3	38	24	46
	Sum	67.4		58.4		69.3		65	13

'Set' represents the simultaneous sequential extraction of three sediment subsamples.

Matrix interference effects in ETAAS

Since FAAS was insufficiently sensitive, except for the determination of iron, manganese and zinc, detection methods based on ETAAS were developed. Interference effects associated with the sediment components extracted at each stage in the protocol were investigated by comparison of calibration and standard additions curves. The calibrant solutions contained the same concentrations of extractants as in the standard additions solutions to ensure that samples and standards were of the same viscosity and produced similar solid deposits when dried in the graphite tube. Thus, any interferences observed result from substances co-extracted with the analytes and not from the reagents used.

Slight enhancements of sensitivity for chromium (+28%) and copper (+17%) are observed in the acetic acid sediment extract (Table 4). In the hydroxylammonium chloride extract (Table 5), the copper additions curve is depressed by 41% relative to the calibration obtained from standard solutions. A suppression is observed for lead which is due to the use of the optimum furnace programme for the extractant (T_{Char} = 600°C) for analysis of the extract (optimum T_{Char} = 850°C). It is likely that matrix components, such as iron and manganese, which are present at high concentrations in the reducible phase of the sediment, are not effectively removed at the lower char temperature and prevent efficient atomisation of the lead. The slopes of additions and calibration curves obtained in the ammonium acetate extract (Table 6) are within ±10% of each other for all elements studied. It has been reported that chromium, lead and nickel can be determined in ammonium acetate extracts of soil without interference from matrix components [12], and the present work indicates that the determination of these metals in sediment extracts is also relatively interference free.

Calibration slopes obtained for the analytes in each of the extractant reagents (Tables 4–6) are similar for chromium and nickel. However, an enhancement in sensitivity for copper and a suppression for lead occurred with ammonium acetate. The suppression, which has been observed previously [12], may be due to a 'carrier' effect in which decomposition of the relatively volatile ammonium acetate removes the analyte from the atom cell before the atomisation step of the furnace programme is reached. The enhancement for copper is more difficult to explain but may, perhaps, be due to alterations in tube surface characteristics caused by the presence of gas phase decomposition products of ammonium acetate. The reason that two relatively volatile ana-

Table 4
Comparison of calibration slopes of analyte elements in acetic acid extract and acetic acid multielement standard solution

Element	Slopes		Difference in slope (%)
	Standard solution ($\times 10^{-2}$)	Extract ($\times 10^{-2}$)	
Cr	1.2240 ± 0.0428	1.5660 ± 0.0381	+28
Cu	0.7023 ± 0.0394	0.8233 ± 0.0923	+17
Pb	0.0811 ± 0.0055	0.0849 ± 0.0043	+5
Ni	0.4639 ± 0.0026	0.5051 ± 0.0074	+9

Peak area measurements were used for all elements.

Table 5
Comparison of calibration slopes of analyte elements in hydroxylammonium chloride extract and hydroxylammonium chloride multielement standard solution

Element	Slopes		Difference in slope (%)
	Standard solution ($\times 10^{-2}$)	Extract ($\times 10^{-2}$)	
Cr	1.3010 ± 0.0267	1.2640 ± 0.0188	−3
Cu	0.7713 ± 0.0198	0.4583 ± 0.0138	−41
Pb	0.0807 ± 0.0042	0.0657 ± 0.0018	−19
Ni	0.4572 ± 0.0213	0.4310 ± 0.0055	−6

Peak area measurements were used for all elements.

Table 6
Comparison of calibration slopes of analyte elements in ammonium acetate extract and ammonium acetate multielement standard solution

Element	Slopes		Difference in slope (%)
	Standard solution ($\times 10^{-2}$)	Extract ($\times 10^{-2}$)	
Cr	1.3410 ± 0.0609	1.4760 ± 0.0331	+10
Cu	0.9179 ± 0.2133	0.9129 ± 0.1325	−1
Pb	0.0338 ± 0.0040	0.0340 ± 0.0035	+1
Ni	0.5122 ± 0.0037	0.5388 ± 0.0117	+5

Peak area measurements were used for all elements.

lytes show such different behaviour in the presence of ammonium acetate is unknown.

In this sediment, analysis by the method of standard additions is necessary to overcome interferences in the determination of chromium and copper in acetic acid extracts and copper and lead in hydroxylammonium chloride extracts. It is feasible to use direct calibration with matrix matched standards for the determination of lead and nickel in acetic acid extracts, for chromium and nickel in hydroxylammonium chloride extracts, and for all four analytes in ammonium acetate extracts. However, in order to increase the robustness of the method, and reduce variations between laboratories which may employ sequential extraction in the study of different sediment matrices, it may be preferable to use the method of standard additions in all cases.

Molybdenum and vanadium

Molybdenum and vanadium are elements which are much studied in the aquatic environment because they are redox-sensitive and can exhibit different behaviour in oxic and anoxic conditions. Investigation of the speciation of these elements may give insight into the processes which occur during sediment diagenesis, as well as providing information on pollutant inputs and mobility. It was therefore decided to extend the present study to include the determination of molybdenum and vanadium in the sediment extracts.

Although both elements are considerably more refractory than nickel, significant interferences were observed when molybdenum and vanadium were determined in the extractants by ETAAS (Table 7). The effects could be overcome by the addition of suitable matrix modifiers. Suppression of the sensitivity for molybdenum in the presence of both hydroxylammonium chloride and ammonium acetate could be overcome by the addition of 200 mg l^{-1} $Pd(NO_3)_2$, and $NH_4H_2PO_4$ was effective in removing an enhancement which was observed in all extractants for vanadium. It is not clear why these modifiers are effective. Both are generally used to reduce chloride interferences, but acetic acid, ammonium acetate and the (freshwater) sediment matrix contain little chloride, and hydroxylammonium chloride (which decomposes at above 151°C [16]) should be removed well before atomisation of the analytes.

Comparison of sequential extraction and pseudo-total digestion

Three subsamples of the sediment were extracted, and the residues remaining after extraction digested with aqua regia in a microwave oven. Analytes were determined in the extracts and digests by FAAS or ETAAS. For ETAAS, some of the extracts were diluted before analysis (with extractant) to bring concentrations within the range of linear calibration.

For each element, the sum of the amount of metal removed by the sequential extraction procedure, plus that released when the residue was digested, was, within error, equivalent to the amount liberated by aqua regia digestion alone

Table 7
Recoveries of molybdenum and vanadium in sediment extracts with and without matrix modification

Extractant	Recovery (%)			
	Molybdenum		Vanadium	
	No modifier ($n = 5$)	With modifier [a] ($n = 5$)	No modifier ($n = 5$)	With modifier [b] ($n = 5$)
Acetic acid	99 ± 17	102 ± 6	112 ± 5	108 ± 9
Hydroxylammonium chloride	89 ± 6	96 ± 8	112 ± 5	105 ± 8
Ammonium acetate	63 ± 7	91 ± 9	117 ± 4	108 ± 10

Concentrations of analytes added to extracts to determine recoveries: 10 μg l^{-1} Mo, 50 μg l^{-1} V.
Volume of extract injected into graphite furnace: 10 μl.
[a] $Pd(NO_3)_2$, 10 μl of a 200 mg l^{-1} solution.
[b] $NH_4H_2PO_4$, 5 μl of a 5000 mg l^{-1} solution for acetic acid and hydroxylammonium chloride, 10 μl of a 1000 mg l^{-1} solution for ammonium acetate.

Table 8
Comparison of results obtained by sequential extraction and pseudo-total digestion ($\mu g\ g^{-1}$ sediment dry weight)

Step	Chromium		Copper		Iron		Lead		Manganese		Molybdenum		Nickel		Vanadium		Zinc	
One	0.12	(11)	2.38	(13)	14	(7.1)	3.34	(5.1)	295	(5.1)	< 0.03		2.58	(16)	< 0.26		20.2	(2.6)
Two	0.31	(16)	0.92	(37)	2100	(13)	35.5	(23)	325	(1.2)	< 0.02		2.36	(5.0)	3.41	(11)	12.9	(5.4)
Three	7.63	(1.7)	5.22	(4.1)	1190	(6.3)	40.0	(5.0)	60.1	(2.9)	< 0.03		3.84	(16)	1.89	(32)	10.9	(5.8)
Residue	9.89		7.51		23000		32.5		183		0.51		18.2		16.8		47.5	
Sum	18.0		16.0		26300		111		863		0.51		27.0		22.1		91.5	
Digest	17.1	(9.1)	14.5	(22)	29200	(19)	124	(5.5)	821	(11)	0.56	(13)	33.1	(44)	21.2	(28)	95.0	(3.2)
% Recovery	105.3		110.3		90.1		89.5		105.1		91.1		81.6		104.2		96.3	

Numbers in brackets are %R.S.D. values for processing of replicate subsamples $n = 3$ for steps 1, 2, and 3 and for digestion of the sediment; $n = 1$ for digestion of the residue. Fe, Mn and Zn were determined by FAAS, other analytes by ETAAS.

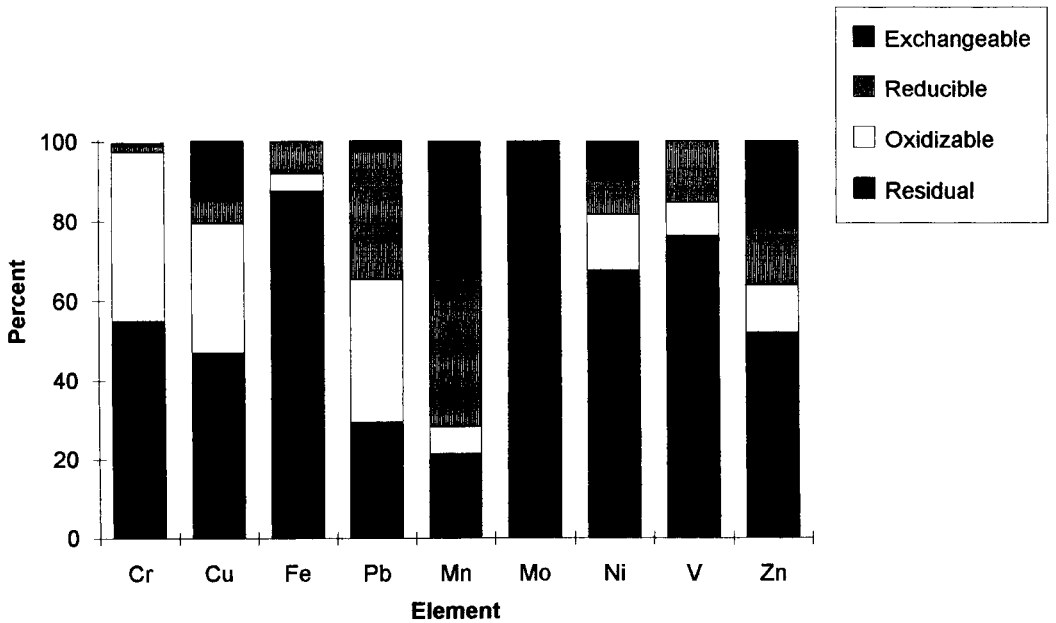

Fig. 1. Operationally-defined partitioning of the analytes between the sediment phases.

(Table 8). The operationally-defined partitioning of the analytes between the sediment phases is shown in Fig. 1. The high proportion of metals in the residual fraction and the generally low levels of extractable metals indicate that the sediment is relatively unpolluted. This is perhaps surprising since the sample was collected from a point downstream of the former Ravenscraig steelworks, and leaching of heavy metals from the site into the river might have been expected. However, at the time of sample collection, the river was in flood and unusually high flow rates could have flushed contaminated sediment downstream and partially replaced them with material from the relatively unpolluted upper reaches of the Clyde.

As expected from a number of previous studies on polluted sediments [11,12,17,18] extractable copper is mainly associated with the oxidizable phase, where it is likely to occur as organically complexed metal species. The presence of chromium in the oxidizable phase has also been reported previously [11,12], although some workers have found a more significant association between chromium and the reducible phase in polluted sediments [7]. The results for iron and manganese are broadly in line with those obtained in the early work on sequential extraction by Tessier et al. [4]. Most of the extractable iron is removed in step two, but some remains in association with the oxidizable phase, and extractable manganese is located principally in the exchangeable/acid soluble and reducible phases. Molybdenum concentrations are below the detection limits except in the residual phase. Vanadium is found in both the reducible and the oxidizable phases. The latter may indicate an association of vanadium with organic matter, which has been reported previously in soils [19] and some types of sediment [20]. Nickel and zinc are extracted at all three stages of the procedure, again as has been observed for other sediments [12,18]. There is little exchangeable/acid soluble lead. This is in contrast to most of the sediments reviewed by Bradley and Cox [18]. However, lead speciation is known to be very variable, perhaps

due to the relatively large proportion of the element which enters the aquatic environment through atmospheric deposition [4].

4. Conclusions

The sequential extraction scheme recommended by BCR is sufficiently repeatable and reproducible for application in speciation studies. The amounts of metals removed by the procedure correlate well with those removed by pseudo-total, acid digestion of the sediment. It has also been demonstrated that, provided sensitive analytical techniques are available, measurement of the extractable metal contents of sediments is feasible even when pollutant levels are low.

Care must be taken, however, to ensure that potential matrix interference effects are characterised and, where possible, compensated for. Analysis by the method of standard additions, although time consuming, may be required if similar results are to be obtained by different laboratories.

With the availability of more sensitive methods of analysis, it may be possible to examine the steps in the BCR procedure in more detail and identify some of the factors responsible for variability between replicate measurements. Errors due, for example, to carry-over from one step of the extraction to the next or to volume differences in step three (when the sample is dried 'to a small volume') could be assessed and, if necessary, eliminated by suitable modification of the protocol.

The application of techniques such as ETAAS in speciation studies will hopefully facilitate the characterisation and production of a sediment reference material certified for its extractable metal content.

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Assessment of the phase selectivity of the European Community Bureau of Reference (BCR) sequential extraction procedure for metals in sediment

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Abstract

Sequential extractions potentially offer useful information on how metals are bound to sediment. For example, reagents used in such extractions may be expected to selectively release metal bound to carbonates, iron and manganese oxides or organic/sulphidic material. However, various criticisms have been levelled at sequential methods, including that of inaccuracy in releasing metal from specific geochemical phases. This study examines specificity by analysing individual mineral phases previously equilibrated with metal-spiked artificial sea water. Substrates were then sequentially extracted according to the three-step BCR procedure. The distribution of recovered metal between extracts was compared to that expected if reagents were acting on a specific phase. Acetic acid released most of the metal associated with calcium carbonate, kaolinite, potassium felspar and ferrihydrite. Hydroxylamine hydrochloride contained most of the recovered metal from montmorillonite and manganese dioxide, as well as nickel from humic acid. Iron oxides are expected to be attacked by this reducing agent, but the majority of the metal had already been removed by the first extract (acetic acid). This may reflect the high adsorption capacity of ferrihydrite. Zinc on humic acid was split between the first two reagents. The third extraction, hydrogen peroxide/ammonium acetate, which might be expected to release metal from organic or sulphidic material, only significantly recovered the added copper from humic acid. Total recoveries of the added metal were high, except from humic acid, felspar and montmorillonite, suggesting strong metal binding by these substrates. Variability between carefully controlled experiments raises some questions about the reproducibility of the extraction procedure which may be exacerbated when applying the method to real sediments.

Key words: Extraction; Metals; Sea water; Sediments; Waters

1. Introduction

A full understanding of sediment contamination requires knowledge of the mineral phase with which metals are associated and the strength of binding involved. Sequential extraction methods use reagents of differing chemical behaviour to release metal bound to the sediment, yielding information on metal speciation. A large number of reagents have been used in such studies since Chester and Hughes [1] first suggested their

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method in 1967. The BCR (European Community Bureau of Reference) proposed method [2] is a recent example. Assessment of the bioavailability of contaminants and ameliorative measures for polluted sediment are two examples where sequential extractions may provide useful information.

However, sequential extraction methods have come under serious attack in recent years [3,4]. Various workers have criticised the widely-used Tessier scheme [5]. Tipping et al. [6] reported readsorption during extraction, where metal initially released by the reagent then re-precipitates or partitions back onto the solid phase. Rauret et al. [7] and Pfeiffer et al. [8] found different concentrations depending upon the solid to solution ratio used, with the most severe effects noted at low ratios. Lack of geochemical specificity was reported in experiments where Kheboian and Bauer [9] studied metal spike recovery from mixed model phases, and Guy et al. [10] used single extractions upon metal sorbed on MnO_2, bentonite and humic acid. The BCR method attempts to reduce the impact of the readsorption and solid to solution ratios, while this paper examines geochemical phase specificity by applying sequential extractions to single mineral phases.

Some workers using sequential extractions have labelled fractions according to the geochemical phase thought to be attacked by a particular reagent. Such terms include carbonate-bound for metals released by sodium acetate; Fe/Mn oxides for those by hydroxylamine hydrochloride; and organic/sulphidic for those by hydrogen peroxide [11]. However, concern over the selectivity of reagents is tending to replace this terminology with operational definitions, e.g., "reducible" for hydroxylamine [12]. This study examined the specificity of reagents to see how accurately they attacked a particular geochemical phase. Adsorption over 24 h or 28 days was used to model a metal contaminant entering a water body and partitioning onto the solid phase.

Metal spikes and model phases were used to assess the specificity of metal release from particular substrates in this work. Ideally, metal should be uniquely released from the phase attacked by that reagent. Data showing this reproducible and comprehensible behaviour would provide evidence for the continued use of sequential extractions. However, if the method is unable to provide clear information on fairly homogeneous substrates, further doubt may be raised concerning the validity of the use of such techniques upon sediments. Data showing non-specific or variable recoveries, or those where metal is released from the same substrate in a series of steps, might suggest that an alternative method of studying metal binding to sediments is required. The BCR method was studied since it attempts to overcome some of the problems ascribed to the Tessier scheme, and as a proposed standard method has the potential for widespread use.

2. Experimental

Fig. 1 shows an outline of the experimental method. Substrates used in the experiment were calcium carbonate (Merck), ferrihydrite, humic acid (Merck), kaolinite, potassium felspar, montmorillonite and manganese dioxide. Ferrihydrite (amorphous Fe(O)OH) was prepared following the method of Benjamin [13] with acidified artificial sea water substituted for the base electrolyte sodium nitrite. It was then aged at room temperature in artificial sea water of pH 8 for 24 h prior to use in the equilibration period. X-ray diffrac-

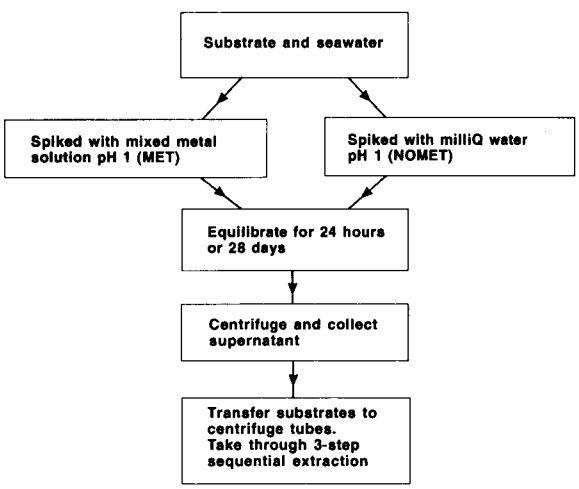

Fig. 1. Outline of experimental method.

Table 1
Mean particle size and surface area of substrates

Substrate	Mean particle size [a] (μm)	Surface area [b] ($m^2\ g^{-1}$)
Calcium carbonate	14	5
Ferrihydrite	27	250
Humic acid	9	12
Kaolinite	3	6
Felspar	20	3
Montmorillonite	8	160
Manganese dioxide	11	26

[a] Analysis by Malvern mastersizer.
[b] Analysis by BET N_2.

tion did not show any evidence of crystalline structures during the experiments. Manganese dioxide was prepared according to the method presented by Van den Berg and Kramer [14]. Table 1 shows particle size and surface area measured by adsorption of nitrogen (BET method) for the substrates. All substrates, with the exception of Fe(O)OH, were pre-equilibrated with sea water before use in the experiment. This was achieved by repeated washing of the substrate with sea water followed by centrifugation. The pH of the supernatant was measured and the process repeated until the reading became constant. Substrates were then stored in sea water before use.

Each substrate was added to an individual 200-ml aliquot of sea water, in the density range 4–69 g l^{-1}. Large variability in the amount added reflects difficulties in pipetting slurries, although densities were more consistent when comparing single substrates (difference = 2.1 g l^{-1}, $\sigma = 2.1$). These samples were then equilibrated overnight at 10–12°C.

Samples (MET) were spiked with a mixed metal solution of copper, nickel and zinc made up from BDH standard solutions; 150 μg l^{-1} for all substrates except ferrihydrite which was spiked at 200 μg l^{-1} (concentrations in the sea water). To avoid any artifacts caused by pH changes from this spike, a spike of milli-Q water of the same pH was added to a control sea water–substrate sample (no metal added, NOMET). Spiking of a sea water sample containing no substrate showed that adsorptive losses to the bottle were negligible. All the samples were then shaken in the dark at 10–12°C, for either 24 h or 28 days.

At the end of the equilibration period, samples were removed from the shaker and centrifuged. The supernatant was collected and the metal remaining in solution determined. In MET samples, the difference between this concentration and that in the control spike (no substrate) was assumed to have been taken up by the solid phase. NOMET samples showed if there was any contribution of metal from the substrate itself. The percentage of added metal found in each extract was calculated as:

$$\frac{\text{Mass of metal in extract (mg/kg)} \times 100}{\text{Mass of metal adsorbed during equilibration (mg/kg)}}$$

The substrate was removed (aerobically) and placed into polyethylene centrifuge tubes, to give a wet weight of 1.2 ± 0.1 g. This quantity may represent an under-estimate of the 1 g dry weight required by the BCR sequential extraction procedure, but significant changes in behaviour caused by varying the solid to solution ratios are associated with lower rather than higher ratios [7,8]. Since all calculations are based on the wet weight solids comparability is maintained.

The substrates (2–4 replicates) were then taken through the BCR extraction. The first step was to shake with 40 ml 0.11 mol l^{-1} acetic acid (HOAc; pH 2.9) for 16 h, followed by centrifugation and collection of the supernatant. The solids were rinsed with deionised water and centrifuged again. The protocol intends the rinse water to be discarded, but here it was analysed to check for losses. The samples were then shaken with 40 ml 0.1 mol l^{-1} hydroxylamine hydrochloride ($NH_2OH \cdot HCl$; pH 2) for 16 h, before repeating the centrifugation and rinse as in the first step. The third step involved digesting with 10 ml 30% (w/v) hydrogen peroxide for 1 h at room temperature, adding a further 10 ml H_2O_2 and digesting for 1 h at 85°C. The solution was then reduced to a few ml before adding 50 ml 1 mol l^{-1} ammonium acetate (NH_4Ac; pH 2) to the cool residue and shaking for 16 h. The supernatant was collected after centrifugation. Samples were stored in a refrigerator prior to analysis.

Saline samples from the equilibration were analysed by HPLC-ICP-AES according to the method of Coffey and Jickells [15]. An anion exchange resin in a Dionex liquid chromatograph (chelation chromatography module) is used to remove anion interferents before on-line uptake into the ICP. Extracts were analysed in triplicate on a Thermo-Jarrell Ash ICP-AES (Polyscan 61E). Matrix-matched standards were used throughout the analyses, with standard additions to check for interferences.

Blanks and controls were run at all stages during the experiment. Method blanks were found to be below the analytical limit of detection. Good recoveries were obtained for a three-point metal spike added to the blank reagent at each stage and taken through that step in the extraction. The standard reference sediment MESS-1 (NRCC) was also sequentially extracted in the current absence of any widely available reference material for such methods.

Artificial sea water and milli-Q water were used throughout the experiment. All plasticware was acid-washed with 10% HNO_3. Reagents were of at least analytical grade.

3. Results

Figs. 2–7 show the percentage of added metal found in each extract for each substrate. Data for some repeated experiments are shown, represented by (i) and (ii). Total metal concentrations recovered from the spiked substrates ranged between 3–23 mg/kg for copper, 1–38 mg/kg for nickel and 3–26 mg/kg for zinc (with the exception of MnO_2 (ii): 40, 65 and 70 mg/kg respectively).

3.1. Copper (Figs. 2 and 3)

The added copper was mostly recovered from all the substrates except calcium carbonate and humic acid. Highest concentrations were found in acetic acid for all but montmorillonite, manganese dioxide, humic acid and MESS-1. There were marked differences between experiments in extraction efficiency for humic acid and felspar.

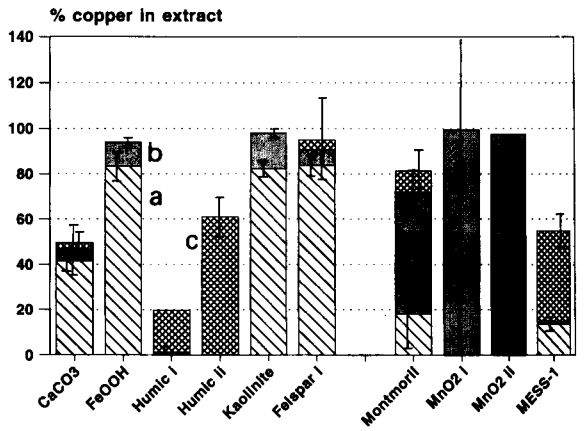

Fig. 2. Percentage of added copper found in extracts after 1 day. Error bars show 2×standard deviation for metal concentrations found in the extracts (mg/kg). Percentage error shown is therefore that in the particular extract, i.e., it has not been calculated from the total metal recovered. Error bars missing where 2×standard error <1%. However, replicate data for the following samples are unavailable: humic acid, 1-day equilibration (third extract); manganese dioxide ii, 1-day equilibration; calcium carbonate, 28-day equilibration (second extract). Key to shaded areas: a = acetic acid (first extract); b = hydroxylamine hydrochloride (second extract); c = hydrogen peroxide–ammonium acetate (third extract).

3.2. Nickel (Figs. 4 and 5)

Added nickel was found in the first and second extracts, with detectable amounts in the third released only into humic acid and MESS-1. Acetic

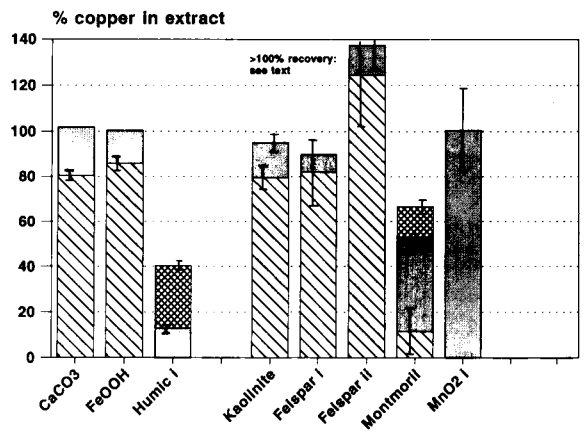

Fig. 3. Percentage of added copper found in extracts after 28 days. For further details see Fig. 2.

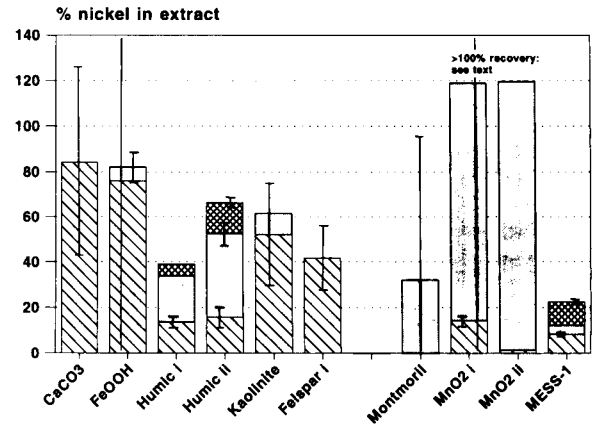

Fig. 4. Percentage of added nickel found in extracts after 1 day. For further details see Fig. 2.

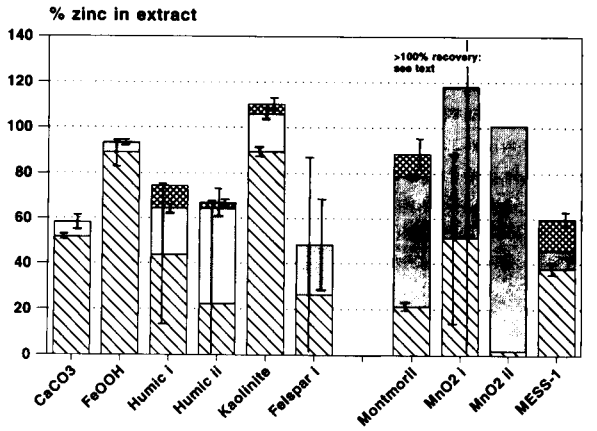

Fig. 6. Percentage of added zinc found in extracts after 1 day. For further details see Fig. 2.

acid contained most of the nickel for calcium carbonate, ferrihydrite, kaolinite and felspar, while hydroxylamine released most nickel for humic acid, montmorillonite and manganese dioxide.

3.3. Zinc (Figs. 6 and 7)

Zinc recoveries showed similar behaviour to those of nickel, although zinc was also found in the third extract for kaolinite and montmorillonite, and acetic acid removed 22–43% of the zinc from humic acid. Acetic acid also sometimes removed zinc from manganese dioxide (2–51%).

4. Discussion

Metal removed by sequential extraction in these experiments had been adsorbed onto the substrates over either 24 h or 28 days. This is an appropriate model for metals partitioning on to particles already equilibrated with sea water, such as when metals are discharged into an estuary. It does not attempt to look at the geochemical

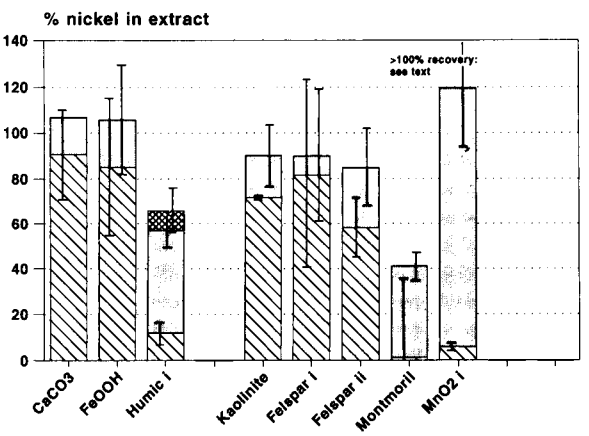

Fig. 5. Percentage of added nickel found in extracts after 28 days. For further details see Fig. 2.

