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SPECIAL ISSUE

6TH SYMPOSIUM ON ION CHROMATOGRAPHY

Sils-Maria (Switzerland), April 9–12, 1989

Guest Editors

P. R. HADDAD
(Kensington)

N. H. VELTHORST
(Amsterdam)

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Journal of Chromatography, 482 (1989) 266
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Foreword

The Sixth Symposium on Ion Chromatography was held under the auspices of the International Association of Environmental and Analytical Chemistry in Sils-Maria, Switzerland, from April 9th to April 12th, 1989.

In the opening session, the late Professor Roland W. Frei, who was to have been the chairman of this symposium, was commemorated. With his death on January 29th, 1989, the analytical chemical community has lost a renowned, stimulating scientist and warm-hearted friend.

The symposium was attended by ninety participants, from both industry and academia. The rapid progress made in the field of ion chromatography was clearly shown in 36 presentations, 24 lectures and 12 posters, concerning sample collecting and treatment techniques, new stationary phases based on silica and polymers, complexation reactions and ligand-exchange phenomena. New detection methods were presented with particular emphasis on those based on luminescence. The growing importance of ion chromatography in environmental chemistry and biochemistry, and for the analysis of inorganic materials, was well illustrated.

The participants felt that the symposium met the demands of scientists doing research in the field of ion chromatography and they concluded in the closing session that continuation of these symposia would be extremely useful.

The organisers of the symposium, staff members of the Department of General and Analytical Chemistry at the Free University of Amsterdam, wish to express their appreciation to those who actively participated by presenting a lecture or poster, and/or by taking part in the discussions during the presentations or in the lobby of the comfortable 'Waldhaus' hotel.

Finally, we hope that the material published in this issue will contribute to further advances in ion chromatography.

N. H. VELTHORST
Chairperson of the symposium

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SAMPLE CLEANUP METHODS FOR ION CHROMATOGRAPHY

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SUMMARY

Sample cleanup procedures for ion chromatography are reviewed. Ion-exchange resins in the batch mode are shown to be useful for pH adjustment, but suffer from some practical problems. The principles of membrane techniques, such as passive dialysis, Donnan dialysis and electrodialysis are discussed and these methods are shown to be especially suitable for ion chromatography. Chemical modification of the sample using disposable cartridge columns or precolumn reactions are discussed, with particular attention to practical aspects. Numerous applications of the above procedures are provided.

INTRODUCTION

Sample handling includes such steps as sample collection, dissolution, cleanup, trace enrichment, and matrix elimination. All of these steps are important and are usually interrelated, but the sample cleanup step often presents difficult practical problems which are unique to ion chromatography (IC). This review will focus on some of the sample cleanup procedures which may be employed in IC. The additional phases of sample handling which are listed above will not be discussed.

After the sample has been dissolved, it is often necessary that some modification of the sample digest be performed before an injection can be made onto the ion chromatograph. This modification may involve a simple filtration step, or it may be more extensive and involve selective removal of the analyte from the sample or removal of interfering matrix components. Alternatively, it may be necessary to change the chemical form of the analyte to improve its separation or detection in the final analysis.

These cleanup procedures often take the majority of the total analysis time and contribute significantly to the final cost of the analysis, both in terms of labour and the consumption of materials. In addition, manipulation of the sample can often introduce a major source of imprecision which can greatly outweigh any variables in the chromatographic process itself. In many cases, the degree of success achieved in the sample cleanup step determines the ultimate success of the analysis.

Sample cleanup can be performed off-line, prior to the chromatographic analysis, or can be incorporated as an on-line process linked with the chromatographic

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hardware. The emphasis of this review will be on the off-line, or batch, methods since these are most commonly used. The goals of cleanup are to achieve: (i) reduction of the overall loading of sample on the column in order to prevent peak distortion and loss of chromatographic efficiency; (ii) removal of matrix interferences; (iii) concentration or dilution of the analyte; (iv) preparation of the sample in the solution most appropriate to the analysis.

SAMPLE CLEANUP PROCEDURES IN IC

Sample filtration

As with all other liquid chromatographic methods, ion chromatography requires that the sample be free from particulate matter to prevent fouling of capillary tubing, column end frits and other hardware components. Fortunately, sample filtration is very straightforward if disposable filter units are employed. Careful attention must be paid to sample contamination[1], particularly by nitrate ion released from the filter membrane. Ultrafiltration devices wherein the sample is forced under pressure through a membrane, can also be applied to difficult samples; for example, the removal of free calcium and magnesium ions from protein material in biological samples such as serum, milk and egg white[2].