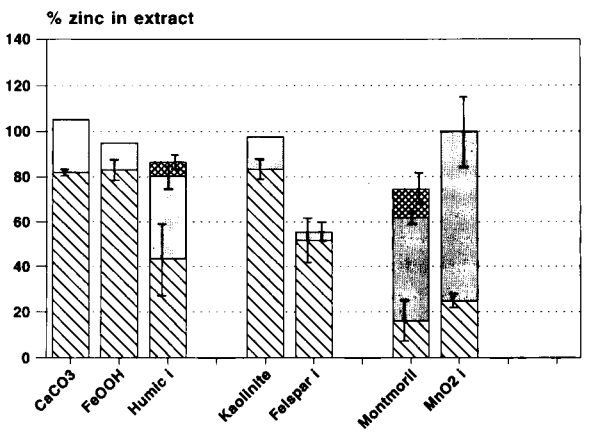

Fig. 7. Percentage of added zinc found in extracts after 28 days. For further details see Fig. 2.

origins of metal in sediment, much of which tends to be found in residual analyses after sequential extractions. Simplifications made include the use of substrates which have not been subjected to environmental modifications such as weathering and organic coatings. The use of single substrates was necessary to avoid any problems caused by redistribution of metal among sediment constituents [16].

Table 2 shows a comparison between substrates and the reagents expected to attack them versus those experimentally determined to release most metal. From this table it can be seen that the specificity of reagents is erratic. Acetic acid does release most of the metal associated with calcium carbonate, despite saturating the system. Metal bound to manganese dioxide was successfully released upon the destruction of the substrate by hydroxylamine hydrochloride. This is in contrast to ferrihydrite, which lost most of its bound metal to acetic acid, while the reducing action of hydroxylamine hydrochloride was of minor importance. The amorphous iron oxyhydroxide was used here instead of a more stable form (e.g., haematite, goethite), to approximate to iron oxides formed where fresh water meets saline. However, no attempt was made to look at release of the co-precipitated metals common in estuarine situations. Since ferrihydrite is noted for its adsorption sites [17], all that can be concluded is that metal release from iron oxides formed in situ may be easier to accomplish by sequential extractions than previously thought.

Metals bound to humic acid should be released in the third extract. This was only true of copper however, with nickel and zinc chiefly being removed by the first two extracts. Since sources of organic matter and metals tend not to be associated, the equilibration method used does represent a reasonable model of their mixing. 'Early' recovery of organically-bound metal suggests that metal release may be determined by relative binding capacities, rather than chemical degradation of the substrate itself. This observation does not come into the generally assumed behaviour of extraction reagents.

Of the minerals examined, kaolinite and potassium felspar released most metal into acetic acid. The bulk of copper and zinc bound to montmorillonite (a double-layer clay) was found in the second extract, but some of these metals were recovered from all three extracts. Only 33–66% of the added nickel was recovered from montmorillonite, and this was mainly in the second extract. Stepped recoveries suggest reagents do not act in a discrete, single-action manner. In these samples, release may be more a result of ion exchange equilibria than of chemical alteration of surface sites.

From these results, it is unclear whether metals are released because reagents have differing chemical properties, or just because a new reagent of lower pH has been added to the system. Whether this observation is considered important depends upon the final use of the information – some indication of the availability of metals un-

Table 2
Comparison between expected and experimentally-determined important reagents for individual substrates

Substrate	Reagent expected to release most metal	Extract found to have highest metal concentration	Assumed mechanism
Calcium carbonate	HOAc	HOAc	Acid–base
Ferrihydrite	$NH_2OH \cdot HCl$	HOAc	Reduction
Humic acid	H_2O_2/NH_4Ac	Variable: $Cu–H_2O_2/NH_4Ac$ $Ni–NH_2OH \cdot HCl$ Zn–HOAc and $NH_2OH \cdot HCl$	Oxidation
Kaolinite	? HOAc	HOAc	Ion exchange
Felspar	? HOAc	HOAc	Ion exchange
Montmorillonite	? HOAc	$NH_2OH \cdot HCl$	Ion exchange
Manganese dioxide	$NH_2OH \cdot HCl$	$NH_2OH \cdot HCl$	Reduction

Table 3
Metal concentrations in unspiked substrates leading to > 100% recoveries in extracts (other substrates < 2 mg/kg)

Metal	Substrate	Concentration [a] (mg/kg)
Copper	Felspar	5 (ii; 28-day)
Nickel	Manganese dioxide	6 (i; 1-day)
		11 (i; 28-day)
		17 (ii; 1-day)
Zinc	Manganese dioxide	4 (i; 1-day)
		32 (ii; 1-day)

* i and ii show data from repeated experiments

der changing pH or redox conditions may be inferred. However, it does appear that some of the more specific qualities attributed to sequential extractions are invalidated by these data.

4.1. Recoveries of added metal

The calculations used here have been made so that the percentage of metal found in the extract should represent just that metal taken up during the equilibration period. If less than 100% of the metal has been recovered, it suggests that some has become strongly bound to the substrate, i.e., binding was not solely physical adsorption. Where significantly more than 100% recovery has been recorded, it was found that the unspiked substrate contained large amounts of that metal (see Table 3). Equilibrium partitioning may have caused metal from the minerals in unspiked sea water to desorb, producing calculations of lower total uptakes from the sea water than actually occurred.

Most of the added copper, nickel and zinc were recovered from ferrihydrite. Total recovery from humic acid was mixed with 19–61% copper; 38–65% nickel; 66–86% zinc being found. In most cases, all the metal sorbed to kaolinite was removed during extraction. While copper recovery from felspar was high, nickel and zinc were less fully released. Removal of added copper and zinc from montmorillonite was in the range 66–88%, while that of nickel release was lower at 33–41%. Nickel recovery was also low in MESS-1 at 22%, while 55–60% total metal was released for copper and zinc.

Metal was recovered well from manganese dioxide and the 28-day equilibration of calcium carbonate – an observation to be expected since both these substrates are dissolved by the reagents. The high loading of carbonate for the method did not prevent most metal being released in the first extraction. However, there was poor recovery of carbonate from the 1-day equilibration. This may be because of sample leakage after gas build-up during extraction – a problem which can be also encountered with organic matter during the peroxide extraction. Gas evolution occurred with both calcium carbonate and humic acid – the method must therefore be very carefully performed. This would present particular difficulties when studying high carbonate anaerobic sediments, as gas release in the first stage would need to be carried out under nitrogen.

An indication of the analytical variability is given by the error bars (± 2 standard error) shown on Figs. 2–7. MESS-1 was sequentially extracted six times over the course of the experiments with good reproducibility. Total concentrations in MESS-1 are copper 25.1 mg/kg, nickel 29.5 mg/kg and zinc 191 mg/kg. On the experimental substrates variability was also generally acceptable. However, nickel in calcium carbonate, ferrihydrite and montmorillonite (1-day); zinc in the first extract (1-day) for humic acid and felspar; and all three metals in manganese dioxide (1-day, replicate i) (Figs. 4–6) showed very high error. Some of this error can be attributed to the analysis: relatively low levels of nickel were taken up by montmorillonite (up to 0.5 mg/kg were found in the acetic acid fraction), so that variability could reflect detection problems. A repeated experiment for zinc on felspar (data not shown) showed lower standard deviations for the analysis (12% instead of 22%), but with similar concentrations found in the extracts. Explanations for error on the other substrates were not found. With the exception of nickel on felspar, the high variability samples were those equilibrated for only one day. This suggests that variability could be caused by uneven metal distribution throughout the substrates. Subsampling into centrifuge tubes may have loaded more metal-concentrated material into one replicate than another. Jannasch et al.

[18] found that metals with low partitioning coefficients also had the slowest equilibration rates; in these experiments, less nickel was sorbed from the sea water than either of the other two metals. Slow kinetics could cause nickel to be more prone to sampling variation than either copper or zinc.

The BCR method requires the substrate to be rinsed with deionised water after the first and second extractions. As this practice may leach some metal from the substrate, rinses were collected and analysed in this experiment. The percentage of metal in the rinse was calculated from the metal concentration in the following extract. Substrates showing leaching of more than 10% thus calculated were ferrihydrite, humic acid and MESS-1. Ferrihydrite and humic acid showed metal concentrations of 10–40% (copper, nickel and zinc) of that found in the following extract. MESS-1 showed zinc concentrations of ca. 20% of that in the extracts. The rinse step therefore overall removed insignificant amounts of metal in comparison with total levels. However, there is evidence of selective leaching, which, while often of high variability, may lead to underestimates of metal found where sediments of high iron oxide or organic content are being studied.

Measurement of the pH of samples after extraction showed values in the range of pH 3–4 for acetic acid extracts ($CaCO_3$ samples pH ~ 6), pH 2–4 for hydroxylamine hydrochloride extracts ($CaCO_3$ samples pH ~ 6) and pH 2–2.1 for the ammonium acetate extracts ($CaCO_3$ samples pH ~ 3.5). The samples were analysed, and in view of the relatively high pH values of the first and second extracts, these were then acidified to 0.2% HNO_3 and the analysis repeated. Table 4 shows that higher metal concentrations were found in the acidified extracts. From these data, copper in hydroxylamine hydrochloride, nickel in acetic acid and zinc in both reagents contained significantly larger amounts at the 5% level in acidified extracts compared to those in non-acidified extracts.

Table 4
Results of paired t-test that samples acidified after extraction contained higher metal concentrations than those analysed prior to acidification (one-tailed test)

Metal	Acetic acid p	Hydroxylamine hydrochloride p	n
Copper	ns	< 0.025	18
Nickel	< 0.025	ns	18
Zinc	< 0.025	< 0.05	17

ns = Not significant.

4.2. Variability between experiments

It was the intention of this study to look at how metal speciation might vary with time, so two sea water–substrate equilibration periods were studied (24 h and 28 days). However, variability associated with repeated experiments over the same time period means that comparison between different experiments cannot be made. Data for repeated experiments upon humic acid, felspar and manganese dioxide are shown here. The major difference between these experiments was that of the mass of sediment present in the 200 ml sea water; humic acid (i) 2.60 g, humic acid (ii) 4.85 g, felspar (i) 1.37 g, felspar (ii) 6.56 g, MnO_2 (i) 2.30 g and MnO_2 (ii) 0.83 g. The metal spike added was constant to the volume of sea water, so that the actual spike mg/kg substrate varied. No association could be found between spike concentration (mg/kg) and proportional recovery: indeed the uptake of metal by the substrates could be remarkably consistent (see Fig. 8). Regression data for these points show a gradient of −2.65 (standard error 0.30); an inter-

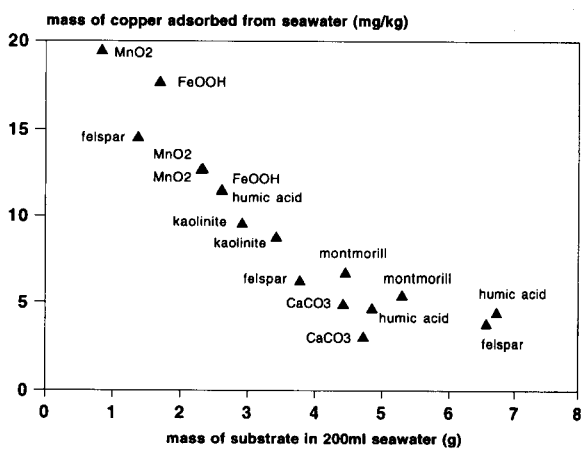

Fig. 8. Copper adsorption from sea water on to all substrates.

cept of 18.79 (standard error 2.08); $r^2 = 0.837$ with 17 samples included in the analysis. This lack of reproducibility between experiments should be viewed with some concern. However, the pattern of metal release into reagents tends to be similar even if total metal recovery is rather different. This is the case both for repeated experiments and for data from all experiments (1-day and 28-day equilibrations).

5. Conclusions

The BCR sequential extraction procedure provides a method for releasing metal from sediment under differing chemical regimes. The geochemical phase specificity has been shown to be of varying quality, as determined upon single substrates. Calcium carbonate and manganese dioxide released most bound metal into the expected reagents (acetic acid and hydroxylamine hydrochloride, respectively), while humic acid generally released metal earlier in the procedure than might be expected. An amorphous iron oxide, ferrihydrite, released most bound metal into acetic acid rather than into the second extract, but this may be a result of a large number of adsorptive sites on this species. Extractions carried out upon felspar and kaolinite removed most metal in the first step, while the second step proved most important in metal release from montmorillonite. Stepped recoveries for humic acid and montmorillonite, where metal is found in more than one extract, suggest metal release is more conditional upon ion exchange equilibria than upon chemical alteration of binding sites.

The method was rather variable when comparing metal release between repeated experiments, leading to difficulties in interpretation. While patterns of release remained similar, the percentage of added metal recovered could vary considerably.

It is recommended that extracts are acidified after separation from sediment to ensure that released metal stays in solution. Significant differences in metal concentration were found in extracts from calcium carbonate, potassium felspar, ferrihydrite and humic acid between samples analysed before and after acidification.

Acknowledgements

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Determination of SO_2, HNO_3, NH_3 and aerosol components at a high alpine background site with a filter pack method

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Abstract

In the time period from November 1991 to November 1992 measurements of SO_2, HNO_3, NH_3 and particulate SO_4^{2-}, NO_3^- and NH_4^+ were conducted at the Sonnblick Observatory in the Austrian Alps. To allow continuous daily measurements a filter pack was used. The precision of the method was checked in respect to the harsh meteorological conditions at the high alpine site and showed acceptable coefficients of variance (< 20%) for particulate matter and nitric acid and slightly higher variations for the gases ammonia (25%) and sulphur dioxide (30%). The annual variations showed large seasonal differences for the aerosol components and for the gases nitric acid and ammonia. Maximum summer/winter ratios were 8, 11 and 17 for the aerosol species NO_3^-, SO_4^{2-} and NH_4^+ respectively and 5 for HNO_3 and NH_3. No marked summer/winter differences could be determined for sulphur dioxide. During winter the absolute concentrations at Mt. Sonnblick were comparable with results reported for measurements of free tropospheric air. In summer a wide scatter of concentrations was determined ranging up to values typically for the planetary boundary layer in remote regions.

Key words: Ammonia; Aerosols; Filter pack method; Nitric acid; Sulphur dioxide

1. Introduction

SO_2, HNO_3, NH_3 and particulate SO_4^{2-}, NO_3^- and NH_4^+ (in the following text referred to as p-SO_4^{2-}, p-NO_3^- and p-NH_4^+) are of major importance in the dry deposition process. They are the main precursors of particulate SO_4^{2-}, NO_3^-, NH_4^+ and the free acidity in cloud droplets and wet precipitation. Increasing data sets are emerging worldwide from measurements at ground based stations in polluted and remote regions [1–7]. In addition vertical profiles over Central Europe and North America are available from various aircraft experiments [8–17]. Such sampling programs were carried out in the warm and the cold season. However aerosol and gas concentrations are often not comparable as respective flights have been performed under different meteorological conditions. Hence we still have little information about the seasonal variation of the compounds of interest in remote regions and at high elevation sites.

As measurements at the Mauna Loa Observatory have shown, mountain observatories can serve as research platforms to investigate the

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atmospheric behaviour of air constituents at tropospheric levels [18,19]. This is the first reported time series of gas and aerosol components over a one year sampling period from a research platform at 3 km above ground level in Central Europe. As a research platform, the Sonnblick Observatory (SBO) was used. Sampling conditions at the 3 km level in the Alps are very harsh. Wind speed ranges up to 180 km/h, temperatures drop down to −25°C. When the site is in the clouds the occurrence of rime has to be expected. Cloud droplets are generally subcooled, with wet precipitation generally in the form of snow. Therefore a sampling method had to be adopted, which allowed continuous measurements under such conditions. Since the Observatory is not equipped with laboratory facilities mailing of the samples had to be possible. The filter pack method employed overcame the environmental demands and yielded acceptable detection limits.

In the present paper we will discuss the application with respect to the obtained results at the Sonnblick Observatory covering the sampling period from November 1991 to November 1992.

The experiment is part of the EUROTRAC (European Experiment on the Transportation and Transformation of Environmentally Relevant Trace Constituents in the Troposphere over Europe) subproject ALPTRAC (High Alpine Aerosol and Snow Chemistry Study). A goal of ALPTRAC is to gain an understanding about the main physical and chemical processes responsible for the atmospheric occurrence of acidic and related components and their subsequent deposition to the High Alpine region.

2. Experimental

2.1. Site

The experimental work reported here was performed at the Sonnblick Observatory (SBO). The SBO is located at the summit of Mt. Sonnblick in an altitude of 3106 m. Mt. Sonnblick is situated in the main ridge of the Austrian Alps (Hohe Tauern, 12°57′E, 47°03′N). As Mt. Sonnblick is among the highest peaks in the area it is exposed to air masses from all directions. The Observatory is surrounded by large glacier fields. Towards the northeast a steep wall descends over 800 m down to a sparsely populated valley. There are no noticeable local emission sources. Since the observatory is supplied with electricity it has no local sources of exhaust fumes. Hence the Observatory has the ideal characteristics of a high alpine background station for air chemistry measurements. The SBO is situated in the center of Europe surrounded by regions with large emission densities at distances of a few hundred kilometres.

2.2. Sampling system

For sampling of SO_2, HNO_3, NH_3 and aerosols an open-face filter pack was used. The apparatus was situated at the sampling platform on the top of the Observatory. A polyethylene funnel was used as a shelter to protect the open-face filter pack [20]. No preseparator for cloud droplets was used. Filter holders were made from polycarbonate and were supplied by NILU (Norwegian Institute for Air Research).

Sampling time was 24 h. The filters were changed at 8:00 am. Air flow was approximately 11 l/min (reduced to 1013 mbar and 0°C). The exact air volume was determined by a dry test gas meter.

The filter pack method used was based on the system described by Fellinger [21]. It is an extended version of the method by Anlauf et al. [22]. They used a triple filter pack consisting of a PTFE front filter, a nylon filter and a potassium carbonate impregnated filter. In addition there was a fourth filter which was coated with oxalic acid/glycerol for the collection of gaseous ammonia [23,24]. Furthermore we used a potassium hydroxide impregnated filter instead of the potassium carbonate impregnated filter. As potassium hydroxide converts to potassium carbonate during contact with air the potassium hydroxide filters are an equivalent replacement for the potassium carbonate filters.

Thus the filter packs we used consisted of a set of four 47 mm diameter filters in series.

The front filter was a PTFE membrane (Gelman "Zefluor", 1 μm pore size) for collection of particulate matter followed by a Nylon membrane (Gelman "Nylasorb", 1 μm pore size) for collection of nitric acid and sulphur dioxide. However, the collection of sulphur dioxide with the Nylon filter is incomplete. The third and the fourth filter were cellulose filters (Schleicher and Schüll "Black Ribbon") impregnated with potassium hydroxide (aqueous solution containing 1% w/w potassium hydroxide) and oxalic acid (aqueous solution containing 3% w/w oxalic acid in 5% w/w glycerol) respectively. The potassium hydroxide filter was used for quantitative sampling of sulphur dioxide, the oxalic acid filter collects ammonia. The impregnation of the cellulose filters was performed in a purified gas atmosphere. Drying took place in a vacuum desiccator.

After sampling the filters were transported to the laboratory in Vienna where they were placed in 4-ml polyethylene vials. The vials were closed with caps to be air-tight and stored in the refrigerator to await extraction and analysis. Taking storage at the site, transportation to the laboratory and storage in Vienna into account the analysis was done within one month after sampling.

For extraction PTFE filters were soaked with 0.2 ml ethanol [25] after which 3.4 ml deionized water were added. The extracts were analysed for anions (Cl^-, NO_3^-, SO_4^{2-}) and cations (Na^+, NH_4^+, K^+, Ca^+). Nylon filters were extracted in 0.2 ml 1% H_2O_2 and 3.4 ml carbonate buffer (2.6 mM Na_2CO_3–3.3 mM $NaHCO_3$). Addition of H_2O_2 was necessary as nylon filters retain SO_2 which is present as SO_3^{2-} and SO_4^{2-} ions. H_2O_2 quantitatively oxidizes SO_3^{2-} to SO_4^{2-}. Thus the extracts of the Nylon filters were analysed for NO_3^- and SO_4^{2-}. Extraction of the potassium hydroxide coated filters was in 0.2 ml 1% H_2O_2 and 3.4 ml deionized water. Again addition of H_2O_2 was necessary to oxidize SO_3^{2-} to SO_4^{2-}. Extracts were analysed for SO_4^{2-}. The oxalic acid/glycerol impregnated filters were extracted with 3.6 ml deionized water. Extracts were analysed for NH_4^+. Extraction took place in an ultrasonic bath, and an extraction time of 30 min was generally employed.

Anions were determined by ion chromatography using a Dionex system (D10) equipped with an AS1 column and a membrane suppressor (AMMS). A Chelex-100 column was used as a guard column to prevent metal contamination of the separator column. The eluent was a carbonate buffer (2.6 mM Na_2CO_3–3.3 mM $NaHCO_3$), the eluent flow was 1.5 ml/min. Injection volume was 300 μl, detector sensitivity was 30 μS full scale. Monovalent cations (Na^+, NH_4^+, K^+) were determined by single column cation chromatography using a Wescan ICM system equipped with a Wescan cation column 269–004. The eluent was 2 mM HNO_3 with a flow rate of 0.8 ml/min. Injection volume was 100 μl, detector sensitivity was 10 μS full scale.

The bivalent cation Ca^{2+} was determined by flame AAS using standard procedures (PE Model 403, N_2O–C_2H_2, 422.67 nm, with addition of 1% v/v saturated KCl).

The continuous collection of daily samples throughout several months was performed as a collaborative programme between the Vienna University of Technology and the Sonnblick Observatory. Preparation and analysis of the filters was done in the authors laboratory in Vienna. The loaded filter packs were sent to the observatory in packs of 12 filters. At the site the daily exchange of samples was carried out by the observatory staff. After sampling the complete set of filter packs was sent back to Vienna for analysis.

3. Results and discussion

3.1. Performance of the filter pack method

First tests of the performance of the applied method were made by Fellinger [21]. These investigations included a comparison of the filter packs with a denuder train [26] to determine the accuracy of the method. Since extremely difficult meteorological conditions prevail at the mountain site and the mailing of the samples could lead to unknown problems, studies were conducted to assess detection limits and the reproducibility of the method at the high Alpine site.

Detection limits

Storage and shipment of the filterstacks resulted in notable blank readings which determined the detection limits of the method for the single components. To check the blank readings routinely additional filterstacks were sent to the observatory. Every set of samples sent to the SBO contained twelve filter packs. While ten filterstacks were used for sampling, two filterstacks were taken as fieldblanks. In respect to preparation, storage and analysis the fieldblanks were treated exactly like the other samples. To consider contamination during the handling of the filter packs at the site the "fieldblank-filter packs" were connected to the sampling system and air was drawn through the filters for 10 s. The detection limits of the method were determined according to 3S.D. of the blank values with an average sample volume of 15 m^3 air. The standard deviation was calculated for the sum of fieldblanks determined within the whole year of sampling. The detection limits are shown in Table 1.

Detection limits are sufficiently low for the determination of aerosol components p-SO_4^{2-}, p-NO_3^- and p-NH_4^+ as well as for nitric acid and sulphur dioxide. Problems occurred in respect to the quantification of ammonia during winter months when the average monthly concentrations were close to the detection limit. The high detection limit for ammonia is a result of the large variation of the field blanks. While after coating of the filters with oxalic acid and storage in the laboratory no notable blank readings occurred, field blanks for ammonia were relatively high. We attribute these variations to contamination of the filters during mailing and storage at SBO. Although the filterstacks were mailed in tight plastic-containers we could not solve that problem completely. Median concentrations of the aerosol components p-Na^+, p-Cl^-, p-Ca^{2+} and p-K^+ were close to the detection limits during most of the months. Therefore we could only conclude that during November to January the average concentrations of p-Ca^{2+}, p-Na^+, p-Cl^- and p-K^+ were below 2.2, 1.2, 0.74 and close to 0.19 nmol/m^3. For p-Ca^{2+} only the restriction was due to the high detection limit resulting from large variations of the blank values. For the other components not even the very small detection limits achieved, could cope with the low concentrations at the background site. In summer several events occurred when concentrations of these aerosol species were significantly higher.

In the case that filters showed a concentration less than three standard deviations of the field blanks no absolute concentrations could be determined. To calculate monthly averages half of the blank value was assumed for these samples according to the procedure by Huebert and Lazrus [9].

Collection efficiency

To determine the collection efficiency of the various filters in respect to the conditions occurring at SBO efficiency checks were performed at the field site by installing two filters in series. The average collection efficiency of the Nylon filters in respect to nitric acid was 98%. In regard to sulphur dioxide the combination of a nylon and a potassium hydroxide coated cellulose filter showed an average collection efficiency of 87%. The ratio of sulphur dioxide collected by the nylon or the impregnated cellulose filter varied widely. No dependence on temperature, humidity or absolute concentration values was noticed. Since sampling of ammonia with a cellulose filter impregnated with oxalic acid showed poor results (average collection efficiency was 78% varying from 40–100%) we added glycerol to the coating solution [23,24]. After which there was an improvement in the average collection efficiency for ammonia (90%).

Measurements with two PTFE filters installed in series showed that aerosol components were

Table 1
Detection limits for the filter pack method

	HNO_3	SO_2	NH_3	NO_3^-	SO_4^{2-}	NH_4^+	Ca^{2+}	Na^+	Cl^-	K^+
DL (nmol/m^3)	0.62	1.1	4.1	0.29	0.14	0.40	2.2	1.2	0.74	0.19

Table 2
Reproducibility of the method

	HNO_3	SO_2	NH_3	NO_3^-	SO_4^{2-}	NH_4^+
During dry conditions (n = 4)						
Mean (nmol/m^3)	18	11	170	3	120	89
R.S.D. (%)	9	16	7	6	2	5
Measurements in cloud (n = 5)						
Mean (nmol/m^3)	4	3	13	8	11	23
R.S.D. (%)	17	30	25	16	13	14

quantitatively sampled on the first PTFE filter. In addition it was noticed that there was no positive artifact resulting from the sampling of reactive gases (e.g. nitric acid) by the second PTFE filter.

Reproducibility

To check the reproducibility of the method while sampling under harsh meteorological conditions triple sampling was performed at the field site. Table 2 shows the mean concentration during the sampling periods and the standard deviation of the three samples divided by the mean concentration. The experiment is based on five successive sampling periods. The results are compared with the performance of the sampling system during dry conditions [21]. The reproducibility test under dry conditions was conducted at the SBO and in Vienna respectively. It comprises the data of four sampling periods.

During dry conditions the coefficient of variance was below 10% for all components except for sulphur dioxide that showed a deviation of 16%. Measuring in cloud the deviations increased reaching values between 10 and 20% for the aerosol components and nitric acid and 25 and 30% for ammonia and sulphur dioxide respectively. In respect to sulphur dioxide the low absolute concentrations have to be taken into account. Sulphur dioxide was the only component calculated from the analysis of two filters. The higher variations for ammonia are due to the scatter of the blank values. Sampling with an open face system in cloud often led to deposition of humidity and droplets on the front filter. This phenomenon can effect the sampling. Reactive components might be scrubbed by this layer. The only possibility to avoid such an effect is to use a preseparator to remove cloud water droplets before sampling of gases and interstitial aerosol. On the other hand the usage of a preseparator may lead to losses of reactive gases such as nitric acid and larger particles. Since the comparison during harsh meteorological conditions showed satisfying good agreement between the single filter packs we decided not to use any kind of preseparator.

Table 3
Arithmetic means of gas (HNO_3, SO_3, NH_3) and particulate NO_3^-, SO_4^{2-}, and NH_4^+ concentrations (nmol/m^3) determined at the SBO

	n	HNO_3	SO_2	NH_3	p-NO_3^-	p-SO_4^{2-}	p-NH_4^+
November 1991	15	1.4(1.4)	13.1(25)	2.7(2.1)	1.0(1.6)	1.8(3.4)	2.4(3.9)
December	30	0.8(0.6)	5.5(9.4)	3.9(3.6)	0.3(0.2)	1.7(1.9)	1.9(3.3)
January	16	1.2(1.3)	2.4(2.5)	2.4(0.8)	0.6(0.9)	2.0(2.2)	2.4(2.3)
February	22	2.7(1.8)	11.0(18)	3.5(2.5)	1.1(1.6)	4.9(3.8)	6.3(4.9)
March	26	6.8(6.4)	9.6(9.1)	11.3(9.7)	2.8(4.3)	10.9(8.2)	16.5(13)
April	24	7.4(4.3)	13.2(25)	15.3(16)	5.3(8.0)	17.5(12)	31.0(25)
May	28	6.8(8.6)	7.3(10)	13.8(14)	5.9(7.2)	29.1(30)	48.6(47)
June	29	6.6(5.3)	7.6(13)	13.5(10)	4.5(5.6)	24.0(25)	41.8(49)
July	10	7.6(7.2)	4.4(3.5)	23.4(20)	2.2(3.1)	17.4(27)	25.2(38)
August	9	7.4(5.6)	3.2(2.5)	8.2(10)	8.8(13)	30.1(30)	52.8(50)
September	13	4.5(10)	3.7(12)	6.7(45)	3.0(7.6)	11.7(34)	16.0(62)
October	24	4.7(4.5)	3.8(3.5)	4.4(6.0)	0.9(1.8)	5.2(4.1)	5.7(4.9)
November 1992	25	2.1(1.9)	2.1(2.0)	3.6(3.4)	0.8(1.5)	3.5(3.5)	3.1(2.8)
Winter period (Nov.–Jan.)		1.4(1)	5.8(12)	3.2(3)	0.7(1)	2.2(3)	2.5(3)
Summer period (May.–Aug.)		7.1(7)	5.6(10)	15(13)	5.4(7)	25(28)	42(47)
Summer/winter ratios		5	1	5	8	11	17

n = Number of samples, standard deviations of the monthly mean values based on daily samples are given in parentheses.

The deviations observed in the triple sampling correspond to various intercomparisons of filter pack techniques reported in the literature [20,23,27–29] which in some cases show substantially higher detection limits. Hence the measurements were found suitable for routine measurements at a remote background station.

Measurement artifacts

Filter packs collect aerosol constituents prior to the sampling of gases. Therefore the method can be subject to several artifacts due to the alteration of the aerosol sampled on the PTFE filter during the sampling period [30–34]. The evaporation of ammonium nitrate (which is forced by an increase in temperature or a decrease of humidity) leads to the formation of nitric acid and ammonia which are subsequently collected as gases. Furthermore the equilibrium between $(NH_4)HSO_4 + NH_4NO_3 \rightleftarrows (NH_4)_2SO_4 + HNO_3$ can cause a positive nitric acid artifact. The opposite effect is the collection of reactive gases on aerosol particles already deposited on the filter. Nitric acid vapour may be retained by previously collected alkaline aerosol, generating a negative nitric acid artifact. Ammonia can be retained at the front filter if acidic ammonium sulphate aerosol is present, generating a negative ammonia artifact.

Evaporation of ammonium nitrate during sampling and during storage (temperatures during storage and mailing can be significantly higher than during sampling) surely is a problem for the measurements. However only minor concentrations of ammonium nitrate are expected at Mt. Sonnblick. Moreover impactor measurements conducted at the Sonnblick Observatory showed that p-NO_3^- is mainly attached to p-Ca^{2+} and is present in a larger particle size range (mass median aerodynamic diameter: 3 μm) than ammonium sulphate (mass median aerodynamic diameter: 0.7 μm) [35]. Because of the insufficient detection limit for p-Ca^{2+} this relation between p-Ca^{2+} and p-NO_3^- could hardly be seen with the filter pack measurements. Since ammonium sulphate tends to be acidic, a negative artifact for ammonia could be expected. As there is no correlation between the neutralisation ratio and the concentration of ammonia we consider this effect as neglectable. It must be born in mind that particulate loadings on the filters are very small. Average particulate concentrations per square centimetre filter surface were 1.5 μg SO_4^{2-}, 0.2 μg NO_3^- and 0.5 μg NH_4^+. Hence we think that our identification of gaseous and aerosol components is substantially correct.

3.2. Seasonal variations of gas and aerosol components

Aerosol components and the gases nitric acid and ammonia show pronounced seasonal cycles with very low concentrations during the winter months and much higher concentrations during summer. To demonstrate the maximum differences within these cycles the months November to January have been averaged as "winter-values" and the time period from May to August was summarized as "summer-values". Calculating the summer to winter ratios factors of 8, 11 and 17 can be seen for p-NO_3^-, p-SO_4^{2-} and NH_4^+ respectively. For ammonia and for nitric acid a summer to winter ratio of 5 could be found.

The average "winter-values" agree well with results reported from aircraft or ground based measurements in the free troposphere [1–3,8–15,18,19,35]. However the clean air status at Mt. Sonnblick is not as clearly defined as for most of the reported aircraft measurements or the sampling at the Mauna Loa Observatory in Hawaii, where three criteria (condensation nuclei counts, dewpoint and relative wind direction) could be determined to select data which represent the free troposphere. Until now we could not define adequate criteria to distinguish between events when the site is in the free tropospheric air or influenced by air masses of the planetary boundary layer. During winter the air masses at the 3 km level are surely decoupled from lower, more polluted air masses due to the formation of stable meteorological conditions. Without additional measurements it is not possible to determine to what extent the site is in the free troposphere or influenced by a modified mixing layer.

The averaged "summer-concentrations" determined at the Sonnblick Observatory range up to

concentration values typically for measurements in remote areas within the planetary boundary layer [4–8,11,12,16–18,35]. Simultaneously conducted filter pack measurements next to Mt. Sonnblick at the valley floor showed aerosol concentrations comparable to the measurements at the mountain site. This indicates that during summer the site often is immersed into the planetary boundary layer.

4. Conclusions

With the filter pack method described a method suitable for continuous daily measurements at a high alpine background site could be introduced. The detection limits provided for SO_2, p-SO_4^{2-}, HNO_3, p-NO_3^-, NH_3 and p-NH_4^+ were sufficient to allow measurements during winter when the concentration values correspond to results from measurements in the free troposphere. The acquired reproducibility was acceptable. The coefficient of variation was below 20% for most of the species except for ammonia and sulphur dioxide (maximum coefficients for these two species were 25 and 30% respectively).

The continuous daily measurements for the observed species showed seasonal trends with very low concentrations in winter and maximum concentrations in summer. Only for sulphur dioxide a different seasonal cycle, revealing a small spring maximum and no discernible differences between the winter and summer concentrations, was determined.

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Permeation denuder for sampling and continuous analysis of gases
Part 1. System configuration, basic studies and application to atmospheric ammonia and sulfur dioxide

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Abstract

The application of tubular and planar permeation denuders for introduction of gaseous compounds into flow-injection systems is presented. Construction details of the permeation denuders are given and different configurations of implementation in flow systems are described. The principles and experimental requirements of concentrating gaseous compounds are outlined. Collection efficiency of the permeation denuders is determined in dependence of gas flow rate, kind of membrane and composition of absorber solution using ammonia and sulfur dioxide as test gases. A brief discussion of calibration procedures is presented. The suitability of the proposed method is exemplified in the determination of ambient ammonia and sulfur dioxide using various detection schemes.

Key words: Flow systems; Ammonia; Permeation denuder; Sulphur dioxide

1. Introduction

Sampling of gaseous species is commonly performed by trapping or sorption of the contaminant of interest in the liquid phase (bubbler/impinger) at impregnated filters or columns filled with solid absorber materials. The actual determination step is subsequently carried out by liquid-phase chemistry using the variety of analytical methods [1]. These techniques, although easy to handle, suffer from several drawbacks. With respect to the ever increasing number of analyses to be performed in environmental laboratories the lack of automation capability and tedious operations involved are possibly the most serious limitations. In addition, these procedures are integrative by nature (sampling times are typically in the order of hours) so that short-term fluctuations of the gas-phase concentration cannot be detected. A further disadvantage is that real-time information is not obtained since samples collected at different sites and/or in a successive manner are analysed with delay of hours or more in the

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analytical lab. Finally, particulate matter present in the air is scrubbed off and may considerably contribute to the concentration of the species of interest. Prefiltration to remove particulates is frequently unacceptable because of potential loss by adsorption of the gas on the filter material or the particulate matter collected on it [2,3]. On the other hand, in the particular case of ammonia determination blow-off of ammonium nitrate from prefilters generating gaseous ammonia leads to significant overestimations [4].

The latter problem can be circumvented by the use of diffusion denuders [5–7] which have been recognized as a valuable approach for sensitive, selective and artefact-free collection of gaseous pollutants. In their simplest form they consist of straight tubes the inside wall of which are coated with a suitable adsorber material acting as a perfect sink to the gas of interest. If air is fed through these tubes the gaseous species are trapped and accumulated at the wall whereas aerosols freely pass the tube and can be collected on a back-up filter. This only holds, however, if laminar flow conditions are maintained. Advantage is taken from the fact that diffusion coefficients of gases are several orders of magnitude higher compared to aerosols. After sampling for any desired period of time the wall coating is washed off and the determination takes place in the liquid extract.

A severe disadvantage of diffusion denuders is the discontinuous nature of operation. Problems are often involved with reproducible coating which is a basic requirement for reliable operation since calibration and sample measurement steps have to be performed successively always using newly coated denuder walls. Finally, coating, drying, washing and re-coating are laborious procedures which carry a high risk of contamination and require considerable skill.

A more recent innovation in gas analysis is the development of the permeation denuder [8–10] (also termed diffusion scrubber [11–13]) which ingeniously combines the attractive features of diffusion denuders with simplicity of operation and the ability to be used for continuous monitoring. The gas collecting elements of permeation denuders are gas-permeable membranes which separate the sample stream from a liquid absorber solution. In the majority of papers published so far hydrophobic microporous tubing with annular jackets were employed [8,12–14]. Here, sample air is forced through the annulus between tube and jacket and a liquid absorber stream is continuously pumped through the microporous tube (in early papers reverse operation has been proposed [12]). The gaseous species diffuse to the wall of the membrane, through the membrane and into the absorber solution where they are trapped. Upon leaving the permeation denuder the liquid phase can be collected for subsequent analysis or, more attractively, directly transported to a suitable detection device [8–10,12–15].

In the present paper recent developments of the coupling of permeation denuders with flow-injection analysis (PD-FIA) are reported. The basic principles of the method are outlined and instrumental requirements discussed. A comprehensive study is undertaken to examine the experimental variables that effect collection of gaseous compounds. In this respect the behaviour of tubular and planar denuders is compared. The preconcentration capabilities of PD-FIA are exploited and a simple means for calibration of the system is proposed. Improvements in the construction and design of permeation denuders will also be presented.

In this first part of a series of papers the application to PD-FIA for the determination of atmospheric ammonia and sulfur dioxide is described.

1.1. Collection efficiency, mass transfer and concentration capabilities. Comparison of diffusion and permeation denuders

Diffusion denuders have originally been developed for selective and quantitative removal of gases from gas–aerosol mixtures [16]. It was not until 1979, however, that the denuder tube was applied to denude a sample of certain gaseous compounds, i.e., to collect on the wall the compounds of interest rather than the interfering ones [5]. Despite the reverse of the role of the denuder it was (and obviously still is) an apparent goal to achieve quantitative gas collection. Based

on theoretical calculations [17] and by empirical means the configuration and design of denuders has undergone considerable changes during the last decade [6,7,18–21]. Annular denuders bearing as much as 12 concentric tubes [18], capillary assays [19], hollow fiber bundles [20] and even coated screens [21] have partially replaced the original straight tube configuration because they all offer enhanced collection efficiency at given gas flow rate. However, the general necessity of quantitative collection in denuder sampling has apparently never been rationalized or called in question.

Permeation denuders in many respects resemble the classical diffusion denuder [11]. The major difference, however, is that the wall coating of diffusion denuders is replaced by an absorber liquid present behind a gas-permeable membrane. This not only makes considerable difference with respect to practicability (e.g., the tedium of coating, eluting and recoating is omitted) but also involves conceptual differences. The requirement of homogeneous coating difficult to warrant in diffusion denuders is inherent in the permeation denuder since the liquid absorber solution is in contact with the entire membrane area. Partial depletion of the coating, always a problem in diffusion denuders at long sampling times or elevated analyte concentrations, can be effectively prevented by continuous renewal of the absorber liquid, i.e., propelling the liquid absorber through the permeation denuder at a reasonable rate. The most important difference, however, is the ability to permanently observe the collection behaviour of the permeation denuder by coupling it to a continuous flow or flow-injection system. The examination of constancy of the collection efficiency is thus possible at regular intervals by introduction of a calibration gas. This in turn permits to apply permeation denuders at experimental conditions were only partial collection of the gaseous species is achieved. In practice this means that arbitrarily short permeation denuders can be used and the gas flow rate can be set to any practical value.

Consideration of the concentration capabilities offered by diffusion denuders reveals that long sampling times and a low volume of eluent are advantageous. The use of high gas flow rates which would result in enhanced mass uptake cannot be allowed for reasons discussed above.

In recent work [22] we have thoroughly treated the concentration capabilities of gas-diffusion separation in FIA systems. Much of the results of the considerations made in that work likewise apply to gas collection with permeation denuders. Accordingly, enhanced mass transfer across the membrane is obtained at high gas flow rates (while concentration efficiency decreases). A limiting value, however, exists where no further benefit can be made by increase of gas flow rate [14]. A second important variable effecting the concentration capability of permeation denuders is the volume of liquid absorber solution within the device or, at continuously flowing absorber stream, its flow rate. Finally, dilution of the absorber liquid by merging reagent streams or dispersion phenomena have to be taken into account when detection methods are coupled online. The importance of the considerations outlined to the application of PD-FIA will be exemplified under Results and Discussion.

2. Experimental

2.1. System configuration

The basic arrangement of the sampling and detection systems employed in the present study are shown in Fig. 1. In the simplest version (Fig. 1A) the liquid absorber solution is continuously pumped through the permeation denuder and further on to the detector, if required reagents can be added to the liquid absorber stream by the use of merging stream manifolds (dashed line in Fig. 1). In a modified version (Fig. 1B) the permeation denuder is made to encompass an integral part of the valve of a flow-injection system (for operational details see Results and Discussion). A further variant is depicted in Fig. 1C where the permeation denuder is located in the supply channel of the FIA system.

Gas samples were introduced into the permeation denuders by means of an air pump (Model GS 312, Desaga, Heidelberg) employed in the

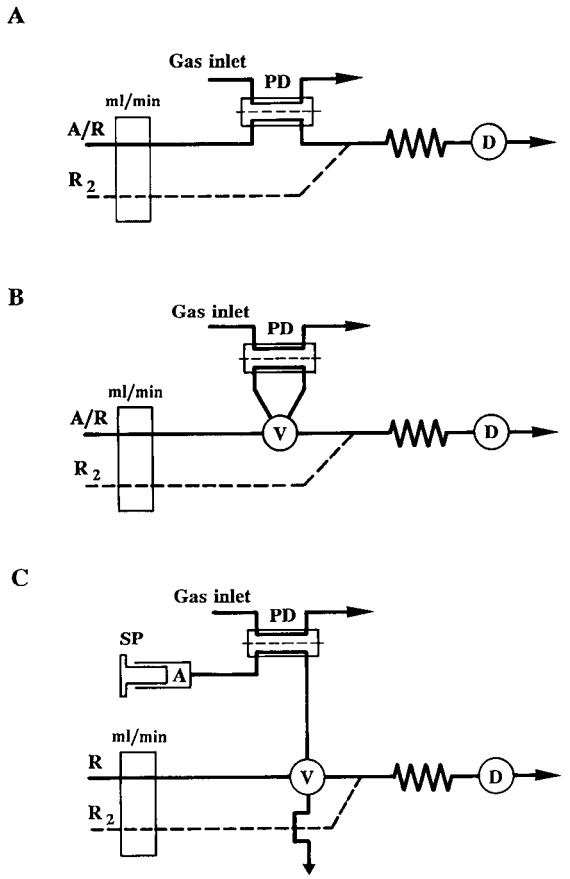

Fig. 1. Configuration of different manifolds for interfacing permeation denuders (PD) to FIA. V = valve; D = detector; A = absorber stream; R = reagent stream; SP = syringe pump. Dashed line indicates optional additional reagent channels.

aspiration mode. In order to reduce adsorption/desorption phenomena at the gas inlet the gas samples (calibration gases and atmospheric samples) were introduced via the shortest practical length of PTFE tubing. The inner diameter of all gas-carrying tubes was 1.5 mm.

A multichannel variable speed peristaltic pump (Type IPS-8, Ismatec, Zürich) was used for liquid propelling of carrier and reagent streams. Common all-PTFE rotary switching and injection valves (Besta, Heidelberg) were employed. A motor driven syringe pump (Braun, Melsungen) was used for stepwise liquid transportation (vide infra). All interconnections of the liquid part of the flow system were made from PTFE tubing (0.5 and 0.7 mm i.d.). The gas-diffusion cell used for the determination of aqueous ammonium was identical to the large size cell used in previous work [23].

The spectrophotometric detector was a Model 5023 variable wavelength photometer (Tecator, Höganäs) furnished with a 10 mm path-length, 18 μl volume, flow-through cuvette. A tubular liquid-membrane ammonium selective electrode [24] was used for potentiometric detection of ammonium. Potential measurements were made with a purpose-made high impedance differential amplifier. The detectors were interfaced to a strip-chart recorder (Type L 6512, Linseis, Selb) and signals were evaluated manually.

2.2. Construction of the permeation denuder

Two basically different configurations have been used. The first one is similar to that described previously [9] and is of tube-in-tube design (see Fig. 2). The outer jacket of the tubular permeation denuder was made from 5 or 40 cm long PTFE tubes with 3.0 and 5.5 mm inner and outer diameter, respectively. To the ends of these tubes perspex blocks were fixed which were machined to accept all necessary connections (see inset in Fig. 2). Microporous polypropylene tubing of 2.6 mm o.d., 1.8 mm i.d., 0.2 μm pore size

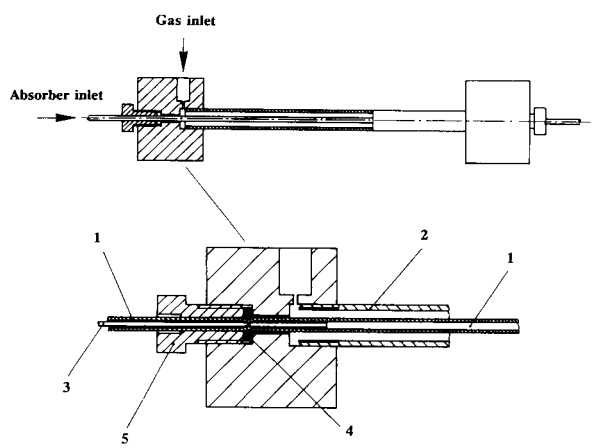

Fig. 2. Schematic depiction of the tubular permeation denuder (one end shown). 1 = gas-permeable membrane; 2 = PTFE jacket; 3 = PTFE interconnection tubing; 4 = Viton O-ring; 5 = male nut.

and 70% porosity (Accurel PP, Typ S6/2, Enka, Wuppertal) was inserted into the jacket leaving approx. 4 cm protrude at each side. PTFE interconnection tubing of 1.6 mm o.d., 0.7 mm i.d. was pushed into the microporous tubing from both sides. Upon fixation of the male fitting the O-ring squeezes the tubes together so that a gas and liquid tight connection is achieved. In order to straighten the microporous tubing inside the jacket and get a uniform annulus it is advisable first to fix one side and while carefully pulling the other end of the microporous tube to fix the other male nut, too.

This way of construction offers considerable advantages over previously used procedures found in the literature. No gluing of the microporous tubing is required which makes membrane change a simple and quick task. More important, however, is that the effective length of the permeation denuder (given by the contact zone between gaseous sample and liquid absorber solution) can be conveniently altered. Though the maximum length is, of course, fixed by the geometrical dimensions of the outer jacket, moving the incoming PTFE interconnecting tubes further inside results in progressively shorter permeation denuder tubes.

The second configuration is a planar gas sampling device with flat membranes similar to that commonly applied in gas-diffusion FIA where dissolved gases are separated by membrane transfer from a donor to an acceptor stream [22–25]. The actual version used here consists of two perspex blocks with engraved channels that are mirror images of each other. The length and width of the two channels was 70 and 3 mm, respectively. The depth, however, was made different (i.e., 4 and 0.5 mm for the gas and liquid carrying channel, respectively) to prevent pressure built up in the gas channel when high gas flow rates are applied. In order to minimize the occurrence of turbulences in the gas stream the entrance and exit bores were arranged at a low angle (approx. 30°) relative to the membrane. This measure was also thought to reduce potential particle impaction. The two halves are separated by flat gas-permeable membranes which also act as the gasket. The actually used membranes are specified under Results and Discussion. Clamps are used to hold the two blocks together.

2.3. Reagents and solutions

Reagents used were of analytical grade quality. Solutions were made up with bi-distilled water from a quartz still. Ammonium standards were made from a 0.1 M ammonium chloride solution by appropriate dilution. Due to the high contamination risk from ambient ammonia, low level standards were prepared under a hood which was continuously flushed with purified air. The standards were generally used immediately after preparation.

The indicator stock solution was made by dissolving 0.5 g bromocresol purple (Merck) in 100 ml of water. To be used as the colour reagent in gas-diffusion FIA [25] the stock was diluted 1:50 (v/v) with water and the pH was adjusted to the desired value (vide infra) by dropwise addition of 10^{-3} M sodium hydroxide.

The buffer solution used in the potentiometric detection method of ammonium was 0.05 M tris(hydroxymethyl)aminomethane adjusted to pH 7.5 with hydrochloric acid [24].

A stock solution of sulfite (1 g/l SO_2) was prepared by dissolving 1.97 g anhydrous sodium sulfite in 1 l of water previously degassed with nitrogen. This solution was standardized iodometrically but was shown to contain the desired concentration and was stable within experimental error for at least 1 month. In order to take account of the low stability of diluted aqueous sulfite solutions [26,27] the working standards were made up with 1 mM ethylenediaminetetraacetic acid which was found to be a reasonable stabilizing agent that does not interfere in the common spectrophotometric detection methods [28,29].

The composition of the reagent solution used for spectrophotometric determination of sulfur dioxide was 0.033 g/l pararosaniline (*p*,*p*′,*p*″-triaminotriphenylmethane, chloride salt; Sigma) in 0.4 M hydrochloric acid containing 0.37% formaldehyde. This reagent solution, though not optimized for maximum sensitivity of the sulfite

assay, is stable and exhibits reproducible response for several days. Absorber solutions used for impinger sampling of ammonia and sulfur dioxide are specified under Results and Discussion.

2.4. Generation of calibration gases

Sulfur dioxide in synthetic air with a certified concentration of 69.0 ppm (v/v) (Messer Griesheim, Frankfurt/Main) was diluted to the desired concentration with purified air by means of a commercial dynamic dilution system (Model SGGU-62, Horiba, Langenfeld). This instrument permits to set dilution factors in the two ranges 25–200 and 200–1000 with high precision and accuracy. Within each range further dilution of up to a factor of 5 can be applied. The actual gas concentrations at the outlet were periodically checked by conventional impinger sampling and subsequent analysis with the standard reference method [30].

Ammonia in nitrogen with concentrations of 21.4 and 300 ppm (v/v) (AGA Edelgas, Düsseldorf) was diluted with a laboratory made dynamic dilution system described in detail elsewhere [31]. Briefly, the system belongs to the family of gas stream mixing devices and relies on the well-defined and theoretically accessible gas-flow through a capillary to which a constant pressure is applied [32,33]. Adjustment of the actual gas-flow can be precisely regulated by changing the pressure and/or using capillaries of variable length and diameter [33,34]. The gas leaving the capillary is admixed with a carrier stream of purified air. Since in our work the absolute gas-flow through the capillary was at least 2 orders of magnitude smaller than the carrier flow the latter was taken as the total flow rate without introducing significant errors. The ammonia concentration leaving the dynamic dilution system was experimentally determined by gas collection in midget impingers and subsequent analysis of the liquid phase for ammonium. Gas-diffusion FIA with spectrophotometric detection [23] was employed as the method of choice because of its inherently high sensitivity and precision.

3. Results and discussion

3.1. Determination of collection efficiency. Effect of experimental parameters

The collection efficiency of the permeation denuders used in the present work was determined by measuring the analyte concentration before and after the gas has passed the denuder and differentiating the results. To this end standard gas mixtures by means of an air pump were aspirated through fritted impingers filled with appropriate absorber solutions which were subsequently analysed for the species of interest by common colorimetric methods. In order to cross-check the results the liquid absorber stream flowing through the permeation denuder was also analysed. This procedure permitted to balance input and output concentrations and thus gave more reliable data.

The influence of gas concentration, gas flow rate, composition and flow rate of the absorber solution and kind of membrane on the collection efficiency was investigated for both, the tubular and the planar permeation denuder. The effect of variable length of the tubular denuder on the collection efficiency was also studied.

In Fig. 3 the collection efficiency of ammonia as obtained with the three different permeation denuders is plotted as a function of the gas flow

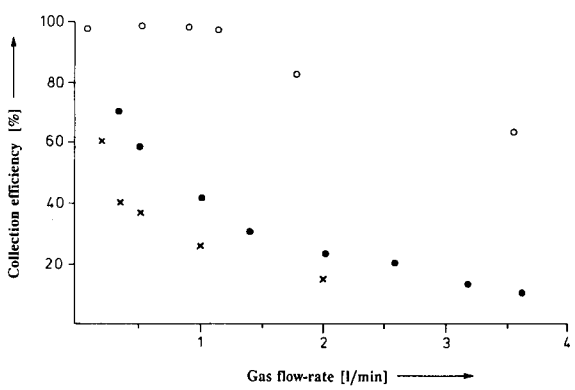

Fig. 3. Collection efficiency of gaseous ammonia as a function of gas flow rate for various permeation denuders. For experimental details see text. ○ = 40-cm tubular denuder; ● = 5-cm tubular denuder; × = planar denuder.

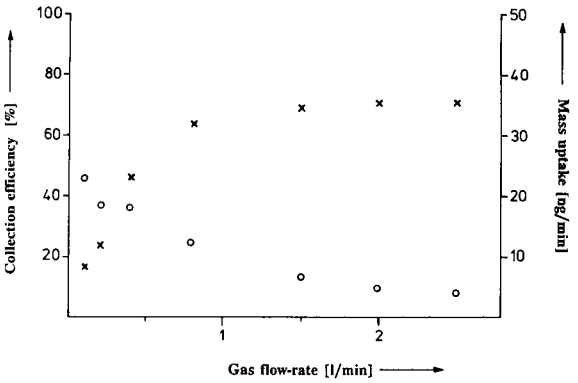

Fig. 4. Collection efficiency (○) and mass uptake (×) of SO_2 as a function of gas flow rate (planar denuder). For experimental details see text.

rate at a NH_3 concentration of 210 $\mu g/m^3$. The absorber stream used was 0.01 M sulfuric acid which was previously shown to behave as a perfect sink for ammonia [31]. As expected lower collection efficiency is achieved at higher gas flow rates and shorter denuder length. The experimental results obtained for the two tubular permeation denuders are in reasonable agreement with theoretical calculations based on a modified equation derived for annular denuders [31,35]. Within experimental error identical results were achieved at ammonia concentrations covering the range 0.005–1 mg/m^3. Lower gas concentrations could not be investigated because of insurmountable problems with blank values introduced by impinger sampling of the influent and effluent ammonia.

The results of the influence of gas flow rate on collection efficiency obtained for the planar unit are depicted in Fig. 4 using SO_2 as test gas. The concentration of SO_2 was 178 $\mu g/m^3$ and a 5% hydrogen peroxide solution was used as absorber. As was shown recently [14] and will be discussed below hydrogen peroxide is an efficient absorber which creates a reasonable sink for sulfur dioxide. Influent and effluent SO_2 was determined using pararosaniline method following gas collection in formaldehyde solution [36]. The concentration of sulfate in the outflowing absorber stream (resulting from oxidation of sulfite by hydrogen peroxide) was determined by high-speed ion chromatography [37].

As for ammonia the collection efficiency decreases with increasing gas flow rate. Closer inspection of the data, however, reveals some inconsistency of the expected exponential decay at flow rates in the range 0.2–0.5 l/min. Since this result was highly reproducible it is believed that the laminar flow conditions established at low gas flow rates turn to turbular flow in this region. Further investigations are in progress and will be reported in due time.

Also shown in Fig. 4 is the mass uptake per time unit of the planar permeation denuder which is simply calculated from collection efficiency, gas flow rate and analyte concentration. It is evident that above a certain flow rate the mass uptake becomes virtually invariant and accordingly the sensitivity of permeation denuder systems can not be further improved in using higher gas flow rates. A similar observation has been made by Dasgupta et al. [14] for tubular denuders.

From a practical point of view this result is interesting in so far as gas flow rate fluctuations in the plateau region do not effect reproducibility of gas sampling. Thus, simple and cheap air pumps can be applied for sample aspiration. Results obtained at SO_2 concentrations covering the range 0.01–20 ppm (v/v) exhibited almost identical behaviour.

The collection of SO_2 was also used to study possible effects of the flow rate and composition of the absorber solution. In initial tests hydrogen peroxide solution has been used as absorber liquid because it was used in previous work [7,13,38] and is believed to behave as a reasonable sink for sulfur dioxide. However, other absorber solutions have to be used if detection methods for sulfite rather than sulfate are employed. For that reason different absorber solutions were selected which were expected not to prevent or to interfere in the determination of trapped sulfite in the common spectrophotometric methods [28–30]. The results of this study summarized in Table 1 reveal some interesting facts. The highest collection efficiency is obtained with aqueous formaldehyde solution. This is probably due to the formation of stable hydroxymethanesulfonic acid which makes

a perfect sink for SO_2. Hydrogen peroxide similarly 'catches' SO_2 by conversion to sulfate but at a slightly slower rate. Acidic acceptor solutions are unfavourable because of the equilibrium reaction between hydrogen sulfite and dissolved sulfur dioxide. The presence of pararosaniline and formaldehyde in acidic solution (which have been included in this investigation because this mixture is used as colorimetric reagent for sulfite in the reference method [30]) has only marginal positive effect. This can be explained by the unfavourably slow kinetics of this reaction [39].

The flow rate of the absorber stream was also changed to examine possible saturation effects which have been supposed by others [13,40]. Using the formaldehyde absorber no effect was observed in the flow rate range studied, i.e., 0.1–3 ml/min. However, with the 0.1 M sulfuric acid absorber solutions the collection efficiency further decreases with decreasing flow rate (reaching only 5% collection efficiency at 0.2 ml/min) which is obviously due to the unfavourable equilibrium reaction.

As far as analytical applications of gas-diffusion separation [22–25] and membrane based gas collection [9–15] is concerned the role of the membrane material and its structure on gas transfer has apparently been a subject of little concern. It seems that in many instances the selection of a particular membrane ensues from accidental availability rather than rational considerations.

Tubular microporous membranes of reasonable size for construction of permeation denuders are rare. Few companies produce such membrane tubing and it is sometimes difficult to purchase the small amount required for analytical applications. The polypropylene membrane tubing used by us in many applications [8–10,28,31,34] is well characterized and offers some favourable characteristics, i.e., high porosity, high water entrance pressure and good mechanical strength, but appears of limited suitability with respect to thickness and available geometrical dimensions.