Chemical modification of the sample using ion-exchange resins

Perhaps the most common chemical modification of the sample performed in ion chromatography is adjustment of the pH of strongly acidic or alkaline samples. Injection of such samples without pH adjustment usually produces an unacceptable chromatogram because of baseline disturbances. In particular, system peaks are often caused by large discrepancies in pH between the sample and eluent. This is especially true when aromatic carboxylate salts are used as eluents with indirect UV absorption detection.

It is usually not possible to adjust the sample pH by simple addition of acid or base because of contamination of the sample by the acid anion or base cation, since these species may be of interest in the sample. In such cases it is often possible to use an ion-exchange resin in the batch mode to perform the pH adjustment. For example, high-capacity cation-exchange resin in the hydrogen form can be added to an alkaline sample in order to lower the pH. Similar procedures can be designed to suit different sample types by varying the form of the resin used to achieve alternative chemical modification of the sample. For example, a cation-exchange resin in the silver form will result in the precipitation of chloride from the sample, or a cation-exchange in the barium form can be used to lower the sulfate concentration in a sample.

This approach is simple and relatively effective, but suffers from a number of drawbacks. First, the sample volume required is large and the reaction time must be adjusted whenever the composition of the sample changes. Second, the resin used must be cleaned thoroughly to prevent contamination of the sample by ions leached from the resin material. Third, the sample volume may change due to uptake or release of solvent from the resin. Finally, some loss of sample components may occur due to adsorption on the resin.

TABLE I

APPLICATIONS OF SAMPLE CLEANUP USING ION-EXCHANGE RESINS

Sample	*Species determined*	*Resin type*	*Resin form*	*Purpose of cleanup*	*Ref.*
Bread	BrO_3^-	Dowex 50W-X8-10	Ag^+	Cl^- removal	3
Brine	Anions	Dionex ICE suppressor	Ag^+	Cl^- removal	4
Brine	SO_4^{2-}	Cation exchanger	H^+	Cl^- removal	5
Water	Aldehydes	Dowex 1X8	Acetate	Cl^- removal	6
Water	Anions	Bio-Rad X-4, X-8, X-16	Ag^+	Cl^- removal	7
NaOH	Anions	Rexyn 101 16–50 mesh	H^+	pH reduction	8
Na_2CO_3 fusion melt	Anions	Bio-Rad AG50W-X12	H^+	pH reduction	9
Water	F^-, SiO_3^{2-}	Dowex, 50W-X8	H^+	Cation removal	10
HCl	Cations	Anion exchanger	OH^-	pH increase	11
Urine	Br^-	Cation exchanger	CO_3^{2-}	Removal of interferences	12

Cleanup with ion-exchange resins in the column mode is also common in IC. Here the resin is packed into a suitable container (which may be as simple as a Pasteur pipet), and the sample passed through. The principles discussed above for cleanup using the batch method apply equally well to the column mode. Some applications of the use of resins for sample cleanup are given in Table I. This table illustrates the common requirement for removal of chloride from samples.

Chemical modification of the sample using membranes

Dialytic techniques, in which selected sample components are transferred across a membrane, may be subdivided into *passive* dialysis and *active* (or Donnan) dialysis procedures. Passive dialysis involves diffusion of particles of a specified molecular weight range through a neutral membrane. On the other hand, active or Donnan dialysis is the transfer of ions of a specified charge sign through an ion-exchange membrane. When the dialysis is performed under the influence of an electric field, it is termed *electrodialysis*. Each of these approaches has been applied to the cleanup of samples for ion chromatography.

Passive dialysis

Passive dialysis is a very slow process which requires appreciable volumes of sample (*e.g.* 5 ml) and normally results in severe sample dilution. These factors have mitigated against its widespread use. Nordmeyer and Hansen[13] have described an automated device for the rapid dialysis of very small samples (*e.g.* 40 μl) which enables direct injection of the dialysate onto an ion chromatograph. This device is shown schematically in Fig. 1, from which it can be seen that the sample is introduced into the annular cavity formed between a hollow dialysis fibre and an external, concentrically mounted, small diameter PTFE tube. The eluent is contained inside the fibre and flow is stopped whilst solute components from the sample dialyse into the interior of the hollow fibre. Because of the small volumes involved, dialysis time is very short (typically less than 1 min), and the sample is then injected directly onto the ion

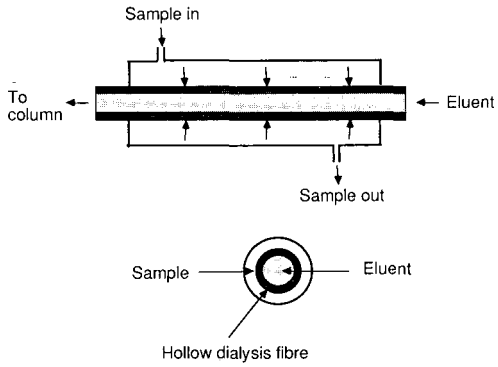

Fig. 1. Schematic representation of a passive dialysis-injection device. Adapted with permission from ref. 13.

chromatograph. When applied to the removal of free calcium from human serum, linear calibration curves were obtained and peak heights showed a relative standard deviation of less than 5% over a two-week period.