Flat gas-permeable membranes are commercially available in great variability as far as base material, thickness, porosity and pore size are concerned. Homogeneous silicon is well-known for its high oxygen permeability and was among the first materials applied in analytical separation of gaseous compounds (e.g., [41]). This material is further characterized by chemical inertness and mechanical strength (though stretchable it is difficult to tear). The advent of microporous hydrophobic membranes has replaced silicon membranes in many analytical applications involving gas separation since it is believed that these materials offer improved gas-transfer and are applicable to a wider range of gases. To the best of our knowledge neither of these qualities has yet been systematically investigated. To our experience [42] at least the generally higher permeability of microporous membranes compared to silicon membranes is more than questionable. Membrane parameters (i.e., thickness, porosity, pore size and way of preparation) as well as experimental conditions play a crucial role and different gases may exhibit sometimes higher and lower permeability when microporous and silicon membranes, respectively, are compared.

The collection efficiency of SO_2 with various membrane materials was investigated using the planar permeation denuder. The membrane characteristics and the results of this study are collected in Table 2. Though these data are surely insufficient to draw final conclusions the data reveal preference for thin microporous membranes of high porosity which is in agreement with earlier findings [25]. In practical applica-

Table 1
Dependence of collection efficiency on the composition of the absorber solution (a planar permeation denuder was used furnished with an Accurel polypropylene membrane. Gas flow rate, 0.8 l/min, liquid flow rate, 0.5 ml/min)

Composition of the absorber solution	Collection efficiency (%)
Bidistilled water	19
5% Hydrogen peroxide	23
1 mM EDTA	21
0.01 M sulfuric acid	11
0.1 M sulfuric acid	8
1% formaldehyde	28
7% formaldehyde	31
Premixed colour reagent [a]	14

[a] The colour reagent constitutes 33 mg/l pararosaniline and 0.37% formaldehyde in 0.4 M hydrochloric acid.

Table 2
Collection efficiency of SO_2 for various membrane materials in permeation denuder gas-sampling (flat membranes were employed in the planar unit. Gas flow rate, 0.8 l/min; flow rate of the hydrogen peroxide stream was 0.5 ml/min)

Base material	Type of membrane	Porosity (%)	Pore size (%)	Thickness (%)	Collection efficiency (%)
Polypropylene	Accurel 1E PP	80	0.2	80	11
	Celgard 2500	45	0.075	100	6
PTFE	Plumber tape	n.s.	n.s.	40–60 [a]	14
	Tecator 5589-001	n.s.	n.s.	30–40 [a]	16
	Gore GSC TB 8098	70–75	0.45	76	11
Poly(vinylidene) difluoride	Millipore GVHP 09050	70–80	0.2	125	9
Silicon	Bran + Luebbe 178-3724-01	none [b]	none [b]	80–100 [a]	8

n.s. = Not specified by the manufacturer.
[a] Evaluated by microscopic inspection.
[b] Homogeneous material.

tions, however, the ruggedness is an important aspect and must be weighed out against collection efficiency considerations. The silicon membrane is shown to offer only reasonable permeability for SO_2 but offers the striking advantage that, in contrast to microporous membranes through which water penetrates at high differential pressure, the seepage of water is impossible. Moreover, silicon membranes are resistant to many organic solvents which is particularly interesting in the collection of organic compounds and subsequent determination by gas or liquid chromatography [43]. Nonetheless, the Accurel polypropylene membrane was used in the remainder of this work.

3.2. Operational procedures of permeation denuder coupled to continuous flow and flow-injection systems

In early work on diffusion scrubbers (synonymous for permeation denuder) Dasgupta [11] stated that "the true potential of the diffusion scrubber is realized only if the scrubber effluent is used as the input sample stream in a microscale continuous flow analysis system". In the simplest configuration (see Fig. 1A) this can be achieved by propelling a reagent solution through the permeation denuder at a reasonable rate and monitor the reaction product with a downstream located detector. A limitation of this approach can be seen in the fact that compromises have occasionally to be made in finding absorber solutions that act as a reasonable sink for the analyte gas of interest and at the same time are optimal as far as selective and sensitive detection is concerned. The application of merging stream manifolds where one or more reagents are added to the effluent offer much higher flexibility, yet sacrifying sensitivity (due to dilution) and response time.

Effluent solutions can also be used to feed the injection loop of chromatographic systems which prevents true continuous monitoring but permits simultaneous determination of several compounds [9,13].

The use of permeation denuder sampling coupled to FIA systems has only recently been recognized to be of great potential [8–10,15,38,44,45]. The most striking feature is the high flexibility with respect to instrumental links and detection schemes involved. The in-line concentration ability has attracted particular attention [8–10,15,45].

Preconcentration manifolds are simply arranged using the configuration schematically depicted in Fig. 5A. The switching valve is used to direct in one position the absorber solution through the denuder towards the detector and to let, in the other position, the absorber stream by-passing the denuder while the absorber solution in the denuder is arrested. In both positions of the valve the sample gas is trapped in the absorber solution. As with continuous flow sys-

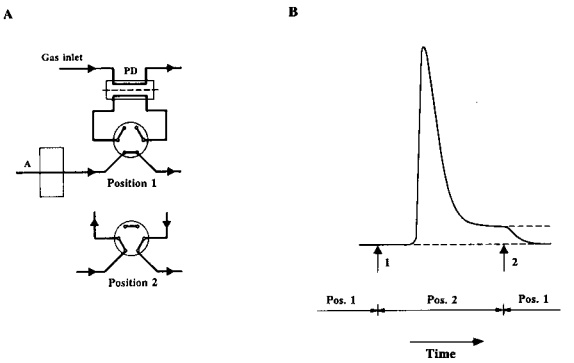

Fig. 5. Principle of in-valve preconcentration. (A) Depiction of the valve positions and liquid pathways during preconcentration (position 1) and elution (position 2). A = absorber solution; PD = permeation denuder. (B) Typical recorder trace as obtained with in-valve preconcentration manifold (see Fig. 1B). Arrows 1 and 2 indicate the moment of switching the valve to positions 2 and 1, respectively. The plateau region after the transient signal is a result of the steady gas diffusion into the continuously flowing absorber stream.

tems a continuous signal arises when the absorber solution runs through the permeation denuder. Upon switching the valve preconcentration of the gaseous analyte occurs and at the same time the detector signal changes to the blank value created by the absorber solution itself. Switching back the valve after appropriate time of preconcentration to the initial position makes fresh absorber solution to push out the arrested volume. As a result a transient signal is obtained which returns to the continuous flow permeation level initially recorded. A typical recorder trace of a preconcentration/elution cycle is shown in Fig. 5B. In Fig. 6 recordings are shown as obtained in sampling and preconcentration of gaseous ammonia using gas-diffusion FIA [23] for detection of trapped ammonium (vide infra). The figure displays the linear relation between absorbance and both preconcentration time and gas-phase analyte concentration. It is also evident that the continuous flow permeation levels of the two experiments differ. At given analyte concentration the same level is always reached after the transient signal irrespective of preconcentration time set whereas for varying analyte concentration the level jointly increases with increasing peak height of the transient signal.

Fig. 6 also impressively indicates the sensitivity enhancement achieved with preconcentration. In this particular experiment for a preconcentration period of only 1 min almost seven-fold higher signals are obtained. In general, the sensitivity enhancement is affected by all the factors governing mass transfer in the permeation denuder, the liquid volume of the absorber solution present in the denuder and the dispersion of the liquid element introduced into the FIA system [22].

3.3. Calibration modes of permeation denuder sampling devices

As with any instrumental method of analysis PD-FIA requires some means of calibration in order to relate the detector signal to gas-phase analyte concentration. In view of the many experimental variables involved it appears likely to be the best to use calibration gases run through the permeation denuder at regular intervals. In fact, this has been done in almost all applications published so far. The advantage of this method is that neither the collection efficiency of the permeation denuder nor the variables effecting sample dispersion and reaction kinetics have really to be known as long as they are reproducible for desired length of denuder operation.

Gas-phase calibration, however, generally is a tedious procedure, requires considerable experi-

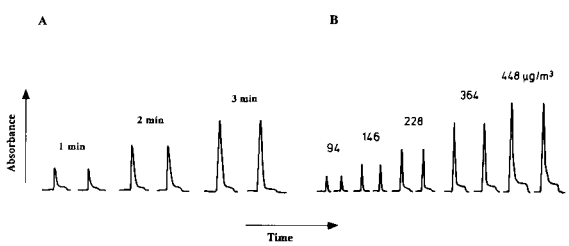

Fig. 6. Recorder trace for determination of ammonia using the preconcentration manifold shown in Fig. 5A. The 5-cm tubular denuder was use. Gas flow rate, 1.5 l/min; liquid flow rate, 0.7 ml/min; absorber solution 0.01 M H_2SO_4. (A) Variation of preconcentration time. (B) Change of analyte concentration. Duplicate analysis is shown in all cases.

ence and is almost impossible to be performed in the field. Therefore, it would be desirable to solely apply aqueous standards for calibration of the detection system. Attempts to achieve this goal led us to examine whether the collection efficiency of a particular denuder once determined under given experimental conditions remains constant [31]. A long-term study was undertaken with the 5-cm permeation denuder using ammonia as a test gas at concentrations in the range 0.1–5 mg/m^3. Collection efficiency was determined over a period of about 6 months on a weekly basis. Between these measurements the denuder was frequently applied to real sample analysis, i.e., evaluation of indoor and atmospheric ammonia concentrations. The surprising result was, that within experimental error (3–5% R.S.D.) no alteration has been observed. It is noteworthy, however, that the permeation denuder has not been dismantled during that time and hence the same piece of microporous tubing was used. Exchange for another piece of the same kind of tubing lead to variations of the collection efficiency of more than 15% deviation from the mean value in unfavourable cases. In conclusion this means that a permeation denuder once prepared and characterized in the laboratory may be applied for extended periods of time without the need for gas-phase recalibration.

In order to perform adequate liquid-phase calibrations of PD-FIA systems some general criteria must be fulfilled:

(i) the component that results from trapping of analyte gas must be known and present in a defined chemical form;

(ii) it must be possible to prepare stable standard solutions of this particular compound in the desired concentration range;

(iii) the reaction conditions for the trapped analyte and the aqueous standard must be identical with respect to composition of used reagents, mixing conditions and kinetic aspects;

(iv) dispersion of the inner volume of the denuder introduced into the flow-injection system must be identical to that of the aqueous standards.

Criteria I and II can simply be met for many gaseous constituents since they are either only dissolved in the liquid absorber phase or undergo stoichiometric reactions resulting in definite species. A relevant exception is given, however, in the determination of nitrogen dioxide where nitrite and nitrate are formed in typical absorber solutions and the relation of the two compounds might be variable [34].

In order to fulfil criterion III the aqueous standards can be prepared in the absorber solution and continuously propelled through the flow channel that passes the permeation denuder. Upon downstream merging with reagents the mixing conditions and reaction times would be identical to those in sample analysis. Problematic situations arise, however, when the reagent solution itself is used as trapping agent and the kinetics of the reaction are slow. This, for instance, is given in the photometric reaction between sulfite and pararosaniline–formaldehyde reagent.

The last criterion only plays a role if the denuder is coupled to FIA in the configuration shown in Fig. 1B since in continuous flow systems sample dispersion does not occur. The determination of dispersion in common FIA systems, though a rather trivial task, is generally not required since standards and samples are treated in an identical and reproducible manner. However, when the preconcentration set-up depicted in Fig. 1B is used difficulties arise from the fact that the void volume of the entire injection loop is larger than the liquid inner volume of the denuder and hence dispersion would not be the same for injection of aqueous standards and trapped analyte solutions.

A solution to this problem is to reconfigurate the FIA system as depicted in Fig. 1C. Here the denuder is located in the entrance line of the FIA valve rather than in the loop. The absorber solution is placed in a syringe pump which operates either in continuous flow or stop-and-go mode. Upon activation of the syringe pump fresh absorber solution is introduced into the permeation denuder and flows through the loop of the FIA injection valve. Successive injections into the FIA system result in a series of transient signals the envelop of which reflects the actual change of gas-phase analyte concentration (see Fig. 7).

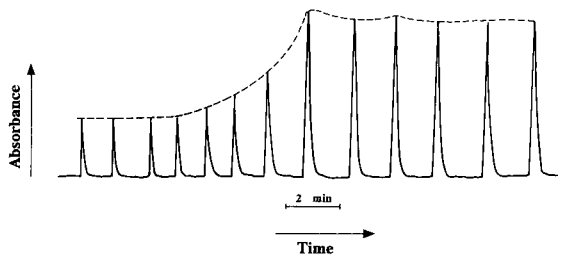

Fig. 7. Continuous monitoring of ammonia by successive injection of the denuder effluent into the gas-diffusion FIA system. Manifold configuration was as shown in Fig. 1C. During the measuring period the gas standard concentration has been changed from 52 $\mu g/m^3$ to 138 $\mu g/m^3$. Experimental conditions were as specified in Fig. 6.

Aqueous standards can be introduced at will for calibration and recalibration.

Preconcentration of gaseous constituents in the absorber liquid is achieved by stopping the syringe pump for any desired length of time. Thereafter the syringe pump is initialized and a heart-cut of the denuder effluent is injected into the FIA system. To do so, precise control of the syringe feed is required and the inner liquid volume of the permeation denuder must be considerably higher than the loop volume of the injection valve.

In practical applications we have used this mode of operation with and without preconcentration with great success. It is highly flexible with respect to the analytical detection system connected, easy to handle, permits frequent recalibration with minimal expense and allows to discern between response changes and detector drift due to the intermittent reading of baseline and analyte signal.

3.4. Analytical systems for determination of atmospheric ammonia

Considerable interest exists in the evaluation of atmospheric levels of ammonia since it plays a prominent role in atmospheric neutralization of acidic gases and elevated levels are known to exhibit adverse effects on the environment [46,47]. From an analytical point of view discrimination of gaseous ammonia and ammonium salts present in suspended matter is a challenging task. Diffusive sampling by means of passive samplers [48,49], diffusion denuders [5,50,51] and permeation denuders [31,52] is probably the most suitable approach. The main advantage of the application of permeation denuders over the two other methods is their continuous monitoring capability.

In the following PD-FIA systems for determination of atmospheric ammonia are described utilizing potentiometric and spectrophotometric detection of trapped ammonium. The basic arrangement of the PD-FIA system was as in Fig. 1B and gas-phase calibration was used. A brief discussion of the performance characteristics of the two methods is presented and the suitability for the intended purpose is discussed. All measurements were made with the 5 cm long tubular denuder.

In the potentiometric detection method the tubular ion-selective electrode was connected with the shortest practical length of tubing to the outlet channel of the switching valve. The Tris–HCl buffer was propelled continuously through the system at a rate of 0.8 ml/min. This flow rate is a good compromise with respect to reagent consumption, wash-out time of the permeation denuder and response behaviour of the liquid membrane electrode [24]. Gas-phase calibration was conducted by aspiration of ammonia standards in the range 0.01 and 4.8 mg/m^3 at a gas flow rate of 2 l/min. The preconcentration time was set to 1 min. A typical recorder trace is shown in Fig. 8. It evidences the potential applicability of the method in the given concentration range. Lower concentrations are accessible at

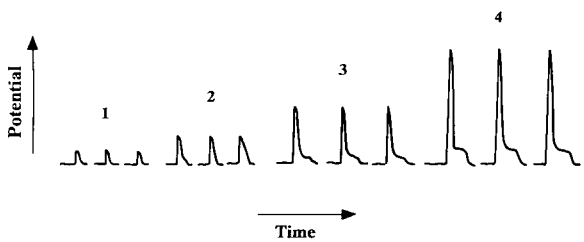

Fig. 8. Recorder trace for potentiometric detection of ammonia in PD-FIA. Numbers 1–4 refer to ammonia concentrations of 0.04, 0.23, 0.85 and 1.74 mg/m^3, respectively. Triplicate measurements are shown at each level. Experimental details are given in the text.

prolonged preconcentration times. The figure also displays the reproducible, yet non-linear (and non-Nernstian) response of the electrode to changing analyte concentrations. The precision of repetitive measurements was generally in the range of a few mV which translates into a relatively high R.S.D. of 7–25% because of the low slope of the calibration plot in this concentration range. This behaviour is typical for the liquid-membrane ammonium selective electrode at low analyte concentrations [24] and remains to be unfavourable in practical applications. Another serious problem with the potentiometric detection method was the high within-day and day-to-day response variability of the electrode to aqueous ammonium standards in the low concentration range (5–100 μg/l) which made frequent recalibration indispensable.

Application of gas-diffusion FIA to the determination of trapped ammonium was considered to be an attractive alternative since the detection limits reported are extremely low, linear calibration plots through the origin are obtained and long-term stability is given [23]. The higher complexity of the flow system (e.g., two-stage gas transfer is involved) was initially thought to be problematic but proved to be highly reliable and of suitable practicability for routine use. The detectability of ammonium using the gas-diffusion method with colorimetric pH detection critically depends on the design of the gas-diffusion unit, the flow rates of donor and acceptor stream and the composition of the indicator solution [23]. With respect to the latter the choice of the indicator substance and the adjustment of solution pH is of utmost importance [53].

In the optimized system the gas-diffusion unit with high membrane area to liquid channel volume used in previous work [23] was applied. The flow rates of the donor and acceptor stream were set to 0.6 and 0.2 ml/min which is a reasonable compromise with respect to sensitivity and sample residence time. The pH of the indicator solution was adjusted spectrophotometrically to give 0.7 absorbance units at maximum wavelength of 590 nm against water using a 1-cm cell.

The performance of the system was evaluated using gas-phase calibration in the range 1.2–150 μg/m^3 ammonia at a gas flow rate of 2 l/min. Linear response curves were obtained at varying preconcentration times in the range 1–5 min. At higher gas concentrations and longer preconcentration times the linear range of the ammonium detection method was exceeded. The precision of repetitive measurements was generally in the range 2–5% R.S.D.. Constant slope values of the calibration plots at varying preconcentration time could be maintained for many hours of operation of the PD-FIA system. However, after renewal of pump tubes and reagent solutions or insertion of a new membrane another, yet again stable response was generally observed.

The detectability of the proposed system can be gathered from Fig. 9. Signals shown for a gas concentration of 0.7 μg/m^3 at 1 and 2 min preconcentration time suggest detection limits in the lower ng/m^3 range. Attempts to actually measure such low levels, however, failed which is believed to be due to irreversible adsorption of ammonia traces in the gas-carrying flow lines of the gas generation system and/or the permeation denuder inlet.

The applicability of the system was evaluated in monitoring ambient ammonia in the vicinity of a piggery over a period of several days. Initially, the collection efficiency of the permeation denuder used was determined and the system was calibrated in the laboratory using gas standards. Upon installation and running preliminary tests it became apparent, that the ammonium level pre-

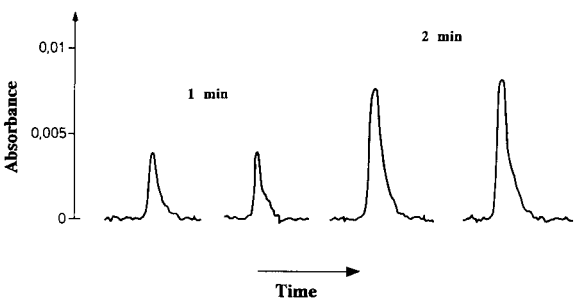

Fig. 9. High-sensitivity detection of ammonia with preconcentration. Gas-diffusion FIA was used to detect trapped ammonium (for details see text). The recording shows duplicate analysis of 0.7 μg/m^3 NH_3 with 1 and 2 min preconcentration.

sent at the measuring site was too high for the system to be operated in the preconcentration mode. Instead, the absorber solution was pumped continuously through the permeation denuder at a rate of 0.2 ml/min and the effluent was used to feed the loop of the gas-diffusion FIA system (configuration as in Fig. 1C). Successive injections were made at a rate of 10–20 h^{-1} with occasional introduction of aqueous standards. In Fig. 10 a section of the recorder trace is shown evidencing the principal applicability of the method. In parallel to the monitoring system, samples were analysed in the conventional manner so far applied, i.e., impinger sampling and determination of trapped ammonium by the standard colorimetric method [54]. Since this standard method can only provide integrative information adequate comparison of the data was of limited utility but the benefits of permeation denuder sampling with respect to performance characteristics and practicability became impressively apparent.

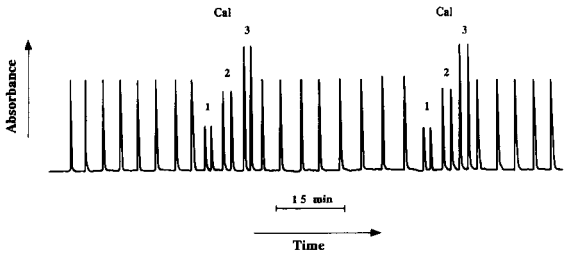

Fig. 10. Section of an original recorder trace of ambient ammonia monitoring. The arrangement used was as given in Fig. 1C with continuously flowing absorber solution and successive injection of the effluent into the gas-diffusion FIA manifold. The two liquid-phase calibration runs indicated by cal in the figure evidence the high stability of the system. The concentrations of the liquid standards were 50, 100 and 150 μg/l ammonium, respectively.

3.5. Analytical system for sulfur dioxide monitoring

Despite the availability of a variety of monitoring systems for ambient sulfur dioxide continued interest exists in the development of novel means that either offer improved performance or are simpler and cheaper to use. In particular the continuous determination of ambient levels of sulfur dioxide is a subject of considerable concern.

Denuders proved to be an affordable approach and when interfaced to flow systems permit monitoring with high sensitivity and selectivity [12–14,38]. The outstanding features attainable have recently been impressively documented by Simon and Dasgupta [55] who were able to detect as little as 500 parts per quadrillion of SO_2 by coupling a wet effluent denuder to ion chromatography.

Our attempts in developing a PD-FIA system were directed towards ready-to-use low cost instrumentation which permits reliable SO_2 detection at concentration levels present in burden atmospheres. The PD-FIA system was configurated as shown in Fig. 1B and furnished with the planar permeation denuder. In initial experiments a premixed pararosaniline–formaldehyde reagent stream was used as absorber solution in a single-line flow system. The advantage of the simplicity of this system was, however, more than counterbalanced by problems related to calibration of the system and gradual deposition of pararosaniline reagent at the membrane surface leading to steadily decreasing collection efficiency. As mentioned above the kinetics of the colour reaction used are slow and detection of the reaction product under FIA conditions takes place at non-equilibrium conditions. Hence, sulfite standards premixed with colour reagent would create considerably higher detector response and useful calibration can only be achieved by gas-phase standardization.

A dual-line manifold with a formaldehyde absorber stream (which proved an ideal sink for SO_2) and downstream merging of pararosaniline was also tested but rejected soon because of the difficulties involved in finding reaction conditions where hydroxymethane sulfonic acid (HMSA) formed upon adsorption of SO_2 in formaldehyde reacts with pararosaniline. Dasgupta et al. [36] have intensively studied the underlying chemistry and proposed to first destroy HMSA in alkaline solution followed by reaction with PRA in acidic media. Though it would obviously be possible to perform these reactions in FIA it was regarded to be too complicated.

The final choice was to use 1 mM EDTA solution as the absorber stream and merging the premixed pararosaniline–formaldehyde reagent downstream. This method has been used in continuous flow and preconcentration mode. The flow rates of the absorber and reagent stream were set to 0.8 and 1.2 ml/min, respectively. The mixing coil dimension was 120 cm × 0.5 mm i.d. The accessible concentration range without preconcentration is 50–500 $\mu g/m^3$ SO_2 with a detection limit of 25 $\mu g/m^3$ SO_2.

In order to improve the detectability preconcentration of SO_2 in the recipient channel of the PD was also used. A linear increase of signal with preconcentration time is achieved permitting to adjust the system to the desired sensitivity. With a 2-min preconcentration time the accessible working range is lowered by almost an order of magnitude compared to the continuous flow configuration.

In order to evaluate the stability of this detection system SO_2 gas standards as well as sulfite standards prepared in 1 mM EDTA were introduced in regular intervals over a period of several weeks. The precision of the gas-collection efficiency of a particular PD with the same membrane in place amounts to 8% R.S.D. Liquid-phase calibrations at the beginning and end of a measuring period (usually 5–8 h continuous sample aspiration) generally gave identical results.

Monitoring of the urban atmosphere of Berlin was conducted repeatedly and results obtained show reasonable agreement with common SO_2 monitoring systems [28].

4. Conclusions

Permeation denuders interfaced to flow-injection systems are shown to be an attractive means for sampling and analysis of gases. Hydrodynamic flow conditions in the gas and liquid stream as well as gas transport across the membrane are sufficiently reproducible to operate the system under dynamic equilibrium conditions, i.e., neither collection of the gas must be complete nor needs the product of the chemical reaction(s) to be fully developed.

Preconcentration of gaseous compounds can simply be accomplished by arresting the absorber solution within the permeation denuder. Upon re-introducing the trapped sample into the flow system a transient signal occurs, the height of which can be taken as a means for quantification.

Gas-phase calibration though initially required to determine the collection efficiency of a particular denuder under given experimental conditions can be omitted later because of the high long-term stability of gas collection efficiency. Hence these systems may be conveniently recalibrated using the liquid phase of the FIA set-up.

Though intentionally developed for selectively removing gaseous species from gas–aerosol mixtures the actual interference of particles in denuder sampling has been a subject of little concern. In the proposed PD system the membrane acts as an efficient barrier for particulate matter but particle impaction on the membrane surface could be a problem. However, in the course of our investigations where denuders have been applied in real sampling situations for periods of several months no such problems have ever been observed.

In conclusion, PD-FIA offers extremely versatile operation with respect to the application of various detection schemes. High flexibility of PD-FIA is not only given in the choice of reaction and detection methods involved but the entire arsenal of separation and preconcentration methods as well as gradient techniques (just to mention a few striking features) offered by FIA can be utilized and turn permeation denuder gas sampling into an outstanding tool for gas analysis.

Acknowledgement

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Application of adsorptive cathodic stripping voltammetry for the determination of Cu, Cd, Ni and Co in atmospheric samples

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Abstract

The application of adsorptive cathodic stripping voltammetry (ACSV) for the determination of Cu, Cd, Ni and Co in acid digested atmospheric samples was carried out. The accuracy of the technique was evaluated and compared with GFAAS (graphite furnace atomic absorption spectrometry) and ICP-MS (inductively coupled plasma mass spectrometry) using a standard reference material (MESS-1). All the considered techniques had the required accuracy. Detection limits were lowest for Cu and Ni using ACSV (0.30 and 0.08 ng ml^{-1} for a 10 × diluted sample and a 1 min collection, respectively) whereas for Co detection limits were lowest by direct determination using ICP-MS analysis (0.03 ng ml^{-1}). GFAAS had the lowest limit of detection for Cd (0.3 ng ml^{-1}). Cd was not determined by ICP-MS in both MESS-1 and aerosol digests due to a higher detection limit. For all considered metals determined in low volume aerosol there were no statistical differences between all three techniques. In addition, there was no observable difference in the metal concentrations in the reference material during ACSV analysis of samples before and after UV irradiation. However a significant difference was observed between ACSV analysis before and after UV irradiation for Cu and Cd in aerosol acid digest samples but no such difference was detected for Ni. This would indicate significant dissolved organic component interference by competitive complexation of Cu and Cd in the aerosol digest samples.

Key words: Atomic absorption spectrometry; Inductively coupled plasma mass spectrometry; Stripping voltammetry; Aerosols; Atmospheric samples; Cadmium; Cobalt; Copper; Nickel

1. Introduction

The first reported polarographic measurements of metals such as Pb, Cd, Zn, Mn and Bi in atmospheric (industrial) samples were by Khlopin [1]. More recently the sensitive electroanalytical technique anodic stripping voltammetry (ASV) has been used to determine Cu, Pb, Cd and Zn, in aerosol samples collected over the North Sea using a sampler onboard an aeroplane flying at various altitudes [2]. ASV, although having very low detection limits (e.g., 10^{-11} M for Cd) in conjunction with a thin film mercury electrode (TFME), is limited to those metals which have a high solubility in mercury. Recently the development of adsorptive cathodic stripping voltammetry (ACSV) has allowed ultra-trace analysis for

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metals other than and in addition to those detectable by ASV. Detection limits have been quoted as low as sub pM for the determination of Pt in sea water by ACSV [3]. Generally, however, detection limits for a 1 min collection period are of the order of 10^{-10} M. Metals such as U, Sb, Fe, V, Ti, Ni, Co, Se, Al and Cr may be determined by ACSV. For a comprehensive review of the applications of ACSV to natural waters, particularly sea water, the reader is referred to the monograph of van den Berg [4].

The aim of the present study was to evaluate the practicalities of applying ACSV analysis to determine the concentrations of Cu, Cd, Ni and Co in a series of low volume aerosol acid digested samples. These are traditionally analysed by graphite furnace atomic absorption spectroscopy (GFAAS) or, if facilities are available, by inductively coupled plasma mass spectroscopy (ICP-MS). However, due to the high capital and running costs of these two techniques some laboratories may not have ICP-MS and GFAAS capabilities. Therefore studies were initiated to apply the documented ACSV technique [5] for the analysis of Ni and Co using DMG (dimethylglyoxime) as the complexing ligand. A similar technique [6] for Cd and Cu was applied using the complexing ligand 8-hydroxyquinoline. The ease of use, time of analysis, detection limits, accuracy and sample pretreatment were investigated and were compared with the more traditional methods of carrying out such metal analyses.

2. Experimental

2.1. Sample collection and acid digestion procedure

Low volume aerosol samples, using a CAPEX 1 (Charles Austin) pump (typical flow rate 10–30 dm^{-3} min^{-1}), were collected from the University of Central Lancashire Campus over a one year period to assess the mean atmospheric concentrations and the extent of variability on aerosol trace metal levels. This is part of an ongoing project to assess the trace metal fluxes to the marine and terrestrial environment in the North West of England. Samples were collected using acid washed Whatman 41 cellulose acetate fibrous filters. The acid washing procedure composed of a 10% HNO_3 (AnalaR, BDH) soak followed by rinsing with Milli-Q water until neutral washings were obtained, then the filters were washed in 10% HCl (AnalaR, BDH) acid followed by a rigorous rinse with Milli-Q water. Filters were then transferred to a laminar flow Class 100 cabinet where they were left to dry. Cleaned filters were stored in resealable plastic bags to await deployment.

Prior to sampling, filters were placed in a polycarbonate filter holder, all manipulations being carried out in the laminar flow cabinet, to minimise contamination, with the operator wearing disposable polythene gloves. The charged filter holders were attached to the low volume pump and sampling was carried out usually for a couple of days. After collection the filters were stored in resealable plastic bags to await analysis.

Samples then underwent a mixed acid digest. The filters were placed in a 50-ml PTFE beaker and then placed on a hot plate in a fume cupboard. Approximately 10 ml of quartz redistilled HNO_3 was added and the beakers were covered with loose fitting PTFE lids. After visual observation showed that the filter material had digested 5 ml of HF (AnalaR, BDH) was added to break down any residual material. Concentrated quartz redistilled HNO_3 was added and allowed to evaporate to a small bead of solution. The evaporation procedure was carried out three times. The resulting bead (approximately 15 μl) of digest was then transferred to an acid washed 25 ml volumetric flask and made up to volume with 0.1 M quartz redistilled HNO_3. The same procedure was carried out on 0.2 g of the standard reference material MESS-1 (supplied by the National Research Council, Canada) to assess the accuracy of the different applied analytical techniques and sample digestion methodology.

2.2. Analysis

ACSV was carried out using a Metrohm E506 polarograph in conjunction with a Metrohm 663A HMDE (hanging mercury drop electrode). For Ni and Co analyses the procedure consisted of pipet-

ting 15 ml of Milli-Q water into the glass polarographic cell to which 150 μl 1 M boric acid (AristaR, BDH) stock solution was added to give a solution pH of 8.6. The boric acid stock solution was initially found to be contaminated with Ni and Co, which were subsequently removed by repeated overnight equilibration with 4×10^{-4} M MnO_2 followed by filtration through a 0.45-μm membrane filter. Then 30 μl of 0.1 M DMG (AnalaR, BDH) stock solution was pipetted into the polarographic cell. The solution was then purged with argon to remove dissolved oxygen. Reagent blank determinations were then carried out by collecting any formed metal complex on the HMDE at -0.6 V (vs. Ag/AgCl) for 1 min. After a 10-s quiescent period a differential pulse cathodic scan was then applied at the HMDE (scan rate -15 mV/s, pulse height 40 mV). During collection a maximum HMDE size (diameter = 0.9 mm) and stirrer rate were used. Reduction of Ni and Co adsorbed at the HMDE occurred at -0.96 V and -1.09 V (vs. Ag/AgCl) respectively. Generally a current sensitivity setting of 6×10^{-9} A/mm was used.

Cu and Cd were determined as above except that the solution optimal pH was 7.7. This was achieved by adding to the Milli-Q water 150 μl of 1 M HEPES (BDH), which had previously been cleaned with 4×10^{-4} M MnO_2. To the buffered solution 30 μl of 4×10^{-3} M (solution concentration of 8×10^{-6} M) 8-hydroxyquinoline (AnalaR, BDH) was added. For Cu analyses, a collection period of the Cu–8-hydroxyquinoline complex onto the HMDE of 1 min was maintained at a potential of -1.4 V (vs. Ag/AgCl). Allowing for a subsequent 30-s quiescent period, the electrode potential was then switched back to -0.2 V (vs. Ag/AgCl) followed by a 20-s period. Afterwhich a differential pulse cathodic scan commencing from -0.2 V (vs. Ag/AgCl) and terminating at -0.6 V (vs. Ag/AgCl) was applied at the HMDE. The Cu reduction potential was observed at -0.39 V (vs. Ag/AgCl). The applied scan rate was 5 mV/s and the pulse potential was 40 mV. A current sensitivity of 1.5×10^{-9} A/mm was used throughout the study.

The procedure for Cd determinations was the same as that for Cu except generally a collection period of 5 min was used at -1.4 V (vs. Ag/AgCl) followed by a 10-s quiescent period and finally a differential pulse cathodic scan commencing at -0.4 V (vs. Ag/AgCl). Commencing the cathodic scan at -0.4 V minimised the interference of the Cu reduction current. The scan rate and pulse height were the same as those used for Cu. A current sensitivity of 2.5×10^{-10} A/mm was used. The Cd reduction potential was observed at -0.58 V (vs. Ag/AgCl).

The aerosol sample or standard reference material was then pipetted into the electrochemical cell (50 μl Cu, 140 μl Ni, Co, 300 μl Cd for the standard sediment and 300 μl Cu, 1.5 ml Ni, Co, Cd for the aerosol acid digests). The collection and stripping procedure was repeated until the reduction current was constant. As the digest solution was strongly acidic 60 μl of 1 M NaOH (AristaR, BDH) per 140 μl of digest had to be added to maintain the optimum solution pH. The current response was then calibrated by two internal standard additions. All stock standards were made up in 0.1 M HCl (AristaR) from Spectrosol (BDH) metal standards (1000 mg l^{-1}).

All samples were determined before and after UV irradiation for 3 h using a 0.5-kW mercury

Table 1
GFAAS operating furnace temperatures and times (For all elements argon gas flow rates of 200 ml/min and 300 ml/min were applied during the vaporisation/ashing and cleaning stage, respectively)

Metal	Wavelength (nm)	Vaporisation temp., °C (time, s)	Ashing temp., °C (time, s)	Atomisation temp., °C (time, s)	Cleaning temp., °C
Ni	232.0	110 (20)	900 (20)	2800 (3)	2900 (3)
Co	240.7	110 (20)	800 (20)	2800 (3)	2900 (3)
Cu	324.8	110 (20)	600 (20)	2700 (3)	2800 (5)
Cd	228.2	110 (20)	450 (20)	950 (3)	2800 (2)

Table 2a
Accuracy of applied techniques (Determination of Ni, Co, Cu and Cd in MESS-1. Mean taken from 6 discrete sediment sample digests. Values in parentheses represent samples determined prior to UV irradiation)

Metal	Certified value ($\mu g\ g^{-1}$)	Observed GFAAS	Observed ACSV	Observed ICP-MS
Ni	29.5 ± 2.7	29.7 ± 2.1	29.9 ± 1.9 (29.3 ± 1.9)	29.9 ± 0.56
Co	10.8 ± 1.9	11.1 ± 1.0	11.0 ± 0.9 (10.9 ± 0.6)	11.23 ± 1.65
Cu	25.1 ± 3.8	27.2 ± 3.1	25.2 ± 1.5 (26.7 ± 1.9)	28.0 ± 2.5
Cd	0.59 ± 0.1	0.6 ± 0.2	0.62 ± 0.1 (0.60 ± 0.1)	-

discharge lamp to remove any dissolved organic compounds.

The above procedure was carried out on a number of low volume aerosol samples ($n = 9$) and the standard sediment sample MESS-1 (six discrete acid digests).

The precision of the technique was determined by replicate determination of a blank solution (containing 0.5 nM of Ni and Co, and 0.2 nM of Cu and Cd). The detection limit was calculated from 3σ of these measurements.

GFAAS aerosol and standard reference material analysis was carried out using a Phillips PU9200 system fitted with an FS90 autosampler. The operating parameters for all considered metals are presented in Table 1. All sample analyses consisted of three replicate determinations. Calibration was by a standard calibration graph (four standards ranging up to 40 mg l^{-1}). The detection limits, see Table 2b, are quoted as 3σ of replicate determination ($n = 6$) of the reagent blank solution. Accuracy was determined from the analysis of the certified sediment digest.

ICP-MS analysis was carried out using a PQ2 system (V.G. Elemental, Winsford). Sample introduction was by a de Galan nebuliser system. Sampler and skimmer cones were standard nickel cones having orifice diameters of 1.0 and 0.7 mm, respectively. Detection limits for ICP-MS analysis were determined as above, i.e., replicate ($n = 6$) determination of the acid digest blanks. Elemental mass dwell time was 320 μs

Table 2b
Instrumental detection limits (ng ml^{-1}) (Comparison of the considered techniques)

Metal	ACSV [a]	ICP-MS	GFAAS
Ni	0.08	0.84	0.4
Co	0.1	0.03	0.5
Cu	0.3	0.3	0.7
Cd	0.6	1.0	0.4

[a] Detection limits quoted for a 1.5-ml injection and a 1-min collection period.

3. Results and discussion

3.1. Accuracy and precision: a comparison

Table 2a and b illustrates the observed accuracy and instrumental detection limits for ACSV, ICP-MS and GFAAS.

The accuracy of the three analytical techniques and efficiency of the digestion procedure was assessed by comparing the observed trace metal concentrations in the standard sediment sample MESS-1 with the certified values. Table 2a illustrates the reliability of all the considered techniques producing observed values within the range of the certified concentrations for Ni, Cu and Co. By applying a pooled or two sample t-test (pooled t-test being applied to populations having the same variance) on the different analytical populations no significant statistical difference (95% confidence limits) were found between the various techniques for the metals Ni, Co and Cu. For Cd analysis ACSV (a 5-min collection was employed) and GFAAS produced observed values for MESS-1 which were not statistically different and which were comparable to the certified values. Cd in MESS-1 was not determined by ICP-MS.

Of particular interest is that there was no significant difference between Ni, Co, Cd and Cu analysed by ACSV before and after UV irradiation suggestion that there is no organic interfer-

ence for the analysis of diluted digested reference material.

Table 2b presents the detection limits for each technique, as defined in the experimental section. The ACSV limits of detection are for a ten-fold dilution on addition of the digest to the electrochemical cell (i.e., a 1.5-ml addition), therefore direct detection limits would be an order of magnitude lower than those quoted in Table 2b. However depending on the sample dissolved metal concentrations the injection volume maybe varied therefore the working detection limits will be altered accordingly. For the defined experimental conditions, comparing the individual set of technique detection limits, ACSV yielded the lower detection limits for Ni and Cu (0.08 ng ml^{-1} and 0.3 ng l^{-1}). However ICP-MS had the lowest detection limits for Co (0.03 ng ml^{-1}). GFAAS was observed to have the lowest limits of detection for Cd (0.4 ng l^{-1}) The detection limits of ACSV may be diminished further by (i) prolonging the collection period (as was effectively done for Cd analyses being increased to 5 min collection), (ii) using greater sample volume injections into the electrochemical cell (this was carried out during the low volume acid digest solution analyses) and (iii) using rapid scan forms (i.e., square wave).

The three techniques (ACSV, GFAAS, ICP-MS) were then used to determine Ni, Cu, Cd and Co in a series of low-volume aerosol samples ($n = 9$). Fig. 1 shows a typical polarogram for the determination of Ni in the low volume aerosol digest samples. Polarograms for the other considered metals were similar with accompanying reported reduction potentials being found in the experimental section. Due to the low concentrations of Co in the collected samples only four samples were detectable using ICP-MS, (only one was above the detection limits of GFAAS) hence statistical comparisons of Co determinations were not carried out. Cd determined by ICP-MS were not used in a comparison of the techniques as the observed values were close to the techniques limit of detection

In addition to the different techniques a comparison of metals determined by ACSV before and after UV irradiation was made.

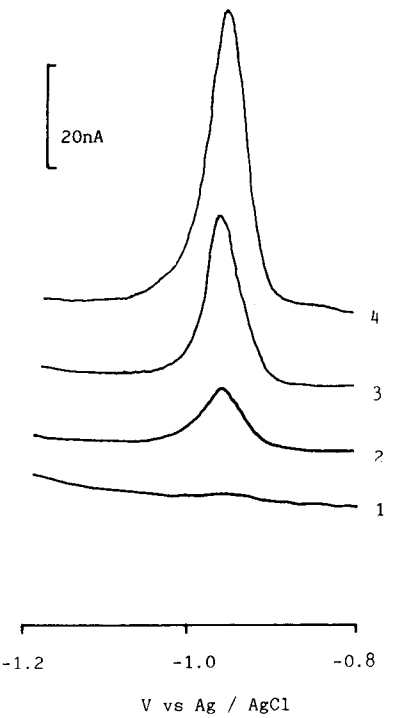

Fig. 1. Polarogram for the determination of Ni in low-volume aerosol samples. Solution conditions: 2×10^{-4} M DMG, 0.01 M boric acid, collection at -0.6 V vs. Ag/AgCl for 1 min, scan rate -15 mV s^{-1}, current sensitivity 10^{-9} A/mm. (1) Baseline scan. (2) Addition of 500 μl of low-volume aerosol digest (library sample No. PLV 19). (3) Addition of 8.5 nM Ni. (4) Further addition of 8.5 nM Ni.

All analytical sample populations for Ni, Cu and Cd analyses in the low-volume aerosol samples were compared using the paired t-test (at $P = 0.05$ with eight degrees of freedom). Comparison of Cu, Cd and Ni concentrations determined after UV irradiation by ACSV, GFAAS and ICP-MS gave calculated t values below the critical t value, indicating no statistical difference between the considered techniques. However for Cu and Cd a significant difference was observed for the none UV irradiated samples determined by ACSV with samples determined after UV irradiation by ACSV, and direct determination by GFAAS and ICP-MS. The ACSV determined Cu and Cd concentrations before UV irradiation being consistently lower, an indication of competitive complexation by residual organic components in the

acid digests. Comparable Ni determinations showed no such difference.

Plots of the determined Ni and Cu concentrations in the low volume samples using ACSV (after UV irradiation) and ICP-MS are presented in Fig. 2a and b, respectively. A corresponding plot for Cd determined by ACSV and GFAAS is also presented in Fig. 2c.

The mean Ni, Cu and Cd aerosol concentrations, calculated from the ACSV analyses (after UV irradiation) in the considered samples, were 12.8 ± 3.8 ng m^{-3} 18.9 ± 7.6 ng m^{-3} and 1.63 ± 0.4 ng m^{-3}, respectively, which are within the literature quoted range for urban levels for these elements. Reported European urban aerosol trace metal concentrations range from 3 to 100 ng m^{-3} for Ni [8], 15 ng m^{-3} for Cu [9] and 5 to 15 ng m^{-3} for Cd [8], illustrating the widely varying reported concentrations. Typical rural/coastal values would be lower generally around 5 ng m^{-3} for Ni, 6.3 ng m^{-3} for Cu and 0.6 ng m^{-3} for Cd [10].

3.2. Interferences

The major interferences encountered by ACSV are (i) competing dissolved organic components which will adsorb onto the HMDE and/or complex with the analyte, both processes diminish the sensitivity of the technique, (ii) the presence of other metals which may compete for the added organic ligand, although this interference is minimal due to a high excess of the added organic ligand and (iii) the addition of nitrate [7] as the major component of the acid digest.

The possible interference of organic ligands other than the added ligand was investigated by determining the various considered metal dissolved concentrations before and after UV irradiation of the acid digests. As already mentioned no significant difference was observed for the considered metals in MESS-1 acid digest samples although a difference was detected for Cu and Cd in the aerosol samples. This is of interest since recent studies have shown organic complexation in rainwaters for dissolved Cu and Cd (Nimmo and Fones, unpublished data). This would indicate the presence of such complexing ligands

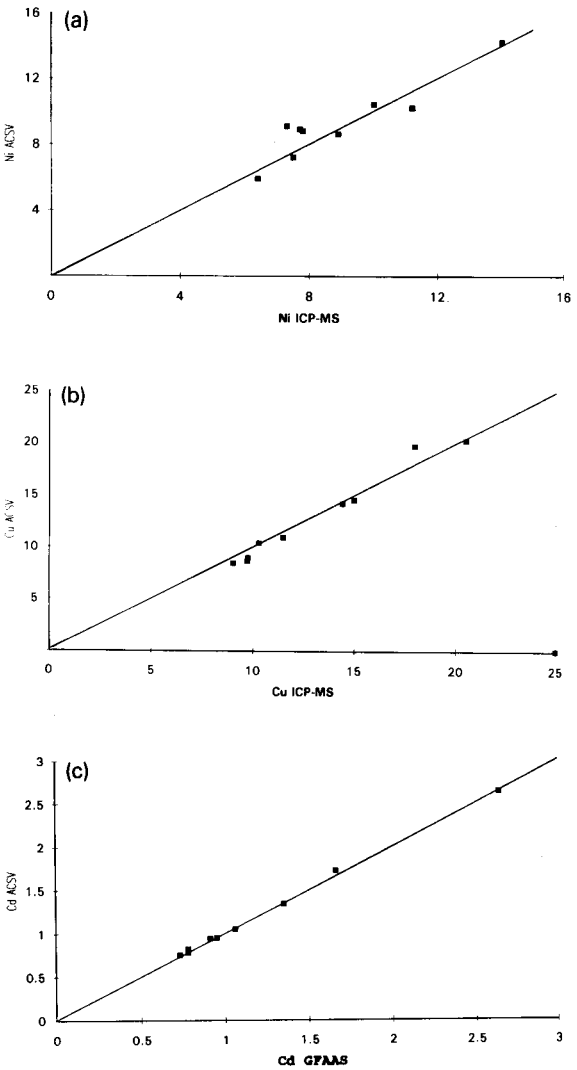

Fig. 2. (a) Plot of ACSV (UV irradiated) determined Ni (ng ml^{-1}) in low-volume aerosol digest samples and Ni determined directly by ICP-MS. (b) Plot of ACSV (UV irradiated) determined Cu (ng ml^{-1}) in low-volume aerosol digest samples and Cu determined directly by ICP-MS. (c) Plot of ACSV (UV irradiated) determined Cd (ng ml^{-1}) in low-volume aerosol digest samples and Cd determined directly by GFAAS.

associated with aerosols which may subsequently be solubilised into the rainwater. Ni and Co organic complexation in rainwaters has also been reported [11].

Other elemental interferences were investigated by adding to a Milli-Q sample containing

0.88 ng ml^{-1} Ni and 0.47 ng ml^{-1} Co, a selection of metals at concentrations which were chosen to represent the maximum levels which may be encountered in the type of atmospheric samples analysed (after a $10\times$ dilution by injection into the polarographic cell). Values were chosen from ICP-MS elemental analysis of the most concentrated aerosol acid digest sample. The following metals were added 6.6 ng ml^{-1} Cu, 1.2 ng ml^{-1} Cr, 2.85 ng ml^{-1} V, 14.6 ng ml^{-1} Zn, 4.9 ng ml^{-1} Pb and 2.9 ng ml^{-1} Mn. No change in the ACSV Ni and Co sensitivity was observed for any of the metal additions.

A similar study was carried out for Cd and Cu. The Milli-Q solution concentrations of Cu and Cd were 0.63 ng l^{-1} and 0.22 ng l^{-1} with the addition of the above mentioned metals at the same concentration. Ni and Co were also added to this solution at the following concentrations 1.7 ng l^{-1} and 0.87 ng l^{-1} respectively. With these metal additions no change in Cu and Cd sensitivity was observed.

Nitrate interference, determined by successive additions upto 2×10^{-2} M of NO_3^- to a sample spiked with Ni (0.88 ng ml^{-1}) and Co (0.47 ng ml^{-1}) did not yield any interfering reduction peak currents near the Ni and Co reduction potentials or any significant reduction in the sensitivity. Similarly no effect was observed on the Cu and Cd sensitivity with NO_3^- additions. The polarographic reduction of nitrate at the dropping mercury electrode (DME) has been reported [7], although the solution pH was much lower than that used in the present ACSV analysis (4.5 cf. 8.6). In addition the reduction current is observed only in the presence of significant concentrations of Yb (1.4×10^{-5} M) at a more cathodic potential (-1.4 V vs. SCE) than the metals considered in this study.

4. Conclusions

The present work has illustrated the practicality of applying ACSV analysis for low concentrations of Ni, Co, Cu and Cd in aerosol acid digest samples. There are several advantages of ACSV over existing techniques. (i) A polarographic system has relatively low capital and running costs. (ii) Lower instrumental detection limits than GFAAS and ICP-MS for Ni and a lower detection limit for Co compared with GFAAS, with an equivalent detection limit to ICP-MS for Cu. The ACSV limit of detection for Cd is lower than that of ICP-MS but higher than that of GFAAS (although with a 5-min collection period a lower detection limit is achieved)

However, the disadvantages of ACSV for this type of application may be summarised as follows: (i) greater time required for analysis compared with ICP-MS – typically to determine Ni and Co or Cu and Cd in an aerosol digest the analysis time would be in the order of 20 min (assuming a 1-min collection period) compared with fractions of seconds per element for ICP-MS and approximately 6 min per element for GFAAS. (ii) The technique is limited to the ACSV detectable metals, whereas ICP-MS and GFAAS will determine a wider range of elements, including non-metallic species (ICP-MS). (iii) Smaller sample volumes are required by GFAAS (μl) and ICP-MS (0.3 ml per element) compared to typically 1.5 ml for ACSV. (iv) Due to changes in the ACSV response with changes in solution pH, this property has to be monitored and adjusted during sample injections. (v) Prior to the analysis of some metals (Cu and Cd) UV irradiation of aerosol acid digested samples is recommended.

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Transport of organic compounds across the air/sea interface of artificial and natural marine aerosols

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Abstract

A modified Gershey apparatus for artificial aerosol formation from salt solutions and sea-water samples was used to study the transfer of selected organic compounds from water to air and the parameters that influence this transfer. These parameters include the surface properties, the vapour pressure and the structure of the organic compounds and the presence of surfactants and suspended particles in the solution. In particular, alkylbenzenes and lower molecular weight *n*-alkanes were transported into the atmosphere while the remaining organic compounds studied were distributed between the liquid aerosol and the depleted sea water. Phthalates were present in the aerosol at high percentages (60–80%) and this behaviour is due to their surface properties towards the air/water interface. The addition of surfactants to the salt solutions generally led to a greater transfer of organic compounds into the aerosol. The results allowed the prediction of the organic composition of natural aerosol collected near Livorno (Italy) during storms in the Tyrrhenian sea.

Key words: Gas chromatography–mass spectrometry; Aerosols; Air/sea interface; Organic compounds; Sea water

1. Introduction

The marine aerosol is an environmental matrix that has become increasingly important in the last few years for the following reasons: it is thought to be responsible for the transport of substances toxic to vegetation present in coastal zones [1–3]; and it is also considered by many environmental researchers to be the means by which several anthropogenic compounds may be transported from sea water to the air [4] even for long distances. Biogenic and anthropogenic surfactants have been found in the marine aerosol [5,6] and the total organic carbon has been determined [7].

No extensive studies have been made concerning the transport of individual organic compounds and very little is known about the mechanism that determines the selective transport of these substances across the air/sea water interface. Therefore a study was instigated using a modified Gershey apparatus that is capable of reproducing, under laboratory conditions, the

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most significant aspects of aerosol formation from sea water under storm conditions, i.e., jet drop formation.

The aim of this work was to verify the precision of the apparatus for artificial aerosol formation, to study the parameters that influence the mass distribution of certain organic compounds in aerosol and sea water and to compare laboratory results with those pertaining to natural aerosol samples. Different classes of organic compounds (*n*-alkanes, polycyclic aromatic hydrocarbons, fatty acid methyl esters, alkyl phthalates and some heterocyclic compounds containing N and S), which are generally found in polluted sea water, were studied.

2. Experimental

2.1. Apparatus

In the past many types of apparatus have been proposed [7–11] for the production of artificial marine aerosol. In this work we considered the whole problem of the natural aerosol formation. The most important marine aerosol generation occurs by means of the breaking wave process. According to the Resch macroscopic model [12], a breaking wave generates gas bubbles which are involved in the rotational wave motion, with the final result that the bubbles scavenge the bulk water mass for several metres while rising to the surface, and collect the surfactant matter and those components which are able to interact with the air/water interface, before breaking. The MacIntyre [13] and Blanchard [5] microscopic model represents the bubble collapse as a three-step process: bubble cap collapse and generation of film drops; jet formation; and fractionation of jet into drops. The jet drops are the factor most responsible for the transfer of organic surfactant matter into the atmosphere from bulk water [14]. The number of film and jet drops is related to the dimensions of the collapsing gas bubbles [6,12], and bubbles of 300–700 μm diameter give only jet drops. Bubbles with the above-mentioned characteristics are also involved in a long path in the bulk sea water induced by the breaking wave motion. Therefore, they represent the largest contribution to the surfactant scavenging process.

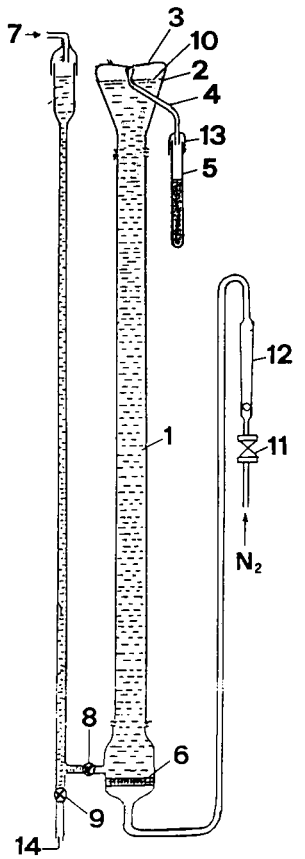

Fig. 1. Apparatus for artificial aerosol production. 1 = Gas bubble column; 2 = column head; 3 = impact surface; 4 = aerosol collector; 5 = graduated measuring tube; 6 = gas sparger; 7 = water solution inlet; 8 = water inlet valve; 9 = outlet valve for depleted water; 10 = water level in the column; 11 = microvalve to control nitrogen flow; 12 = flow meter; 13 = nitrogen outlet; 14 = depleted water.

To reproduce the above conditions in the laboratory, a modified Gershey [15] marine organic matter collector was used. Initially, the apparatus was used as a "jet drop impactor" in well defined conditions. Then the aerosol formation process was carried out on a specific water volume, instead of operating on a continuous countercurrent flow. The latter condition permitted the evaluation of the mass balance of the organic matter involved in the aerosol process. A scheme of the apparatus is shown in Fig. 1.

Pyrex glass was used as the material for all parts of the apparatus. The water column was 150 cm high with a diameter of 4 cm. The column head was expanded to an external diameter of 9 cm and the underhead wall acted as the impact surface. The expanded head prevented the foam formation that occurs when natural sea water is used. The surface of the column head is conical with an angle of 15° and collects the liquid film, formed by the ejected jet drops, in the tube which is in contact with the central point of the conical impactor surface. Because the tube also acts as output of the gas (nitrogen), the recovery of the liquid is highly enhanced and virtually quantitative. The sparger was a glass frit with a surface area of 20 cm^2 placed in the enlarged terminal section at the bottom of the column. The glass frit, whose pores had a nominal diameter of 20–40 μm, produces bubbles 500–700 μm in diameter.

Pure nitrogen was used to avoid any oxidation phenomenon. The gas flow was rigorously controlled by means of a precision flow meter at a rate of 7 ± 0.05 dm^3 h^{-1} at 21°C in artificial sea water. Under the above-mentioned conditions, a laminar flow of bubbles was achieved without appreciable coalescence in marine water. At the beginning of each aerosol process the water level and therefore the total volume used were controlled. The extraction power of the aerosol-forming device was experimentally detected using sodium dodecylbenzene sulphonate at a given concentration (1 mg l^{-1}). As described previously [16], 1 h is necessary for a total recovery of the surfactant from natural sea water with a 7 l h^{-1} flow of nitrogen. In the present experiments 2 h were adopted in view of the lower surfactant nature of humic components.

By using a highly purified sodium chloride solution (3.4%), it was verified that the volume of liquid aerosol was constant with time also for water level variations of about 2 mm. It was also noted that the contribution from film drops, formed during the first step of bubble collapse, was very small owing to the above mentioned bubble diameters. Under these conditions, the gas bubble flow is laminar so the axial diffusion is very small even for a 4-cm column diameter.

The apparatus was cleaned by treatment with warm potassium dichromate–concentrated sulphuric acid mixture and then washed with ultrapure water, generated by a Millipore Milli-Q device (organic-free medium provided) and distilled under sodium permanganate.

2.2. Reagents and material

Two standard mixtures of organic compounds dissolved in *n*-hexane at a concentration of 50 μg ml^{-1} for each phthalate and 100 μg ml^{-1} for the remaining compounds were used. A 0.1 ml volume of these solutions was added separately to the same volume (2.4 l) of different samples of doubly distilled water containing 34 g l^{-1} of sodium chloride pretreated at 450°C for 1 h or of sea-water samples collected from the Tyrrhenian sea 2 km from the coast at a 0.5-m depth. The resulting solutions were homogenized by magnetic stirring in a closed glass container for 12 h at room temperature.

All standard solutions were prepared using chemicals supplied by Supelco (Bellefonte, PA).

2.3. Artificial and natural aerosol

The apparatus in Fig. 1 produced an aerosol volume ranging from 16 to 20 ml in 2 h, depending on the presence of surfactants, using 2.4 l of doubly distilled water containing 34 g l^{-1} of sodium chloride. The five samples of Tyrrhenian sea water produced 18–20 ml of aerosol in the same time.

The aerosol obtained was treated with 2 ml of *n*-hexane and the extract, after passage through a dried sodium sulphate microcolumn, was analysed by gas chromatography. The organic compounds remaining in the depleted salt solutions and sea-water samples were extracted with the same volume of *n*-hexane. A microcolumn containing 500 mg of purified XAD-2 (100–200 mesh) was used to adsorb organic vapours transported by nitrogen.

The samples of natural marine aerosol were collected with a system using a PTFE cyclone bottle (9.5 cm external diameter, 12 cm high) and a fibre-glass filter (8 cm diameter) of nominal porosity 0.45 μm, pretreated at 450°C. The cy-

clone, at an air flow of 5 m^3 h^{-1}, collected all aerosol particles with a diameter greater than 1 μm. The filter collected the remaining particles below 1 μm. This apparatus was designed to test the distribution of organic compounds in the different particles according to the MacIntyre–Blanchard aerosol formation model [6,13].

The filter was extracted with a micro-Soxhlet system for 2 h with 5 ml *n*-hexane and the resulting extract was concentrated to 0.1 ml under nitrogen. The contents of the PTFE cyclone bottle were treated with 5 ml of *n*-hexane to determine the organic compounds adsorbed on the larger particles. This extract was also concentrated to 0.1 ml. The total air volume collected was 7.5 m^3.

2.4. Gas chromatographic (GC) and gas chromatographic–mass spectrometric (GC–MS) analysis

For the determination of organic compounds an HRGC 5160 Mega Series gas chromatograph (Carlo Erba) equipped with a flame ionization detector was used. Injection was made using a cold split–splitless injector, according to the following programme: the sample liquid was introduced into the closed vaporizing chamber maintained at 40°C, then the temperature was rapidly increased to 300°C and after 30 s the injector was purged through the split exit in order to flush out sample residues, especially remaining traces of solvent. The column used was a Supelco SPB-5 capillary column (30 m) and the carrier gas was hydrogen. The chromatographic peaks were analysed with a Mega-2 computer system (Carlo Erba) with Spectra-Physics software. The identification of organic compounds was based on dedicated software using the GC retention indices with eight *n*-alkanes (C_8, C_{12}, C_{16}, C_{20}, C_{24}, C_{28}, C_{32}, C_{34}) as internal standards.