Donnan dialysis

Active or Donnan dialysis involves the transfer of ions through membranes which carry an ion-exchange functionality[14–16]. The process can be illustrated by reference to a dialysis system comprising 0.1 *M* NaCl (solution 1) separated from 0.001 *M* KCl (solution 2) by a cation-exchange membrane. This experimental arrangement is shown in Fig. 2. It can be shown that, at equilibrium, the following equation holds:

$$\frac{[Na^+]_1}{[Na^+]_2} = \frac{[K^+]_1}{[K^+]_2} \tag{1}$$

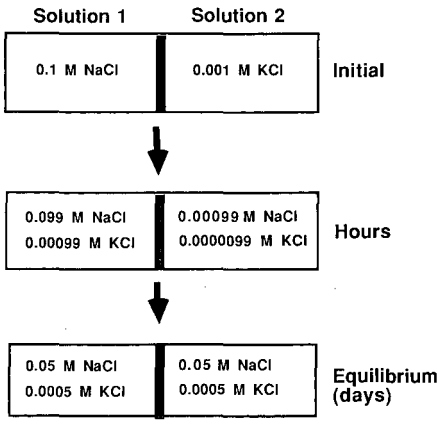

Fig. 2. Schematic representation of Donnan dialysis. The solid central line represents a cation-exchange membrane which separates solutions 1 and 2.

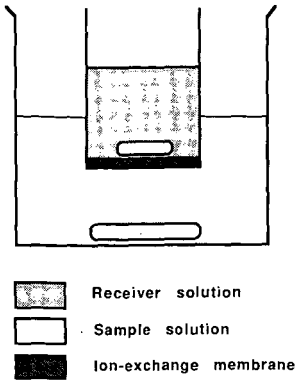

Fig. 3. Simple apparatus for Donnan dialysis.

where the brackets represent molar concentrations and the subscripts refer to the two solutions.

There is a strong tendency for the sodium ions to diffuse from the high concentration zone (solution 1) to the low concentration zone (solution 2). As this process occurs, corresponding transfer of potassium ions from solution 2 to solution 1 proceeds in order to preserve electroneutrality. Thus diffusion of 1% of the sodium into solution 2 is accompanied by transfer of 99% of the potassium into solution 1. If the volume of solution 1 is less than that of solution 2, then sample preconcentration can be accomplished. Eventually the system will attain chemical equilibrium, but this state is achieved only slowly because tranfer of chloride across the membrane is hindered. In the short term therefore, sample modification occurs. A simple form of apparatus for Donnan dialysis is shown in Fig. 3.

In terms of ion chromatographic sample cleanup, Donnan dialysis provides both matrix normalization and sample preconcentration. That is, moderate amounts of potential interferents, such as suspended solids, neutral solutes and ions of opposite charge sign to that of the analyte, neither influence the rate of Donnan dialysis nor are transported to a significant degree into the receiver[16–18]. At this stage, we will focus on the matrix normalization capabilities of Donnan dialysis, for which two distinct possibilities exist. First, Donnan dialysis can be used to selectively add an ion to a sample, or second, to remove a selected species from a sample.

Selective addition of an ion to the sample. This is the most commoonly employed application of Donnan dialysis in IC. It will be noted from Fig. 2 that an ion from the receiver solution enters the sample solution during the dialysis. Thus use of an acid as the receiver will result in transfer of hydrogen ions into the sample, which can be useful if the sample is highly caustic. This treatment is, in effect, the same process by which chemical suppression of the eluent is achieved in suppressed IC. Sample treatment using this method can be illustrated by the dialysis of sodium hydroxide solution using sulfuric acid as the receiver solution. Here hydrogen ions from the sulphuric acid solution exchange with sodium ions from the sodium hydroxide through a cation-exchange membrane. The pH of the sample is therefore lowered, whilst the anion content is theoretically unaltered, allowing subsequent determination of these anions by IC.