GC–MS analyses were performed on a Varian Model 3400 gas chromatograph coupled with a Finnigan ion-trap mass detector. The carrier gas was helium. The temperature programme was 40°C for 1 min followed by a linear increase to 300°C at 4°C/min. Organic compounds were identified by an NBS library search.

For the determination of the individual compounds, the gas chromatogram obtained by extracting with 2 ml of *n*-hexane a volume of solution equal to that obtained from the aerosol process and containing the standard mixtures was used as a reference.

2.5. Surface quantities measurements

Measurements of the surface viscoelastic modulus (ε_0) and the equilibrium surface tension (γ_{eq}) were done with a time-resolved surface viscoelastomer [17,18] on aqueous solutions containing 3.4% sodium chloride (pretreated at 400°C) and 1 mg l^{-1} of each phthalate.

3. Results and discussion

The study was performed using both natural sea water and salt solutions containing sodium chloride after the removal of any interfering surfactant. The same experiments were repeated on salt solutions after the addition of known concentrations of different detergents.

3.1. Precision and accuracy of the method

By using the apparatus shown in Fig. 1, artificial aerosol was produced from five identical volumes of doubly distilled water containing 34 g l^{-1} of sodium chloride and, after the addition of the same mixture of dialkyl phthalates, the content of organic matter in the aerosol was determined.

Table 1
Experimental means of five replicate measurements (peak area), standard deviations (s) and relative standard deviations (R.S.D.s) for the phthalates found in liquid aerosol

Dialkyl phthalate	Mean	s	R.S.D. (%)
Ethyl	34749	3556	10.2
Propyl	203684	17209	8.4
Isopropyl	174496	13189	7.5
Butyl	153515	14961	9.4
Isobutyl	161605	16483	10.2
Pentyl	133101	15288	11.5
Hexyl	107521	13415	12.5
Heptyl	114989	14290	12.4
Octyl	125638	15594	12.4

Table 1 reports the GC data together with their standard deviations and relative standard deviations (R.S.D.s). The results confirmed the precision of the method as the R.S.D.s were between 7.5 and 12% according to the type of phthalate. These compounds are suitable for this experiment as their percentages in the liquid aerosol vary considerably depending on the alkyl group.

The accuracy of the method can be evaluated from the mass balance reported in Table 2, which shows the percentages of organic compounds found in liquid aerosol, in the depleted salt solution and/or transported directly into the air by nitrogen during the artificial aerosol process. The mass balance indicates that $> 90\%$ of the standard was accountable in the two phases.

3.2. Distribution of organic compounds in aerosol and sodium chloride solution

The data in Table 2 show that several compounds with higher volatility, such as the alkylbenzenes and the lower molecular weight *n*-alkanes (up to C_{16}), are transported into the atmosphere in considerable amounts. The polycyclic aromatic hydrocarbon (PAHs) with two and three rings, such as naphthalene, methylnaphthalene, fluorene and anthracene, are partially transported into the atmosphere and are not found in the aerosol. Other compounds (acenaphthene, acenaphthylene, quinoline, naphthoquinoline, carbazole), in contrast, are not affected by the aerosol formation and remain in the salt solution.

The remaining organic compounds studied are distributed between the liquid aerosol and salt solution. These include $\geq C_{20}$ *n*-alkanes, high-molecular-weight PAHs, steroids, fatty acids and phthalate esters. The only exception is dimethyl phthalate, which is the most volatile and therefore is transported completely into the air. The distribution of these compounds in the liquid aerosol and salt solution changes according to the class and its components. For example, the phthalate esters are present to a greater extent in the aerosol with percentages of 60–80% for the average-molecular-weight compounds such as propyl, isopropyl, butyl and isobutyl phthalate. PAHs show the lowest values but methylpyrene and triphenylbenzene, which have very different structures, are present in high percentages in the aerosol. The higher molecular weight alkanes and fatty acid esters behave in a similar way and their percentages are between those for the above-mentioned classes.

We conclude that several organic compounds are present at high percentages in the liquid aerosol even in the absence of surfactants in the salt solution. This behaviour may be due to the different structures and surface properties of the organic compounds concerned.

Table 3 shows the equilibrium surface tension (γ_{eq}) and the surface viscoelastic modulus (ε_0) of aqueous solutions containing sodium chloride and alkyl phthalates measured at 26.5 ± 0.5°C. On the basis of these data, the surfactant nature of phthalates depends on their size and the structure of the alkyl groups (linear or branched). The surface viscoelastic modulus increases when the carbon atoms number of the alkyl groups increases from one to four, then decreases for the higher components. Branched phthalates show a higher surface activity than the compounds with linear alkyl groups, i.e., diisobutyl phthalate is the strongest surface-active agent. Comparison of the data in Table 3 with the percentage of each phthalate in the liquid aerosol (see Table 2) indicates that the transport of these compounds from water to aerosol is due to their surface properties towards the air/water interface.

To explain the behaviour of organic compounds which show low surface-active properties, the following factors should be considered. The phenomenon of bubble breaking and aerosol formation is complex even in a simple salt solution. Blanchard [19] showed that apart from the transport of organic matter into the aerosol, there was a separation of charge between positive and negative ions during the process. The cations are more mobile and are found in higher percentages in the jet formation, creating a positively charged aerosol. Sakai [20] demonstrated that some anions are distributed differently in the water/air interface owing to the different effect of their electrical polarization on the microstructure of the water at the interface.

Of the organic compounds studied, their inter-

Table 2
Percentage of organic compounds in liquid aerosol, in depleted sodium chloride solution and transported directly by nitrogen in the air

Compound	Liquid aerosol	Depleted sodium chloride solution	Transported by nitrogen
Octane	bdl [a]	bdl	97
Isopropylbenzene	bdl	bdl	96
1,3,5-Trimethylbenzene	bdl	bdl	97
p-Isopropylmethylbenzene	bdl	bdl	97
n-Butylbenzene	bdl	bdl	96
n-Hexylbenzene	bdl	17	80
Dodecane	bdl	7	88
Naphthalene	bdl	84	13
1-Methylnaphthalene	bdl	85	11
2-Methylnaphthalene	bdl	80	16
Acenaphthene	bdl	97	bdl
Acenaphthylene	bdl	97	bdl
Quinoline	bdl	98	bdl
Naphthoquinoline	bdl	98	bdl
Carbazole	bdl	98	bdl
Benzothiophene	bdl	92	5
Fluorene	bdl	92	5
Dibenzothiophene	bdl	93	5
Anthracene	bdl	94	4
Hexadecane	12	59	24
Eicosane	29	68	bdl
Methyl palmitate	29	68	bdl
Methyl stearate	29	68	bdl
Methyl oleate	28	68	bdl
Tetracosane	30	67	bdl
Fluoranthene	8	88	bdl
Methylpyrene	25	74	bdl
Crysene	9	88	bdl
Triphenylbenzene	23	74	bdl
Benzo[*a*]anthracene	19	78	bdl
5α-Androstan-17β-ol-3-one	15	81	bdl
5α-Androstan-3,17-dione	10	87	bdl
Cholesterol	28	69	bdl
Benzo[*b*]fluoranthene	16	80	bdl
Benzo[*k*]fluoranthene	12	85	bdl
Octacosane	28	68	bdl
Indeno[1,2,3-*cd*]pyrene	17	78	bdl
Dibenzo[*a*,*h*]anthracene	8	88	bdl
Dotriacontane	23	73	bdl
Benzo[*ghi*]perylene	9	87	bdl
Tetratriacontane	20	76	bdl
Dimethyl phthalate	2	bdl	92
Diethyl phthalate	22	70	bdl
Di-*n*-propyl phthalate	81	17	bdl
Diisopropyl phthalate	78	19	bdl
Di-*n*-butyl phthalate	58	38	bdl
Diisobutyl phthalate	66	28	bdl
Di-*n*-pentyl phthalate	39	54	bdl
Di-*n*-heptyl phthalate	22	72	bdl
Di-*n*-octyl phthalate	23	72	bdl

The initial solutions contained 5 μg of each phthalate and 10 μg of the other compounds.
[a] bdl = Below detection limit.

action with the water microstructure becomes extremely important in the water/air interface, owing to their volatility. It should be noted that the phenomenon regarding the water–air exchange of organic compounds dissolved in water and in the gas form is strongly affected by the air and water hydrodynamics at the interface [21]. The compounds which have a greater affinity towards the gas bubble are present at higher concentrations in the forming jet drops and as a consequence in the aerosol. The trend for *n*-alkanes, whose transport into the liquid aerosol decreases as their molecular weight increases, may be connected to their different volatilities, while the behaviour of PAHs seems to be attributable to their different structures. The high percentages of methylpyrene and triphenylbenzene in the aerosol compared with the other PAHs may be due to their more symmetrical structures, which increase the stripping efficiency of the gas bubbles. The three fatty acid esters have almost identical structures and are transported into the aerosol in the same amount. Androstanes are present at low concentrations in the aerosol whereas cholesterol is found in an appreciable amount.

3.3. Influence of surfactants on the distribution ratio between aerosol and salt solution

Anionic, cationic and non-ionic surfactants were added separately to the salt solution after the addition of the standards to obtain a concentration of 1 mg l^{-1} for each component. The detergents used were sodium dodecylbenzene sulphonate (DBS), *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTA) and polyethylene glycol (PEG). The effects of the three kinds of surfactants on the content of organic matter in the aerosol are shown in Figs. 2, 3, 4 and 5 for PAHs, *n*-alkanes, fatty acid esters and steroids, respectively, and Fig. 6 shows the influence of PEG on dialkyl phthalates. The following observations can be made from the reported trends.

Table 3
Values of equilibrium surface tension (γ_{eq}) and surface viscoelastic modulus (ε_0) of aqueous solutions containing sodium chloride (3.4%) and alkyl phthalates (1 mg l^{-1})

Alkyl phthalate	γ_{eq} (mN m^{-1})	ε_0 (mN m^{-1})
Dimethyl	68.4	7.2
Diethyl	63.0	18.0
Diisopropyl	58.5	27.0
Di-*n*-butyl	58.2	27.8
Diisobutyl	56.9	30.4
Di-*n*-pentyl	58.8	26.6
Di-*n*-hexyl	66.3	11.6
Di-*n*-heptyl	68.0	8.0
Di-*n*-octyl	68.3	7.7

Relative humidity: 89±2%. The data are reproducible to within 3–5%.

The percentage of PAHs in the aerosol increases considerably (up to five times) in the presence of any surfactant. Even those PAHs with three rings, such as fluorene and anthracene, which were not present in the aerosol obtained from salt solutions without detergents, were found in appreciable amounts in this liquid aerosol.

The percentages of *n*-alkanes also increase in the liquid aerosol after the addition of surfactants. The only exception is eicosane, which is present in lower concentrations in the presence of non-ionic and anionic detergents. It should be noted that the presence of DBS increases the percentage of *n*-alkanes in the aerosol when the

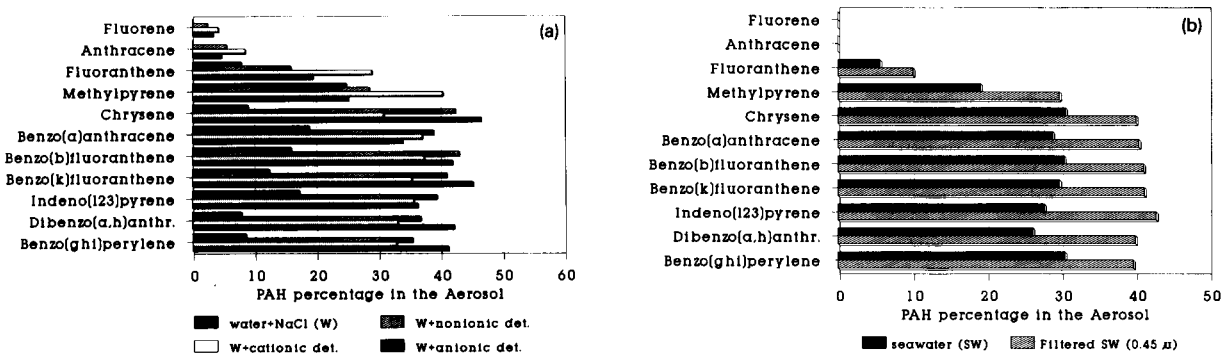

Fig. 2. Percentages of PAHs in the artificial aerosol formed from different solutions. (a) Salt solution alone and after the addition of surfactants; (b) untreated and filtered sea water. Fluorene and anthracene are not transported into the aerosol from the salt solution. Amount of each PAH = 10 μg.

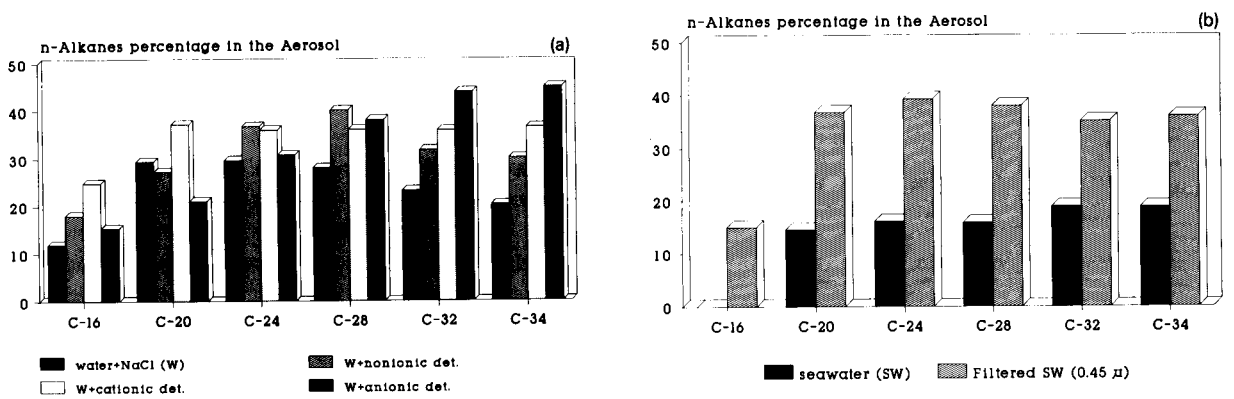

Fig. 3. Percentages of *n*-alkanes in the artificial aerosol formed from different solutions. (a) Salt solution alone and after the addition of surfactants; (b) untreated and filtered sea water. Amount of each *n*-alkane = 10 μg.

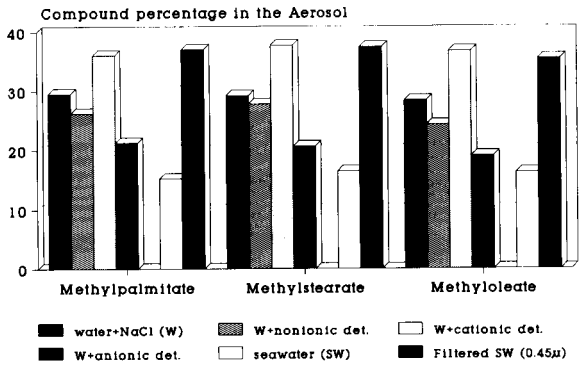

Fig. 4. Percentages of fatty acid methyl esters in the artificial aerosol formed from different salt solutions and sea-water samples in the following sequence: salt solution alone and after the addition of non-ionic, cationic and anionic surfactants, untreated and filtered sea water. Amount of each ester = 10 μg.

number of carbon atoms increases. The addition of the detergent favours the transport of dodecane concentrations ranging from 2 to 4% according to the type of surfactant.

The three fatty acid esters show an unusual behaviour in that their percentages in the aerosol decrease in the presence of anionic and non-ionic surfactants but increase considerably with the cationic surfactants.

The behaviour of cholesterol is similar to that of fatty acid esters; the other steroids differ from

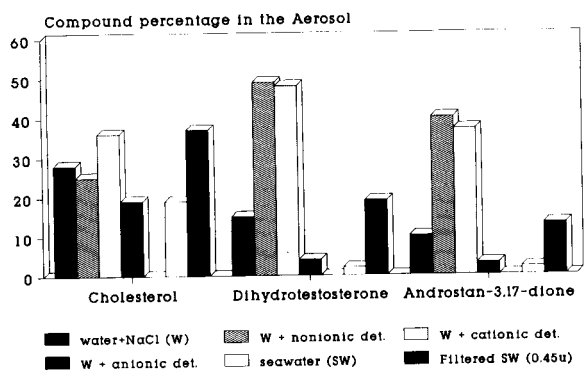

Fig. 5. Percentages of steroids in the artificial aerosol formed from different salt solutions and sea-water samples in the following sequence: salt solution alone and after the addition of non-ionic, cationic and anionic surfactants, untreated and filtered sea water. Dihydrotestosterone = 5α-androstan-17β-ol-3-one. Amount of each steroid = 10 μg.

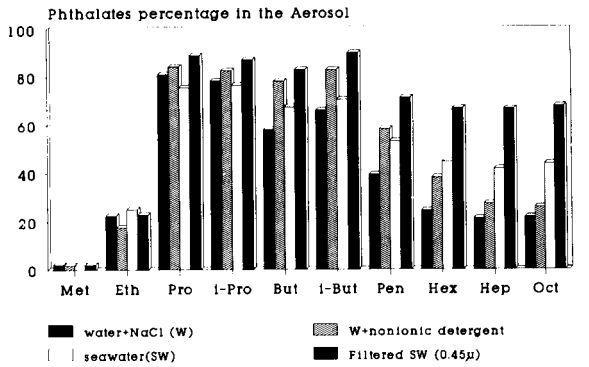

Fig. 6. Percentages of dialkyl phthalates in the artificial aerosol formed from different salt solutions and sea-water samples. Amount of each phthalate = 5 μg.

the above compounds as their percentages in the aerosol decrease only in the presence of anionic detergents.

Dialkyl phthalates, which are present in very high percentages in the aerosol from salt solutions, increase their transport slightly on the addition of PEG.

These results agree with the experiments of Hoffman and Duce [7], who noted an increase in the organic carbon content in the aerosol after the addition of surfactants to sea water. The detergent present in the salt solution determines a higher or lower transport of the organic matter depending on the chemical characteristics of the surfactant and of the compounds involved.

3.4. Aerosol formation from sea-water samples

Samples of Tyrrhenian sea water (collected as described under Experimental) were tested after the addition of the standards without any pretreatment or after filtration through a 0.45-μm fibre glass filter.

As a preliminary step, a sea-water sample was used to produce artificial aerosol without the addition of standard mixtures. The results showed that the concentrations of organic compounds present in the sea water were not high enough to interfere with the measurements of the standards. The data obtained from untreated and filtered sea water and those from salt solutions, with and without surfactants, are compared in

Figs. 2–6. The amount of particulate matter was 3–4 mg l^{-1}.

PAHs with a higher molecular weight show greater transport from sea water than from the salt solution (cf., Fig. 2a and b), while a similar behaviour is observed for the first components (fluorene, anthracene, fluoranthene and methylpyrene). The percentages of the more abundant PAHs in the aerosol are of the same order (30%). This behaviour can be attributed to the presence of natural or synthetic surfactants in the Tyrrhenian sea water, as shown by surface tension measurements. Fig. 2b also shows the data pertaining to the same sea water prefiltered on a 0.45-μm filter. In this instance the transport of PAHs into the aerosol increases considerably and is about 40% for most compounds. This percentage corresponds to that obtained from the salt solutions containing surfactants. The smaller amount of PAHs found in the aerosol from unfiltered sea water may be due to the presence of suspended particles that adsorb these organic compounds and decrease their transport.

n-Alkanes show a lower transport from sea-water (Fig. 3a and b). Their amount in the aerosol decreases as the number of carbon atoms decreases. For example, C_{16} is not found in the sea-water aerosol and the percentages for C_{20}, C_{24} and C_{28} are about half of those from the salt solutions. The prefiltered sea water produces an aerosol containing much higher percentages (30–40%) of *n*-alkanes and similar to that obtained from salt solutions in the presence of surfactants. This behaviour demonstrates the important role of suspended particulate matter in strongly decreasing the transport of alkanes into the aerosol.

Fatty acid esters are transported into the aerosol from sea water in smaller amounts than from the salt solutions (Fig. 4). The transport increases considerably when using prefiltered sea water (30–35%).

The behaviour of steroids is similar to that of fatty acid esters (Fig. 5).

For dialkyl phthalates, the transfer of the first components of this series is similar for both sea water and salt solution (Fig. 6), whereas that of the remaining compounds is greater for sea water (from 22 to 44%). Filtration increases considerably the percentage of the highest molecular weight phthalates.

3.5. Mass distribution ratio between particles and sea water

The artificial aerosol process can be used to determine the mass distribution ratio of organic compounds between the liquid aerosol and sea water and between sea water and the suspended particulate matter. It is not possible to propose a strict treatment owing to the presence of kinetic factors and the transport of solution during the aerosol formation. The data pertaining to the distribution ratio between the amount of compound in the liquid aerosol and that remaining in the depleted solution can be useful as an indication of the specific behaviour. In fact, the amount of each compound present in the solution transported by the liquid aerosol is at most 0.075 μg and is always small in comparison with the total amount of the same substance found into the aerosol at high concentrations. Within these limits, if we assume that the transfer of organic compounds into the liquid aerosol is expressed by the following equations for untreated and filtered sea water:

$$Dm_1 = Q_{\text{aerosol}}/Q_{\text{sea water}} + Q_{\text{particulates}}$$

$$Dm_2 = Q_{\text{aerosol}}/Q_{\text{sea water}}$$

it is possible to calculate the ratio $Q_{\text{particulate}}/Q_{\text{sea water}} = (Dm_2/Dm_1) - 1$, where Q_{aerosol} is the amount (μg) of the compound found in the aerosol, $Q_{\text{sea water}}$ is the amount (μg) of the same compound remaining in the depleted sea water and $Q_{\text{particulate}}$ is the amount (μg) adsorbed on the suspended particulate. The above equations allowed the determination of $Q_{\text{particulate}}/Q_{\text{sea water}}$ for those compounds not transported directly into the atmosphere by nitrogen from the distribution ratio relative to untreated and filtered sea water.

For C_{20}–C_{34} *n*-alkanes the $Q_{\text{particulate}}/Q_{\text{sea water}}$ values are between 1.3 and 2.4, which shows a partition in favour of suspended particles. Similar values (1.8–2.2) were found for the three fatty acid esters whereas larger values (2.4–3.4) were encountered with dialkyl phthalates for the largest

components. The PAHs have values between 0.5–0.9, indicating a partition in favour of sea water.

3.6. Organic compounds found in the n-hexane extract of natural aerosol

The two samples of marine aerosol, collected according to the procedure described under Experimental, were taken off the Tyrrhenian coast near Livorno, Italy, during storms in June and July 1993.

To establish the composition of the natural aerosol it is useful to keep in mind the compounds found in Tyrrhenian sea water and reported in a previous paper [22]. Numerous linear and branched alkanes, several dialkyl phthalates, some PAHs with two and three rings (naphthalene, fluorene, anthracene, phenathrene), a few alkylbenzenes and heterocyclic compounds such as benzothiazole, quinoline and naphthoquinoline were observed in the sea water. Other compounds, such as steroids and fatty acid esters, and also some cyclic ethers, alcohols and alkylphenols which were not examined in this work, were also present in the Tyrrhenian sea water. As described under Experimental, two extracts for each sample of natural aerosol were obtained, one for particles smaller than 1 μm and the other for larger particles. Fig. 7 shows the gas chromatograms for these two extracts. Chromatogram A pertains to the filter extract and B refers to the contents of PTFE bottle. The comparison between the two chromatograms indicates that the extract of the larger particles contains a greater number of compounds and at higher concentration than the other. The filter extract holds several compounds with lower retention times and also lower molecular weight in general. These chromatograms are representative of the natural aerosol samples taken in June and July 1993.

Table 4 shows all the organic compounds found in the *n*-hexane extracts of the natural marine aerosol in order of their elution from the GC column. The presence of almost all of these compounds was predictable on the basis of the results obtained from the artificial aerosol process. Examination of the organic compounds according to their classes leads to the following observations.

All the linear and branched alkanes present in the Tyrrhenian sea water were found in the natural aerosol, including the C_{16}–C_{34} *n*-alkanes. The concentration of C_{21}–C_{30} *n*-alkanes was exceptionally high and represents more than 80% of the organic matter. There are also small amounts

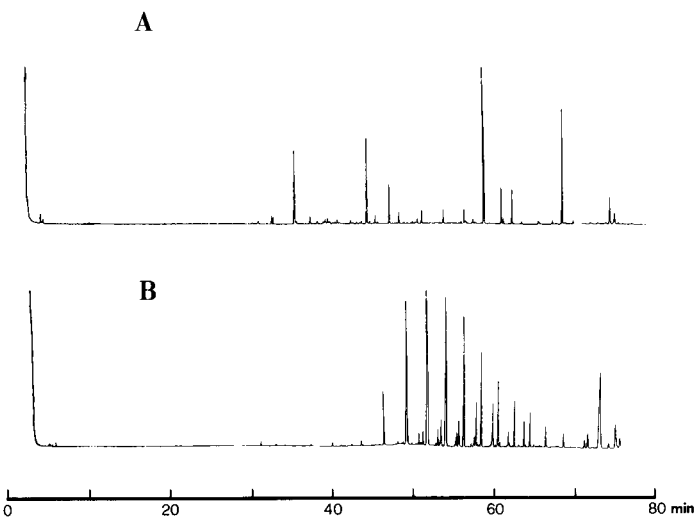

Fig. 7. Gas chromatograms of the *n*-hexane extracts of natural marine aerosol collected near Livorno during storms in June and July 1993. (A) Filter extract; (B) extract of PTFE bottle contents. Attenuation = (A) 32 and (B) 64.

of some linear and branched compounds smaller than C_{16}; according to the laboratory result, these compounds were completely transported into the atmosphere by nitrogen. Their presence in the natural aerosol is probably due to their adsorption by the particulate.

Alkylbenzenes are present in small amounts and their behaviour is similar to that of low molecular weight alkanes as they are transferred into the air by nitrogen.

The PAHs with two or three rings were not found in the natural marine aerosol, even though they are present in sea water, in agreement with the laboratory results, where these compounds remain in the depleted salt solution after the aerosol formation process. Benzothiophene, quinoline and naphthoquinoline show a similar behaviour.

Even for dialkyl phthalates the results obtained correspond to the prediction based on the laboratory experiments. The average-molecular-weight compounds show the highest concentrations and are the most easily transported into the aerosol. The slight presence of dimethyl phtha-

Table 4
Organic compounds found in *n*-hexane extract of natural marine aerosol collected during a storm near Livorno

Retention time (min s)	Compound	Retention time (min s)	Compound
4.34	Branched alkane	39.00	Di-*n*-butyl phthalate
4.40	Cyclic ether	39.24	Alcohol
4.48	Cyclic ether	39.50	*n*-Nonadecane
5.08	Tetrachloroethene	40.08	Butyl, isobutyl phthalate
6.26	Ethylbenzene	41.20	Diisobutyl phthalate
6.40	1,2-Dimethylbenzene	42.18	*n*-Eicosane
7.36	*n*-Nonane	42.54	Fatty acid methyl ester
8.26	Alcohol	44.12	Fatty alcohol
10.42	1,2,3-Trimethylbenzene	44.34	*n*-Heneicosane
12.06	Branched alkane	45.22	Fatty acid ester
15.44	Aliphatic acid ethyl ester	45.34	Branched alkane
17.38	Aliphatic acid ethyl ester	45.58	Di-*n*-hexyl phthalate
22.52	Branched alkane	46.44	Fatty acid ester
23.44	Alcohol	46.58	*n*-Docosane
24.14	Branched alkane	47.46	Branched alkane
24.30	Alcohol	49.08	*n*-Tricosane
27.00	Dimethyl phthalate	50.12	Dialkyl phthalate
28.56	Alkylphenol	51.02	Fatty acid ester
29.08	Phosphoric acid ester	51.06	*n*-tetracosane
30.58	Branched alkane	54.12	Diisooctyl phthalate
31.22	Diethyl phthalate	54.16	*n*-Pentacosane
31.36	*n*-Hexadecane	55.14	*n*-Hexacosane
31.54	Branched alkane	57.08	*n*-Heptacosane
33.04	Phosphoric acid ester	58.54	*n*-Octacosane
33.26	Di-*n*-propyl phthalate	59.30	Squalene
33.54	Alcohol	60.38	*n*-Nonacosane
35.56	Alkylphenol	62.02	*n*-Triacontane
36.34	Diisopropyl phthalate	64.12	Hentriacontane
37.12	*n*-Octadecane	66.04	*n*-Dotriacontane
37.28	Branched alkane	67.54	Steroid
37.34	Butyl, isopropyl phthalate	68.14	Steroid
37.54	Fatty acid methyl ester	68.36	*n*-Tritriacontane
		70.52	*n*-Tetratriacontane

late may be due to its adsorption by particulate material.

The presence of fatty acid esters and steroids agrees with the prediction from the laboratory experiments.

4. Conclusions

The experiments on artificial aerosol composition allow the prediction of the transport behaviour of the organic compounds dissolved in sea water into the natural aerosol during storms. Only those compounds transferred into the liquid aerosol or directly into the air by nitrogen were found in the natural marine aerosol; similarly, those compounds that were not transported during the artificial aerosol process were not present in natural aerosols.

It should be noted that alkanes and dialkyl phthalates present in high concentrations in sea water were found in the natural marine aerosol in even higher amounts, again in agreement with the enrichment process predicted by laboratory studies.

The proposed apparatus has been used to study the parameters that influence the transport of organic material from water to air. The more important parameters are the surface properties, vapour pressure and structure of the organic compounds and the presence of suspended particles and surfactants. The influence of the last factor varies according to the type of surfactant (anionic, cationic, non-ionic) and the structure of the organic components considered.

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ANALYTICA CHIMICA ACTA

The validity of determination of α-naphthol in urine as a marker for exposure to polycyclic aromatic hydrocarbons

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Abstract

The actual individual uptake of polycyclic aromatic hydrocarbons (PAH) depends on a multitude of factors, and it may therefore be advantageous to supplement environmental measurements of airborne PAH compounds with biological monitoring of PAH metabolites to obtain a more reliable assessment of exposure and health risk. In the present study the applicability of determination of the urinary α-naphthol concentration as a marker for exposure to airborne naphthalene and total PAH was scrutinized. The validation is difficult since PAH compounds are widespread in the environment, and it is extremely difficult to find individuals exposed to one source of PAH compounds only. A preliminary study on five smokers employed in an office work place with no occupational PAH exposure revealed that the urinary α-naphthol concentration was closely related to the degree of smoking, i.e., heavily smoking resulted in the highest urinary α-naphthol concentrations (11.8 μmol/mol creatinine). The close correlation between smoking and elevated concentration of urinary α-naphthol was subsequently confirmed on group basis by comparison of smokers and non-smokers at four different work places, (i.e., two iron foundries with low airborne PAH exposure and two work places with unknown PAH exposure). At all work places the median values of urinary α-naphthol of smokers were higher than the median values of non-smokers, indicating that smoking may be a strong confounder when measurements of α-naphthol are used to monitor low dose of PAH exposure. In a detailed study of iron foundries the urinary α-naphthol concentration was clearly associated with the low-dose airborne total PAH exposure of the workers. This study also demonstrated the need for a careful registration of smoking habits, i.e., regardless of the level of PAH exposure. Smokers in average tended to have higher urinary α-naphthol concentrations than non-smokers. The present study has demonstrated that a newly developed liquid chromatographic method for measurement of α-naphthol in urine may be applied in biological monitoring of low dose PAH exposure.

Key words: Liquid chromatography; Environmental analysis; Biological monitoring; α-Naphthol; Smoking; Urine

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment. For cen-

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Table 1
PAH exposure in different work environments

Work environment	Total PAH concentration ($\mu g/m^3$)	Ref.
Creosote-impregnation plants	1.2–22.3	[2]
	2.3–130	[3]
	0.1–106	[4]
	< 960	[5]
Aluminum reduction plant	52–268	[6]
	3.5–380	[7]
	11.3–854	[8]
	14.5–1383	[9]
	< 79	[5]
Road paving	0.004–2.5	[10]
	13–106	[9]
	9.5–94	[11]
	10–21	[12]
Coke ovens	< 1500	[5]
	6–570	[13]
Iron foundries	3.6–52	[14]
	4.1–41.7	[15] and unpublished data
	< 31	[16]
Chimney sweeping	2.27–5.08	[17]
Smoke curing fish	2.2–2472	[18]
Smoke curing meat	0.09–25.5	[19]

turies PAHs have been formed by incomplete combustion or pyrolysis of any organic materials containing carbon and hydrogen [1]. In different work environments PAH compounds are present in various concentrations (Table 1) [2–19]. The composition of PAH compounds present in the work environment are depending on which raw materials were used and the work process. However, various studies have demonstrated that naphthalene is the dominant PAH compound of the samples, i.e., 70% in samples from meat smoke houses [19], 65% in samples from smoke curing fish [18], 80% in samples from aluminum reduction plants [9], 68% in samples from creosote impregnation [5].

Several PAHs have both mutagenic and carcinogenic properties and it has been demonstrated that some of the volatile polycyclic aromatic hydrocarbons, e.g., pyrene, potentiates the effect of the carcinogenic PAH compound benzo[*a*]pyrene [20]. An increased incidence of lung cancer has been demonstrated in workers exposed to high levels of airborne PAH, and exposure to PAH is considered a major environmental and occupational health problem. Humans may be exposed to PAH compounds from diet, medical treatment, car exhaust, fumes, and different types of occupational exposure. In food PAHs are present in smoked fish and meat [21]. For several years, coal tar has been used in the treatment of patients with atopic dermatitis, chronic eczema and psoriasis. Furthermore, active as well as passive smokers are exposed to PAHs [22].

The assessment of occupational exposure to PAHs by measurement of airborne PAH compounds is often used. However, the actual individual uptake of these hazardous compounds is depending on a multitude of factors, and it may therefore be advantageous to supplement environmental measurements of airborne PAH compounds with biological monitoring to obtain a more reliable assessment of exposure and health risk.

Aromatic hydroxylation is a common reaction catalyzed by the microsomal cytochrome P-450 system. Two mechanisms have been described, one mechanism involves direct insertion of an oxygen to the carbon–hydrogen bond. Another mechanism involves addition of the oxygen to carbon–carbon double bond to produce arene oxide intermediates, which rearrange to form an aromatic hydroxyl compound. The arene oxide intermediates are important in determining the possible toxicity of aromatic compounds [23]. The major metabolite of naphthalene is α-naphthol [24].

More than 300 components have been identified in coal tar and it has been estimated that as many as 10 000 components may exist [25]. Detailed knowledge on the metabolism of several PAH compounds is described by the IARC [20], but only a limited number of PAH metabolites are commercially available. Consequently, biological monitoring of PAH exposure is restricted to a few PAH compounds for which the metabolites are available as standards. This limitation may, however, partly be overcome by using these metabolites as markers for total PAH exposure. The metabolite α-naphthol has been measured in urine from workers in creosote impregnation plants [26] and iron foundries [15].

The purpose of the present study was two-fold. First to evaluate the use of a newly developed highly sensitive liquid chromatography (LC) method for determination of α-naphthol in urine from smokers and non-smokers. Secondly to evaluate to what extent smoking influences urinary α-naphthol as a marker for naphthalene and total PAH exposure.

2. Experimental

2.1. Chemicals

The acetonitrile used was LiChrosolv (Merck, Darmstadt). Water was obtained from a Milli-Q water purification system (Millipore Waters, Taastrup). A 7.000 mmol/l α-naphthol stock solution was prepared by dissolving α-naphthol in acetonitrile. α-Naphthol was pro analysi (Merck) from two different batches, i.e., one batch for the method evaluation samples and one batch for the five different standards.

Helix pomatia β-glucuronidase with sulfatase activity, 100000/5000 units/ml (G-7017) and metabolites of α-naphthol, i.e., α-naphthyl-β-D-glucuronide sodium salt and α-naphthyl sulfate potassium salt were obtained from Sigma (St. Louis, CA). The metabolites were dissolved in pooled urine from non-exposed persons and used to test the efficacy of the enzyme treatment.

2.2. Study population and exposure groups

The study population consisted of 115 workers (i.e., workers from 2 iron foundries located in rural Danish towns) with known occupational exposure to PAH, and 115 workers without known occupational exposure to PAH (i.e., workers from drinking water supply plants, cotton workers and workers from garbage recycling plants located all over the country). All workers were examined between May 1989 and October 1990.

2.3. Collection of air samples

Twenty-six personal air samples of 600–800 l of air were collected at an air flow rate of 1.9 l/min (Model P2500 Dupont constant flow sampler, Wilmington, DE) during the entire working period (approximately 6–7 h) to obtain estimates of PAH exposure for different iron foundry workers. Particulate matter was collected on a 37-mm glass fiber filter (Millipore AP 4003-705) in a standard cassette of polystyrene connected by a 10-cm PVC tube in series to a styrene–divinylbenzene adsorption tube (ORBO 43 from Supelco, Gland) for the collection of volatile PAH compounds. The filter cassettes and tubes were wrapped in aluminum foil to protect against light. The employed air sampling method was described earlier [5] and used in the investigations of fish and meat smokehouses in Denmark [18,19]. Urine samples were collected in September 1990 to have the same season for the air samples as for the collection of urine samples. Samples were stored in a refrigerator (−20°C) and extracted within 24 h.

2.4. Determination of airborne PAH compounds collected on filters and tubes

Reversed-phase LC was used for the quantitative analysis of PAH compounds as described previously [27]. For each sample the concentration of 15 selected PAH compounds (similar to the standard PAH compounds) was estimated by comparison with the chromatogram of the standard PAH preparation. Before each series of analysis the standard PAH preparation was injected at five different concentrations to obtain a standard curve. The stability of the apparatus was controlled by injection of a standard preparation for each fifth sample analyzed. In the concentration ranges observed in iron foundries the standard plots were linear for all compounds. Total PAH concentration was calculated as the sum of the concentrations of the 16 PAH compounds.

2.5. Collection of urine samples

The urine samples from iron foundry, cotton, garbage incineration and water supply workers were collected as the second urine of the day at the end of the work week (Friday). The samples were frozen at −20°C until analyzed. Urine creatinine [28] was used to standardize the result.

2.6. Determination of α-naphthol in urine

RPLC was used for the quantitative analysis of α-naphthol in urine as described previously [29]. Before each series of analysis the standard α-naphthol preparation was injected at five different concentrations to obtain a standard curve. The stability of the apparatus was controlled by injection of a standard preparation for each fifth sample analyzed. In the concentration ranges observed in the study the standard plots were linear.

2.7. Sample preparation

The five different standards were prepared by serial dilution of the α-naphthol stock solution in pooled urine from unexposed persons to produce standards in the range 0.174–1.000 μmol/l.

Aliquots of 10.0 ml of the standard urine samples as well as the collected samples were buffered with 10.0 ml 0.2 M sodium acetate buffer (pH 5.0) and hydrolyzed enzymatically with 200 μl β-glucuronidase–sulfatase (26400 units β-glucuronidase and 440 units sulfatase) for 20 h at 37.5°C in a water bath/shaker. Hereafter 10.00 ml of the buffered samples were extracted with 10.00 ml cyclohexane–diisopropanol (9:1) by shaking for 20 min. The cyclohexane–isopropanol phase was finally extracted with 300 μl 0.1 M sodium hydroxide, followed by addition of 100 μl 0.4 M hydrochloric acid. All phases were separated by centrifugation. Samples of 25 μl of the final neutralized water extracts were injected to the column.

2.8. Statistics

A Mann-Whitney test and a Wilcoxon rank sum test were used to test differences between the groups concerning exposure levels of smoking and non-smoking individuals [30] and a Hotelling-Pabst test, a Spearman rank correlation and Pearson's r test were calculated to estimate to what extend α-naphthol could be a marker for naphthalene or total PAH exposure [31].

3. Results and discussion

Levels of α-naphthol were measured in urine from five smoking office workers. Smoking habits varied from heavy smokers (> 10 cigarettes a day) to only a few cigarettes a day. Smoking more than 10 cigarettes a day resulted in concentrations of urinary α-naphthol between 9.6–13.4 μmol/mol creatinine (3 workers). Two workers smoking less than 10 cigarettes a day had no detectable amounts of urinary α-naphthol.

Table 2 represents 10 and 90% median values for urinary α-naphthol of smoking and non-smoking workers with known occupational exposure to PAH, (i.e., iron foundry workers) and workers without known occupational exposure to PAH compounds. Smokers showed a significant elevated level of α-naphthol as compared to non-smokers, for group 1 ($P = 0.002$) and 3 ($P = 0.0009$). For the two other groups the smokers showed an elevated level of α-naphthol, but the difference was not significant probably due to the

Table 2
Median values, 10 and 90% of urinary α-naphthol from smoking and non smoking individuals in different work environments

	α-Naphthol in urine (μmol/mol creatinine), N [a], median values, 10% and 90%		Mann-Whitney P-value
	Smokers	Non-smokers	
Iron foundry workers (1)	$N = 26$, 1.78, 0.00, 4.11	$N = 19$, 0.45, 0.00, 2.27	0.002
Iron foundry workers (2)	$N = 45$, 1.99, 0.51, 5.46	$N = 25$, 1.43, 0.00, 6.72	0.518
Cotton workers	$N = 30$, 2.31, 0.94, 7.02	$N = 19$, 0.80, 0.00, 3.59	0.001
Garbage recycling and drinking water supply workers	$N = 47$, 1.78, 0.00, 6.11	$N = 19$, 0.94, 0.00, 4.31	0.443

[a] Number of individuals.

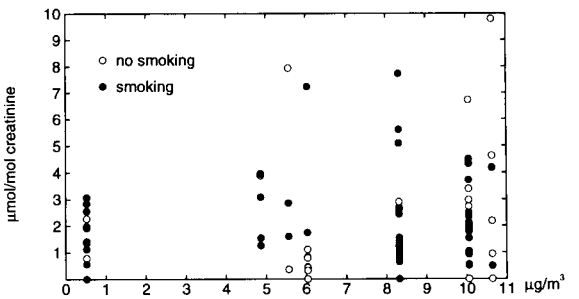

Fig. 1. Relation between urinary α-naphthol concentration and naphthalene exposure for smokers and non-smokers.

presence of a few non-smokers who showed un explainable high α-naphthol levels.

Fig. 1 represents urinary α-naphthol levels of individuals with known occupational exposure plotted versus naphthalene exposure. For all workers regardless of smoking habits urinary α-naphthol tends to increase with increasing level of airborne naphthalene exposure. This tendency is also obvious when looking at smokers and non-smokers individually. The calculated statistics for the relationship between these groups are presented in Table 3 giving the Spearman's rank correlation and Pearson's r values for α-naphthol in urine versus airborne naphthalene and total PAH exposure in personally collected air samples. A non-parametric T^2 test (Hotelling-Pabst test) for trends in the observations and a Spearman's rank correlation revealed a clear connection between urinary α-naphthol and air concentrations of naphthalene and total PAH of non-smokers. Naphthalene was present in all samples and represented at least 65% of the total calculated content of PAH compounds.

3.1. Validation

Biological monitoring of exposure to hazardous chemical compounds is widely used in combination with environmental measurements to provide a more reliable assessment of exposure and health risk. In this context it is extremely important to validate the analytical methods concerning detection limit and quantification limit, but also to validate the ability of the method to detect low exposures, i.e., study of dose response relationship at low exposure levels.

In the present study the use of urinary α-naphthol was validated as a marker for exposure to airborne naphthalene and total PAH. A previous paper describes in detail the evaluation of a newly developed fast and easy-to-handle method for determination of α-naphthol in urine [29]. The employed method was statistically controlled, i.e., intercept of the linear functional relationship between the 'true' concentration and the measured concentration was not significantly different from zero (i.e., the 0.95 confidence interval included zero) and the slope was not significantly different from 1, indicating that the recovery of the method is not significantly deviating from 100%.

The validity of measurements of α-naphthol as marker for PAH exposure is difficult to study since PAH compounds are widespread in the environment. It is extremely difficult to find individuals that are exposed to one source of PAH compounds only without confounding with expo-

Table 3
Pearson's r values and estimated association of Spearman's rank correlation

		Spearman's rank correlation		Pearson's r
		$y = ax + b$	ρ	
Naphthalene	Smokers ($N = 54$)	$y = 0.24x + 6.19$	0.054	0.109
	Non-smokers ($N = 35$)	$y = 0.669x + 6.04$	0.481 [a]	0.398
Total PAH [a]	Smokers ($N = 54$)	$y = 0.29x + 6.91$	0.047	0.119
	Non-smokers ($N = 35$)	$y = 0.669x + 6.04$	0.471 [b]	0.396

[a] Sum of fifteen selected PAH compounds, i.e., naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene and indeno[1,2,3-*cd*]pyrene.

[b] $P < 0.01$ for sum of squares of rank differences.

sure from smoking, wood burning, medical treatment, food etc.

3.2. Effect of smoking

In a preliminary screening of employees in an office work place, where the exposure to PAH compounds is restricted to non-occupational sources, (i.e., smoking, diet, medicine), we studied the effect of smoking habits on urinary α-naphthol concentrations. Smoking habits varied from heavy smokers to workers who only smoked a few cigarettes a day. The heavy smokers showed the highest levels of urinary α-naphthol. In order to elucidate to what extend smoking may influence the α-naphthol concentration in urine from workers with known and unknown PAH exposure, the urinary α-naphthol concentrations of smokers and non-smokers were compared for iron foundry workers as well as for workers with unknown occupational exposure (workers from drinking water supply plants, garbage recycling plants, and cotton workers). Table 2 shows that for all four working environments the median values of urinary α-naphthol were higher for smokers when compared with non-smokers. This was highly significant for two of the work environments. For the two other working environments the results of a few non-smokers with unexplainable high α-naphthol levels may have masked the difference. Nevertheless the present study has demonstrated that smoking may be a strong confounder, and that it is very important to register smoking habits carefully when assessing low dose PAH exposure.

3.3. Iron foundry study

The relation between low dose PAH exposure and urinary α-naphthol concentration was studied in two iron foundries. The workers were engaged at seven different tasks. The airborne PAH exposure at each task was determined using personal air sampling collection. A clear connection was observed between urinary α-naphthol and airborne PAH exposure as well as airborne naphthalene exposure (Table 3 and Fig. 1). The relation was dependent on the smoking habits. Smoking tends to mask the level of occupational exposure and hence smokers working at the seven tasks had approximately the same levels of urinary α-naphthol. By contrast, non smokers showed distinct difference in urinary α-naphthol dependent on their working task. Consequently, if effects of low dose PAH exposure is to be studied using α-naphthol in urine as a biomarker, then it may be advantageous to exclude smoker from the study group.

4. Conclusions

In conclusion, this study has demonstrated that urinary α-naphthol is an attractive candidate as a biomarker for PAH exposure measurements. The newly employed LC method is suitable for screening of urinary α-naphthol in a large number of samples from low-dose PAH-exposed individuals. The method is fast and sensitive and is able to detect even small differences in exposure, i.e., different smoking habits, environment or other sources.

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Flow injection amperometric detection of aniline with a peroxidase modified carbon paste electrode

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Abstract

A horseradish peroxidase modified carbon paste electrode, useful for the determination of aniline at low ppb levels, is described. The enzyme was dispersed in the carbon paste and immobilised on a Nafion membrane. Cyclic voltammetry and differential pulse polarography were used to characterise the enzymatic electrochemical process, and several reaction products were identified. The electrode has been utilised for the flow injection amperometric detection of aniline at potentials down to +100 mV (Ag/AgCl), giving fast responses ($t < 30$ s), a measurement precision of 0.75% R.S.D. ($n = 8$) and a sampling frequency of 20 h^{-1}. Application of the electrode to the analysis of aniline in spiked vegetable oil samples, using liquid–liquid extraction, resulted in a procedure giving no matrix interferences and a mean recovery of 99.4 ± 3.2%.

Key words: Amperometry; Flow injection; Aniline; Peroxidase modified carbon paste electrode

1. Introduction

Aniline and other aromatic amines are important industrial chemicals. They are used in the manufacture of dyes, polymers, pesticides, pharmaceutical products, etc. Many of them are suspected carcinogens in humans. Moreover, certain aniline derivatives are classified as priority organic pollutants and their content in waste effluents is regulated. Aniline is also added as a denaturing agent to vegetable oils destined for industrial use to prevent its illegal selling in the food market. Consequently, the determination of aniline and its derivatives is very important from a toxicological and environmental point of view.

Aniline and associated compounds can be determined by gas or liquid chromatography, where high specificity and low detection limits are obtained [1]. Spectrophotometric methods, which involve diazotization and reaction with a suitable coupling agent, followed by measurement of the absorbance of the azo dye formed, are also frequently used [2–6].

Reactions catalyzed by enzymes have long been used for analytical purposes in the determination of different analytes such as substrates, activa-

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tors, inhibitors and also the enzymes themselves [7]. The recent availability of highly active enzyme preparations, at reasonable costs, has extended the use of enzymes as routine reagents. Also, the methods developed for the immobilisation of the enzymes onto solid matrices have increased the versatility of their use. Enzyme electrodes, which combine the selectivity of enzymes with the high sensitivity of electrochemical measurements, can be easily integrated into flow systems, providing an excellent tool for the analytical chemist.

Horseradish peroxidase (HRP), a key enzyme in analytical systems, catalyses the oxidation of a variety of organic and inorganic compounds using H_2O_2 or other peroxo compounds of the ROOH type. Generally, the enzyme reacts according to the following cycle:

$$H_2O_2 + HRP \longrightarrow 2H_2O + \text{compound I}$$

$$\text{compound I} + AH \longrightarrow \text{compound II} + A$$

$$\text{compound II} + AH \longrightarrow HRP + A$$

For the above reaction various substances can act as cofactors, such as hexacyanoferrate(II) [8], *o*-phenylendiamine [9], hydroquinone [10], ferrocene [11], etc. However, there are only a few reported examples of employing aniline as a cofactor. Scheller et al. [12] describe a substrate competition electrode for the determination of aniline and phenol. Aniline competes with hydroquinone for the pseudo-peroxidatic activity of haemoglobin. The decrease of the electrochemical reduction current of benzoquinone in the presence of the alternative substrate serves as the measuring signal. Kulys and Vitziunaite [13] coimmobilised HRP and glucose oxidase (GOD) on the electrode, using ferrocyanide as a cofactor. The ferricyanide formed was reduced at the electrode. Addition of aromatic amines increased the reaction rate and thus the steady current.

Instead of using isolated, purified enzymes, various publications have recently appeared that have utilised biocatalytic active systems, such as microbial cells, cell organelles and tissue slices. Schubert et al. [14] report an organelle electrode for aniline based on immobilised liver microsomes. The microsomal fraction of liver contains a monooxigenase system consisting of cytochrome P-450, NADPH–cytochrome P-450 reductase and phospholipid. Aniline is converted to *p*-aminophenol, which is electrochemically oxidised at the electrode at a potential of +250 mV.

In the present work, an enzymatic electrode for aniline, based on a HRP-modified carbon paste electrode, is described. Different parameters were evaluated and optimized for use in flow-injection systems. Moreover, the method has been applied to the determination of aniline in spiked vegetable oils.

2. Experimental

2.1. Reagents

Horseradish peroxidase (EC 1.11.1.7, type VI A) was purchased from Sigma. Aniline and hydrogen peroxide (30%) were purchased from Merck. Aniline solutions were made daily by dilution of a concentrated solution with the background electrolyte. Nafion solution (5% solution, 1100 equivalent weight) was obtained from Aldrich. The stock solution was diluted with a mixture of 2-propanol and water (1 : 1, v/v). Carbon paste was prepared by mixing 1.8 ml of paraffin oil (Uvasol, Merck) with 5 g of spectroscopic grade graphite powder (Ultra Carbon, Dicoex, Bilbao). All other reagents (hexane, phosphoric acid, sodium hydroxide) were of analytical-reagent grade. The sample examined was sunflower oil denatured with variable amounts of aniline.

2.2. Apparatus

Cyclic voltammetry was performed with an EG & G Princeton Applied Research Model 273 potentiostat, controlled by an IBM-PS/2 computer. Measurements were done in a Metrohm glass cell, using a carbon paste working electrode of 7.1 mm^2 of geometric area. Potentials were measured and referred to a silver/silver chloride/saturated potassium chloride reference electrode. A platinum wire (15 × 1 mm) was used as auxiliary electrode. Differential pulse polarographic measurements were achieved with a Metrohm

E-506 Polarecord coupled with a Metrohm E-505 polarographic stand.

Flow injection amperometric measurements were performed using a twelve cylinders Spetec Perimax 12 peristaltic pump and a six-port rotary valve (Rheodyne 5060) as carrier propulsion and injection systems, respectively. Amperometric detection was carried out in a home-made thin-layer flow cell (Kissinger design) equipped with a carbon paste electrode of 7.1 mm^2 of geometric area. A downstream compartment coupled to the thin-layer cell outlet was put in place containing the reference electrode (silver/silver chloride/saturated potassium chloride) equipped with a low resistance liquid junction, and a stainless-steel waste tube acting as a counter electrode. A Metrohm 641-VA potentiostat was used as amperometric detector and its output was recorded in a Houston OmniScribe D5000 strip chart recorder.

2.3. Procedures

Preparation of HRP modified electrode

The lyophilized HRP preparation was thoroughly mixed with a graphite–paraffin oil carbon paste in the proportion of 5 mg of enzyme preparation and 95 mg of carbon paste. The resulting paste was packed in the well of the thin-layer amperometric cell and covered with a Nafion layer, applied as previously described [11], in order to avoid losses of HRP by solubilisation, under operational conditions.

Aniline extraction procedure

Aniline was extracted from oil samples with 2 M hydrochloric acid. 25 ml of oil, previously diluted with 25 ml of hexane, were shaken with two portions of 10 ml of acid. After phase separation, the aqueous extracts were combined and made up to the desired volume with the supporting electrolyte.

The supporting electrolyte was 0.1 M phosphate buffer (pH 8.0). For flow experiments, the carrier solution and samples contained 1×10^{-4} M H_2O_2.

3. Results and discussion

3.1. Study of the enzymatic process

According to the overall reaction scheme describing the oxidation of organic substrates catalysed by peroxidase enzymes:

$$H_2O_2 + 2DH \xrightarrow{HRP} 2H_2O + 2D$$

aniline (DH) is oxidised yielding a product (D) which is electrochemically reduced providing an amperometric signal related to aniline concentration.

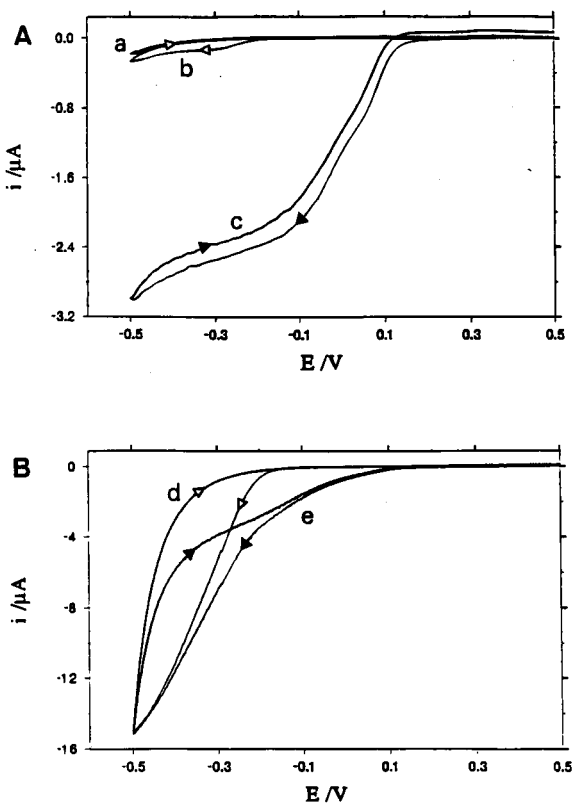

Fig. 1. (A) Cyclic voltammograms using a carbon paste electrode in pH 8.0 phosphate buffer, (a) H_2O_2 1×10^{-3} M; (b) after addition of HRP (25 μg/ml) to the solution and (c) after addition of aniline (1×10^{-4} M). (B) Cyclic voltammograms using a horseradish peroxidase modified carbon paste electrode in pH 8.0 phosphate buffer, (d) H_2O_2 1×10^{-3} M and (e) after addition of aniline (1×10^{-4} M). Sweep rate: 50 mV/s.

Fig. 1A shows the cyclic voltammetric curve at an unmodified carbon paste electrode of a pH 8.0 phosphate buffer containing 1.0×10^{-3} M H_2O_2 (curve a). After addition of 25 μg ml^{-1} of HRP to this solution, the only Faradaic process observed within the scanned potential range (+500 to −500 mV) was the irreversible reduction of HRP with a half peak potential of −240 mV (curve b). Further addition of 1.0×10^{-4} M aniline to the solution containing H_2O_2 and HRP, produced the cyclic voltammogram c, recorded with a time delay of 1 min after aniline addition. It becomes apparent the large catalytic reduction current, starting near +150 mV and showing two overlapping waves, merging slowly with time. An analogous behaviour was observed using a peroxidase-modified carbon paste electrode instead of the soluble enzyme (Fig. 1B). Using the same background electrolyte, and an unmodified carbon paste working electrode, aniline produced an anodic peak at 760 mV (Ag/AgCl) which was not affected in any way by the presence of a large stoichiometric excess of hydrogen peroxide. Hence, the catalytic reduction current observed in the presence of HRP may be useful for the amperometric detection of aniline at low applied potentials. In fact, the available potential range for such detection lies between +150 and −200 mV (Ag/AgCl). At more negative potentials, a rapid loss of HRP activity, associated with the above mentioned cathodic process, was observed.

Some experiments were performed in order to elucidate the nature of this catalytic current. It is well known [15,16] that the anodic reaction of aniline involves a 1 electron oxidation to produce a cation radical, which undergoes some ECE processes yielding *p*-aminodiphenylamine (via head-to-tail coupling) and benzidine (via tail-to-tail coupling). Benzidine is not formed at pH values above 4. Moreover, in alkaline media (pH 7–10) azobenzene is a major product of aniline oxidation. The formation of polymeric products such as emeraldine and nigraniline via a cascade of head to tail coupling and further electrooxidation is also well documented [15]

Fig. 2A shows the curve corresponding to the second cyclic potential scan applied to a carbon paste electrode in an aniline solution in phosphate buffer (pH 8.0). In addition to the aniline oxidation (E_p = 760 mV), a redox system, with peak potentials of +94 mV and +45 mV (oxidation and reduction processes, respectively), can also be observed. This may be assigned to *p*-aminodiphenylamine, since benzidine is not formed at this pH and the couple azobenzene/hydrazobenzene appears to be irreversible at carbon electrodes [17]. Neither the aniline peak nor the +94 mV peak are affected by the presence of hydrogen peroxide in the solution.

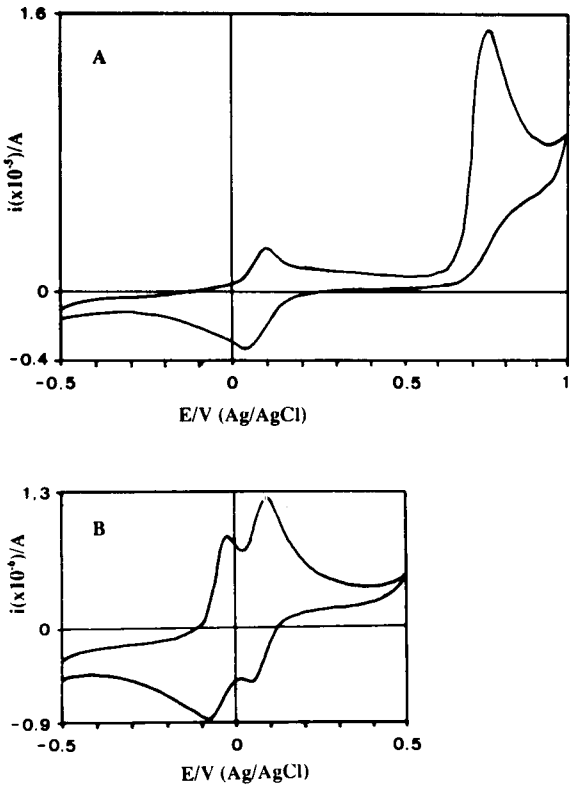

Fig. 2. (A) Cyclic voltammogram of 5×10^{-4} M aniline in the 0.1 M phosphate buffer, pH 8.0; 2nd scan. (B) Cyclic voltammogram of 5×10^{-4} M aniline in the 0.1 M phosphate buffer, pH 8.0, with 1×10^{-3} M H_2O_2 and 2.5 μg/ml HRP in solution; 1st scan. Sweep rate: 50 mV/s.

The enzymatic oxidation of aniline might follow an analogous path as the electrochemical oxidation. Cyclic voltammetry of 5.0×10^{-4} M aniline added to a solution containing 1.0×10^{-3} M H_2O_2 and 2.5 μg/ml of HRP (Fig. 2B) shows two redox couples which appear in the first scan

between −500 mV and +500 mV, where no electrochemical oxidation of aniline takes place. The peak potentials of the second couple (+95 mV and +53 mV for the anodic and cathodic peaks, respectively) are in close agreement with those corresponding to *p*-aminodiphenylamine. The colourless solution becomes brownish after the addition of aniline and exhibits an absorption band at 451 nm.

Peroxidases are known to effect the oxidation of substituted anilines with the formation of appreciable amounts of azobenzenes [18]. It is also known that azobenzene undergoes a two electron reduction at the dropping mercury electrode. Its half-wave potential is found to be dependent on the concentration of the azo compound, the background composition and the presence of surfactants. A half-wave potential of −367 mV (Ag/AgCl) was calculated for azobenzene at pH 8.0 using the $E_{1/2}$–pH profiles provided by Florence et al. [19]. A differential pulse polarogram of the enzymatically oxidized aniline solution shows a cathodic peak at −370 mV (Ag/AgCl). Hence, it can be deduced that azobenzene is also formed in the HRP-catalyzed oxidation of aniline.

The nature of the first redox couple in Fig. 2B (peaks at −21 mV and −72 mV for the anodic and cathodic processes, respectively) remains unknown. The fact that both redox couples (at −21 and +95 mV) show a progressive loss in definition with time, while the colour of the solution becomes more intense and dark, probably indicates that the formation of polymeric products via radical intermediates occurs.

3.2. Flow injection amperometric detection of aniline

Preliminary experiments demonstrate that amperometric detection of aniline in flow systems with a HRP-modified carbon paste electrode is feasible under a wide range of experimental conditions. Stable and low-noise baselines and very low background currents were obtained using carrier streams of acetate or phosphate buffer in the pH range 5.5–8.5, containing different concentrations of hydrogen peroxide. The carrier solutions were degassed by purging with helium prior to starting the operation. Sensitive amperometric signals for aniline standard solution injections were obtained in this pH range with a slight increase in peak currents at pH values below 7. However, 0.1 M phosphate buffer of pH 8.0 was chosen because better long-term stability of the electrode response was obtained using this buffer.

The detection potential was found to influence strongly the electrode sensitivity and stability. The highest sensitivity was achieved at applied potentials near +100 mV. At potentials more negative than 0 mV a rapid decrease in the amperometric signal was observed. Operation below −100 mV produced increasing background currents and led to a rapid and irreversible degradation of electrode performance. Obviously, the electrochemical reduction of immobilized HRP is responsible for the observed background currents and produces a total loss of enzymatic activity.

The influence of the H_2O_2 concentration on the amperometric signal of aniline was examined. No response was obtained below 1.0×10^{-6} M and the highest responses were obtained at the 5×10^{-5} M level. However, a slightly greater concentration (1×10^{-4} M) was routinely used because it gave sharper, well defined peaks.

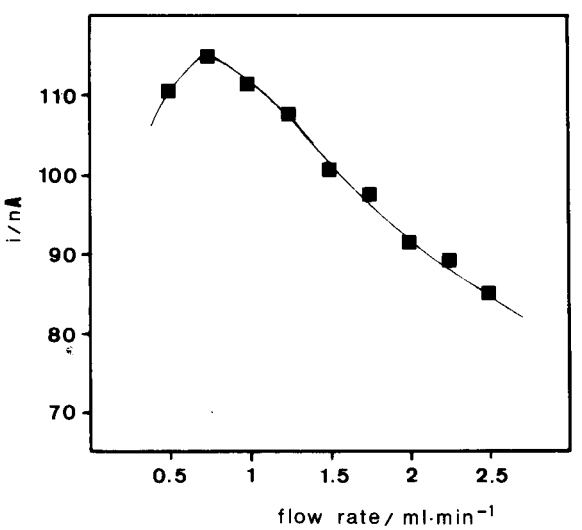

Fig. 3. Variation of the electrode response, *i*, with the flow rate. Applied potential: +100 mV vs Ag/AgCl. Aniline concentration: 5×10^{-5} M.

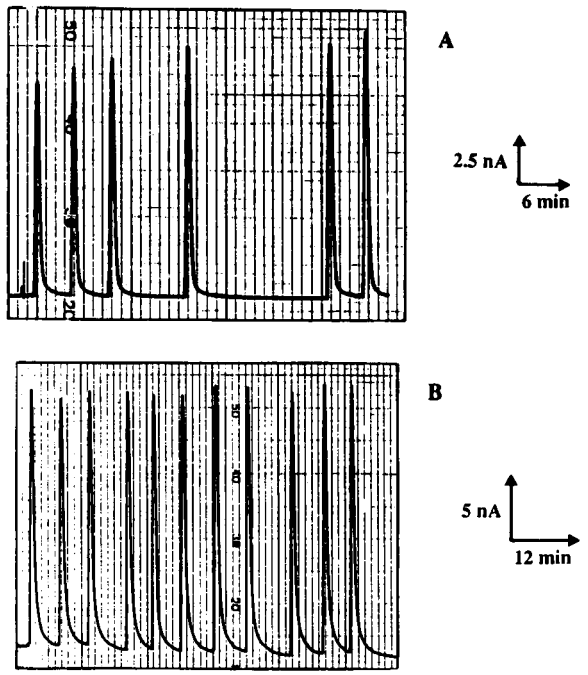

Fig. 4. Effect of the addition of triethylamine to the carrier stream on the FIA signal, (A) no triethylamine added and (B) with 1×10^{-3} M triethylamine added. Samples: 5×10^{-6} M aniline.

The highest sensitivity for repeated injections of 1×10^{-5} M solution of aniline was obtained at a flow rate of 0.75 ml min^{-1}, with significant loss in sensitivity at higher values (Fig. 3). However, at low flow rates, the working electrode showed slight memory effects. This caused an increase in peak current of about 4% of its initial value for each repeated injection of a constant amount of aniline standard solution (Fig. 4A). The magnitude of this effect decreased when a high flow rate (> 3.0 ml min^{-1}) combined with a smaller sample loop (< 350 μl) was used. Unfortunately, such experimental conditions produced a decrease in sensitivity. Otherwise, long washing times, needed to completely eliminate memory effects (over 10 min), dramatically decreased the sampling frequency.

Although the origin of these memory effects is not completely understood, it seems to be related to the adsorption of some unreacted aniline and/or intermediate product onto the modified electrode surface. Thus, the addition of a basic component, in a fairly high concentration, to the carrier stream may block the adsorption sites and suppress the memory effects. This hypothesis was verified by adding triethylamine (TEA) to the carrier buffer. An effective suppression of memory effects was achieved operating at low flow rates (1 ml min^{-1}) with a 500-μl injection loop, when TEA was added to the carrier stream at a 1 mM concentration (Fig. 4B). No adverse effects of TEA were observed on the background current, baseline drift or baseline noise, and good precision was obtained (a R.S.D. of peak current of 0.75% for 8 repeated injections of an aniline standard solution 5.0×10^{-6} M).

The calibration characteristics of the electrode were examined under two sets of experimental conditions. First, using a carrier solution containing no TEA, at a flow rate of 3.0 ml min^{-1} and an injection loop of 350 μl internal volume. Secondly, using a carrier containing TEA 1.0×10^{-3} M at a flow rate of 1.0 ml min^{-1} and a sample volume of 500 μl. In both cases, no memory effects were observed. From the experimental

Table 1
Calibration characteristics

Set-up	Slope (nA mol^{-1} l)	Intercept (nA)	r	Detection limit (mol l^{-1})	Linear dynamic range (mol l^{-1})
1	1.65×10^6	0.62	0.9997 (n = 6)	8.1×10^{-7}	6×10^{-7}–3×10^{-5}
2	7.61×10^6	1.12	0.9994 (n = 4)	1.2×10^{-8}	5×10^{-8}–6×10^{-7}
	2.51×10^6	7.42	0.9996 (n = 6)	–	1×10^{-6}–6×10^{-5}

Set-up 1: carrier composition, 1.0×10^{-4} M H_2O_2 in 0.1 M phosphate buffer (pH 8.0); flow rate, 3 ml min^{-1}; sample loop, 350 μl. Set-up 2: carrier composition, 1.0×10^{-4} M H_2O_2 and 1.0×10^{-3} M TEA in 0.1 M phosphate buffer (pH 8.0); flow rate, 1 ml min^{-1}; sample loop, 500 μl. Data corresponding to the first and second portions of the calibration plot. Detection limit was calculated as the corresponding concentration to three times the standard deviation of the estimate.

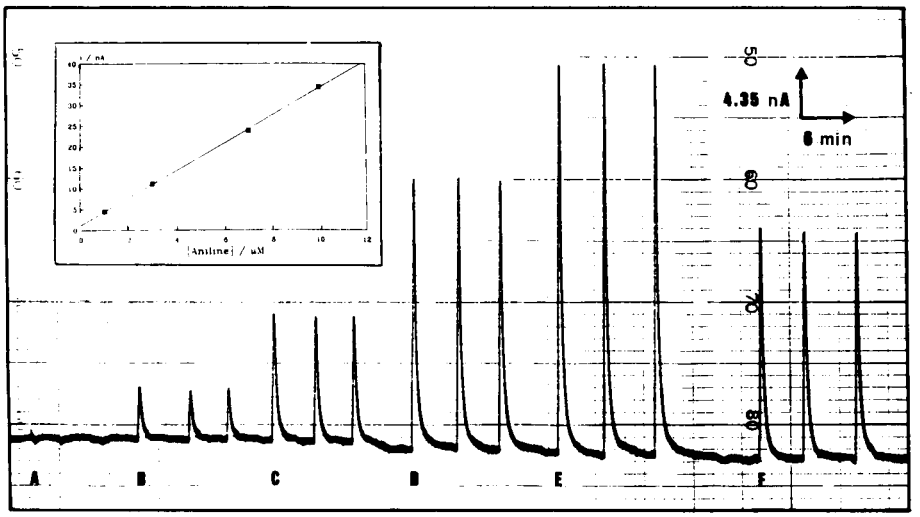

Fig. 5. Flow injection amperometric responses to the aniline standards. Aniline concentrations: (A) 0, (B) 1×10^{-6}, (C) 3×10^{-6}, (D) 7×10^{-6}, (E) 1×10^{-5} M, and (F) oil extract containing 6×10^{-6} M aniline. Conditions: carrier: 0.1 M phosphate buffer, pH 8.0; flow rate: 3 ml/min; loop: 350 μl; applied potential: +100 mV vs Ag/AgCl.

results (Table 1) it becomes apparent that using TEA-containing carrier streams higher sensitivity and lower detection limit (0.4 $\mu g\ l^{-1}$) were obtained, but the calibration plot has two linear portions of different slopes, and the first linear range is restricted to one order of magnitude. Wider linear dynamic ranges were obtained using high flow rates and carriers containing no TEA, but at the cost of lower sensitivity (detection limit of 28 $\mu g\ l^{-1}$). The measurement precision was similar for both experimental set ups. Thus, the selection of appropriate operating conditions is a matter of convenience determined by the expected aniline level in the sample.

2.3. Application to oil samples

Spiked oil samples containing different amounts of aniline were analysed with the HRP-modified electrode under the optimum conditions described above. As aniline is usually added to vegetable oils in small quantities (around 1%), the method was applied to oil samples containing between 0.01% and 1% of aniline.

The extraction of the aniline from the oil was carried out as described in procedures section. The aqueous extract, once made up to the desired volume and the pH adjusted, was directly injected into the flow system and the concentration of aniline was calculated from a calibration plot. Fig. 5 shows typical signals for triplicate injections (350 μl volume) of a blank (A), standard solutions (B–E) and an aniline containing oil extract (F). A blank of oil sample was tested in the same way and did not show any signal. Eight samples of spiked oils containing different concentrations of aniline were analysed, and the results are shown in Table 2. Good recovery (average recovery: 99.4 ± 3.2%) was obtained.

Table 2
Aniline determination in spiked oil samples

	Aniline added (mg/ml)	Aniline found (mg/ml)
Sample 1	0.0	0.0
Sample 2	0.0	0.0
Sample 3	0.100	0.098
Sample 4	0.100	0.095
Sample 5	1.00	1.03
Sample 6	1.00	1.02
Sample 7	10.0	10.17
Sample 8	10.0	9.73

Acknowledgements

Financial support from the Dirección General de Investigación Científica y Técnica DGICYT (PB90/0381) and a bursary from the Analytical Division of the Royal Society of Chemistry from the Anal. Chim. Acta sponsored session at Euroanalysis VII, are gratefully acknowledged.

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Calendar of forthcoming meetings

★ *indicates new or amended entry*

July 31–August 5, 1994
Ottawa, Ont., Canada
8th International Symposium on Molecular Recognition and Inclusion. *Contact:* Mrs. Hguette Morin-Dumais, 8th ISMRI, Steacie Institute for Molecular Sciences, National Research Council Canada, Room 1157, 100 Sussex Drive, Ottawa, Ont., Canada K1A 0R6. Tel.: +1 613 990-0936; Fax: +1 613 954-5242; E-mail: ismri@ned1.sims.nrc.ca.

August 2–6, 1994
Changchun, P.R. China
The Second Changchun International Symposium on Analytical Chemistry (CISAC). *Contact:* Prof. Qinhan Jin, Department of Chemistry, Jilin University, Changchun 130023, P.R. China. Tel.: 0431-822331, ext. 2433; Fax: 0431-823907.

August 22–26, 1994
Hong Kong
ICORS '94. XIV International Conference on Raman Spectroscopy. *Contact:* Prof. Nai-Teng Yu, ICORS '94, c/o Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

August 23–26, 1994
Guildford, UK
QSA-8. International Conference on Quantitative Suface Analysis: Techniques and Applications. *Contact:* Doreen Tilbrook, Division of Materials Metrology, National Physical Laboratory, Teddington, Middlesex TW11 0LW, UK.

August 23–28, 1994
Sapporo, Japan
International Trace Analysis Symposium '94 (ITAS '94). *Contact:* Prof. Hiroto Watanabe, Laboratory of Analytical Chemistry, Faculty of Engineering, Hokkaido University, Sapporo 060, Japan. Tel.: +81 11-716-2111, ext. 6743; Fax: +81 11-726-4454.

August 24–26, 1994
York, UK
International Symposium on Capillary Electrophoresis (jointly with the Chromatographic Society and the British Electrophoresis Society). *Contact:* Dr. T.L. Threlfall, Industrial Liason Executive, Department of Chemistry, University of York, York, YO1 5DD, UK. Tel: +44 904 432576 or 904 432511; Fax: +44 904 432516.

★ ***September 4–7, 1994***
Warsaw, Poland
East European Furnace Symposium. *Contact:* Dr. Ewa Bulska, University of Warsaw, Department of Chemistry, Ul. Pasteura 1, 02-093 Warsaw, Poland. Fax: +48 (22) 22 59 96.

★ ***September 5–6, 1994***
Graz, Austria
Workshop on Evaluation of Measurement Uncertainty in Chemical Analysis. *Contact:* Prof. W. Wegscheider, Technische Universität Graz, Technikerstrasse 4, A-8010 Graz, Austria.

★ ***September 5–9, 1994***
Friedrichshafen, Germany
GCL '94. Tenth International Symposium on Gas Flow and Chemical Lasers. *Contact:* Cologne Communication Management GmbH, Kreuzgasse 2-4, Postfach 18 01 80, D-50504 Koln, Germany. Tel.: +49 221-925793-0; Fax: +49 221 925793-2.

★ ***September 6, 1994***
Glasgow, UK
The Royal Society of Chemistry — Pre-Doctoral Symposium. *Contact:* Dr. John F. Gibson, Secretary (Scientific), The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK. Tel.: +44 71 4378656; Fax: +44 71 7341227.

★ ***September 7–9, 1994***
Glasgow, UK
The Royal Society of Chemistry — 1994 Autumn Meeting. *Contact:* Dr. John F. Gibson, Secretary (Scientific), The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK. Tel.: +44 71 4378656; Fax: +44 71 7341227.

September 11–16, 1994
Essen, Germany
EUCMOS XXII. XXII European Congress on Molecular Spectroscopy. *Contact:* Congress Secretariat, Gesellschaft Deutscher Chemiker, Abt. Tagungen, P.O. Box 900440, W-6000 Frankfurt 90, Germany. Tel.: +49 69 7917-366; Fax +49 69 7917-475; Telex 4 170 497 gdch d. *(Further details published in Vol. 272, No. 2).*

September 12–14, 1994
Paris, France
Workshop on Biosensors and Biological Techniques in Environmental Analysis. *Contact:* Prof. M.-C. Hennion, ESPCI, Lab. Chimi Analytique, 10 rue Vauquelin, 75005 Paris, France.

September 12–15, 1994
Portland, OR, USA
108th AOAC International Annual Meeting and Exposition. *Contact:* Margaret Ridgell, AOAC International, 2200 Wilson Blvd., Suite 400, Arlington, VA 22201-3301, USA. Tel.: +1 703-522-3032; Fax: +1 703-522-5458.

September 12–17, 1994
Madrid, Spain
Frontiers in Analytical Chemistry: Surface and Interface Analysis. A course organized with the ERASMUS Network "Analytical Chemistry". *Contact:* Prof. Dr. A. Sanz-Medel, Universidad di Oviedo, Quimica Fisica e Analitica, E-33006, Oviedo, Spain. Tel.: +34 8 510-3474; Fax: +34 8 50-3480.

★ ***September 14–15, 1994***
Manchester, UK
Waterborne Coatings and Additives — Joint Symposium of The Royal Society of Chemistry and Society of Chemical Industry. *Contact*: Mrs. C.L. Sharp, Conference Secretary, 41 Exeter Road, Davyhulme, Manchester M41 0RF, UK. Tel./Fax: +44 61 7474961.

★ ***September 18–22, 1994***
Ambleside, UK
Geoanalysis 94 — An International Conference on the Analysis of Geological and Environmental Materials. *Contact*: Geoanalysis 94 Conference Secretariat, Analytical Geochemistry Group, British Geological Survey, Keyworth, Nottingham NG12 5GG, UK. Tel.: +44 (0)602 363349; Fax: +44 (0)602 363200; Telex: 378173 bgskey g; E-mail: k_snrc@uk.ac.nerc-keyworth.vaxa.

September 18–22, 1994
Chambéry, Savoy, France
14th International CODATA Conference. Data and Knowledge in a Changing World: The Quest for a Healthier Environment. *Contact:* Prof. J.-E. Dubois, ITODYS, Université Paris 7, 1 rue Guy de la Brosse, 75005 Paris, France. Fax: +33 1 42881466. E-mail: codata@paris7. jussieu.fr (Internet).

September 19–22, 1994
Turin, Italy
International Ion Chromatography Symposium 1994. *Contact*: Century International, P.O. Box 493, Medfield, MA 02052, USA. Tel.: +1 508/359-8777; Fax: +1 508/359-8778.

September 21–23, 1994
Stockholm, Sweden
5th International Symposium on Pharmaceutical and Biomedical Analysis: *Contact:* Swedish Academy of Pharmaceutical Sciences, P.O. Box 1136, S-111 81 Stockholm, Sweden. Tel.: +46 8 245085; Fax: +46 8 205511.

September 22–24, 1994
Constanta, Romania
12th Conference on Analytical Chemistry. *Contact:* Dr. Gabirel-Lucian Radu, Romanian Society of Analytical Chemistry, 13 Blvd. Carol I, Sector 3, 70346 Bucharest, Romania.

September 25–30, 1994
Goa, India
ISMEBC '94. International Conference on Molecular Electronics and Biocomputing. *Contact*: Dr. Ratna S. Phadke, Scientific Secretary for ISMEBC '94, Chemical Physics Group, Tata Institute of Fundamental Research, Homi Bhabha Road, Bombay 400 005, India. Tel: +91 (22) 215 2971; Fax: +91 (22) 215 2110 e-mail: mebc@tifrvax.bitnet mebc@tifrvax.tifr.res.in.

September 25–30, 1994
Bristol, UK
1994 European Workshop in Chemometrics. *Contact*: Janice Green, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK. Tel: +44 (0)272 303030 ext. 4421 (ansaphone) or +44 (0)272 303672; Fax: +44 (0) 272 251295.

September 26–28, 1994
Basel, Switzerland
Chemometrics: Multivariate Analysis and Design in Chemical Research and Development. *Contact*: S. Morgenthaler, ASS, EPFL, Dept. de Mathématiques, CH-1015 Lausanne, Switzerland.

October 3–7, 1994
St. Peterburg, Russia
ISCMS '94. International Symposium: Chromatography and Mass Spectrometry in Environmental Analysis. *Contact:* ISCMS '94, Dr. Alexander Rodin, State Institute of Applied Chemistry, Dobrolubov Ave. 14, 197198, St. Petersburg, Russia. Tel.: +7 812 2389786; Fax: +7 812 2338989; Telex: 121345 ptb sigma.

October 11–13, 1994
Amsterdam, The Netherlands
6th International Colloquium Solid Sampling with Atomic Spectroscopy. *Contact*: Prof. Dr. R.F.M. Herber, Coronel Laboratory, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

★ ***October 14–15, 1994***
Hong Kong
CITAC '94 Hong Kong Symposium on Traceability and Comparability of Analytical Measurements. *Contact*: Dr. T.L. Ting, CITAC 94 Hong Kong Symposium Secretariat, c/o Government Laboratory, Ho Man Tin Government Offices, 88 Chung Hau Street, Homantin, Hong Kong. Tel.: (+852) 7623706; Fax: (+852) 7144083

October 17–19, 1994
Strasbourg, France
3rd International Symposium on Supercritical Fluids: Thermodynamics, Physico-chemical Properties, Technology and Applications. *Contact:* ISASF, Mle. Brionne, ENSIC, P.O. Box 451, F-54001 Nancy Cedex, France. Tel.: +33 83175003; Fax: +33 83350811.

★ ***October 19–22, 1994***
Pasadena, CA, USA
Personal Identification. Meeting of the Forensic Science Society. *Contact*: Forensic Science Society, Clarke House, 18a Mount Parade, Harrogate, UK. Tel.: +44 423-506068; Fax: +44 423-530948.

October 31–November 2, 1994
Minneapolis, USA
Anabiotec '94. 5th International Symposium on Analytical Methods, Systems and Strategies in Biotechnology. *Contact*: Anabiotec Conference Secretariat, Elsevier Advanced Technology, Mayfield House, 256 Banbury Road, Oxford, OX2 7DH, U.K., Tel: +44 (0)865 512242, Fax: +44 (0)865 310981.

★ ***November 6–12, 1994***
Venezuela
Third Rio Symposium on Atomic Spectrometry. *Contact*: José Alvarado, Departamento de Química, Universidad Simón Bolívar, Apartado 89000, Caracas 1080-A, Venezuela. Fax: +58-2 9621695/938322.

★ ***November 7–8, 1994***
Montreux, Switzerland
Short Course on LC/MS, SFC/MS and CE/MS (preceding 11th Montreux Symposium). *Contact*: Dr. A. Donzel, Scitec S.A., Lausanne, Switzerland. Tel.: +41 21-241533; Fax: +41 21-241549.

★ ***November 8–10, 1994***
Helsinki, Finland
KEMIA 94. The Finnish Chemical Congress and Exhibition. *Contact*: Ms Ritva Becker. Tel.: +358-0-1509 211.

November 9–11, 1994
Montreux, Switzerland
11th Montreux Symposium on Liquid Chromatography–Mass Spectrometry (LC/MS; SFC/MS; CE/MS; MS/MS). *Contact:* M. Frei-Häusler, Postfach 46, CH-4123 Allschwil 2, Switzerland. Tel.: +41 61-4812789; Fax: +41 61-4820805.

November 21–22, 1994
Enschede, The Netherlands
μTAS'94. Workshop on Micro Total Analysis Systems. *Contact:* Dr. Albert van den Berg, University of Twente, MESA Research Institute, P.O. Box 217, 7500 AE Enschede, The Netherlands. Tel. +31 53 892 691; Fax: +31 53 309 547.

★ ***January 22–25, 1995***
Yokohama, Japan
International Symposium on Chromatography. 35th Anniversay of Research Group on Liquid Chromatography in Japan. *Contact*: Dr. Toshihiko Hanai, International Institute Technol. Analysis, 3-492 Marsumi, Eclairer 2-913, Kanagawaku, Yokohama 221, Japan. Fax: +81 45-402-6361.

March 6–10, 1995
PITTCON '95. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. *Contact:* Pittsburgh Conference, Suite 332, 300 Penn Center Blvd., Pittsburgh, PA 15235-9962, USA.

★ ***April 4–7, 1995***
Rome, Italy
Short Course on Chiral Resolution. *Contact*: Dr. Salvatore Fanali or Dr. Massimo Sinibaldi, CNR, Istituto di Cromatografia, C.P{. 10, 00016 Monterotondo Scalo (Roma), Italy. Tel.: +39 6-90625328/90625836; Fax: +39 6 90625849; Telex: 624809 CNR MLI.

May 9–12, 1995
Jülich, Germany
6th International Hans Wolfgang Nürnberg Memorial Symposium on Metal Compounds in Environment and Life, 6: Analysis, Speciation and Specimen Banking. *Contact:* Dr. H.W. Dürbeck, Institute of Applied Physical Chemistry, Research Center, Jülich (KFA), P.O. Box 1913, D-5170 Jülich, Germany.

June 5–8, 1995
Singapore
Fifth Symposium on Our Environment and First Asia-Pacific Workshop on Pesticides. *Contact:* The Secretariat, 5th Symposium on Our Environment, c/o Department of Chemistry, National University of Singapore, Kent Ridge, Rep. Singapore 0511. Fax: +65 779-1691.

July 9–15, 1995
Hull, UK
SAC 95. *Contact:* Analytical Division, The Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN, UK. Tel.: +44 71 437-8656; Fax: +44 71 734-1227.

August 27–September 1, 1995
Leipzig, Germany
CSI XXIX. Colloquium Spectroscopicum Internationale XXIX. *Contact:* Gesellschaft Deutscher Chemiker, Abt. Tagungen, P.O. Box 90 04 40, D-60444 Frankfurt/ Main, Germany.

August 27–September 1, 1995
Budapest, Hungary
10th International Conference on Fourier Transform Spectroscopy. *Contact:* Mrs. Klára Láng/Mr. Attila Varga, Conference Office, Roland Eötvös Physical Society, P.O. Box 433, H-1371 Budapest, Hungary. Tel./Fax: +36 1 201-8682.

★ ***September 3–8, 1995***
Villeneuve d'Ascq, France
6th European Conference on the Spectroscopy of Biological Molecules. *Contact*: Professor J.C. Merlin or Dr. S. Turrell, ECSBM'95, LASIR, Université des Sciences et Technologies de Lille, Bât. C5, 59655 Villeneuve d'Ascq Cedex, France. Tel.: +33 20436988 (JCM) or +33 20434920 (ST); Fax: +33 20436755; E-mail: ECSBM95@univ-lille1.fr.

September 4–5, 1995
Paris, France
Sample Handling of Pesticides in the Aquatic Environment. Short course preceding the 5th Workshop on Chemistry and Fate of Modern Pesticide. *Contact:* Prof. M.-C. Hennion, ESPCI, Lab. Chimi Analytique, 10 rue Vauquelin, 75005 Paris, France.

September 6–8, 1995
Paris, France
5th Workshop on Chemistry and Fate of Modern Pesticides. *Contact:* Prof. M.-C. Hennion, ESPCI, Lab. Chimi Analytique, 10 rue Vauquelin, 75005 Paris, France.

September 12–15, 1995
Leuven, Belgium
5th International Symposium on Drug Analysis. *Contact:* Prof. J. Hoogmartens, Drug Analysis '95, Institute of Pharmaceutical Sciences, Van Evenstraat 4, B-3000 Leuven, Belgium. Tel.: +32 16 283440; Fax: +32 16 283448.

★ ***February 6–9, 1996***
Bruges, Belgium
HTC 4. Fourth International Symposium on Hyphenated Techniques in Chromatography: Hyphenated Chromatographic Analyzers. *Contact*: Royal Flemish Chemical Society, Working Party on Chromatography, c/o Dr. R. Smits, BASF Antwerpen N.V., Central Laboratory, Haven 725, Scheldelaan 600, B-2040 Antwerp, Belgium. Tel.: +32 3-5612831; Telex: 31047 basant b; Fax: +32 3-5613250.

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	S'93	O'93	N'93	D'93	J	F	M	A	M	J	J	A
Analytica Chimica Acta	281/1 281/2 281/3	282/1 282/2 282/3	283/1 283/2	283/3 284/1 284/2	284/3 285/1–2 285/3	286/1 286/2 286/3	287/1–2 287/3 288/1–2	288/3 289/1 289/2	289/3 290/1–2 290/3	291/1–2 291/3 292/1–2	292/3 293/1 293/2–3	294/1 294/2 294/3
Vibrational Spectroscopy		6/1			6/2		6/3		7/1		7/2	

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Edited by **R.F.M. Herber** and **M. Stoeppler**

Techniques and Instrumentation in Analytical Chemistry Volume 15

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