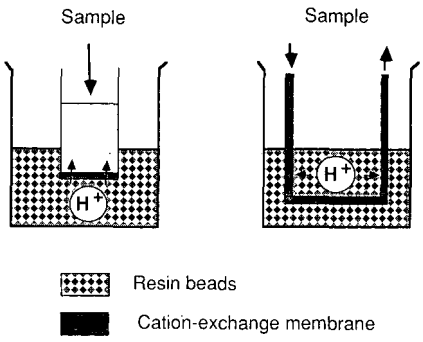

Fig. 4. Apparatus for dual ion exchange.

This method suffers from a practical limitation which seriously detracts from its routine use. This limitation is that the cation-exchange membrane is not entirely impervious to sulphate ions from the receiver solution, which means that the sample ultimately becomes contaminated with sulphate during analysis. This problem can be minimised by increasing the permselectivity of the membrane (*i.e.* its ability to permit the transfer of ions of only one charge sign), or by using an acid whose anion shows little tendency to penetrate the membrane.

An attractive alternative to the use of an acid as the receiver solution has been reported by Cox and Tanaka[19], who used a slurry of ion-exchange resin in the hydrogen form in place of the receiver solution. Since the counter anion is therefore the resin bead itself, transfer across the membrane is eliminated for physical reasons. This process has been called "dual ion-exchange" and Fig. 4 shows the apparatus used. It should be noted that the ion-exchange membrane may also be used in the form of a tube inserted into the resin slurry[20].

Selective removal of an ion from the sample. The second type of application of Donnan dialysis to sample cleanup in IC involves the extraction of the analyte ion(s) into a suitable receiver. This process accomplishes sample normalization, since the analyte ions are ultimately collected in a solution of known composition. A potential problem exists with this method in that determination of the analyte(s) by IC may be precluded by interference from the high concentration of ions in the receiver electrolyte. One possible solution to this problem is to use a carbonate salt solution as the receiver and to further treat this solution by the dual ion-exchange procedure discussed above. The carbonate and bicarbonate in the receiver are converted in the dual ion-exchange step to carbonic acid, following which the sample can be injected directly or the dissolved carbon dioxide removed prior to sample injection. The combination of Donnan dialysis and dual ion-exchange is a powerful method for the treatment of complex samples.

In both of the above methods of sample treatment, the membrane can be in sheet or tubular form[21]. It has been demonstrated that transfer of solutes across the membrane surface is improved if the sample is recirculated around the outside of the membrane tubing during dialysis[22]. Table II shows some applications of Donnan dialysis sample cleanup in ion chromatography.

TABLE II

SOME APPLICATIONS OF SAMPLE CLEANUP FOR IC USING MEMBRANE TECHNIQUES

Sample	*Analytes*	*Process*[a]	*Membrane*	*Receiver*	*Ref.*
NaOH	Cl^-, NO_3^-, SO_4^{2-}	DIE	Nafion 811 cation	Dowex 50WX4 (H^+)	20
NaOH	NO_3^-, SO_4^{2-}	ME	Nafion 901 cation	–	23
NaOH	PO_4^{3-}	DIE	Nafion 117 cation	Dowex 50WX4 (H^+)	19
Na_2CO_3	Cl^-, NO_3^-, SO_4^{2-}	DIE	Nafion 811 cation	Dowex 50WX4 (H^+)	20
NaCl	SO_4^{2-}	DIE	Nafion 811 cation	Dowex 50WX4 (H^+)	20
Sugar[b], syrup[b]	Anions	DD	RAI R-1035 anion	Na_2CO_3–$NaHCO_3$	17
		DIE	Nafion 117 cation	Dowex 50WX4 (H^+)	
River water	Anions	DD	RAI R-1035 anion	Na_2CO_3–$NaHCO_3$	17
		DIE	Nafion 117 cation	Dowex 50WX4 (H^+)	
Coal[c]	S (as SO_4^{2-})	DIE	Nafion 117 cation	Dowex 50WX4 (H^+)	17
Coal[c]	Cl (as Cl^-)	DD	RAI R-1035 anion	Na_2CO_3–$NaHCO_3$	17
		DIE	Nafion 117 cation	Dowex 50WX4 (H^+)	
Leaves[b]	Cl^-, NO_3^-, SO_4^{2-}	DIE	Nafion 117 cation	Dowex 50WX4 (H^+)	17
$AgNO_3$	Cations	DIE	RAI R-1035 anion	Dowex 50WX4 (H^+)	19
Polyelectrolyte	Anions	DD	Homemade anion	Na_2CO_3–$NaHCO_3$	24
Serum	Ca^{2+}	PD	Cuprophane C1IM	H_2O	13

[a] DIE = dual ion-exchange, DD = Donnan dialysis, PD = passive dialysis, ME = membrane electrolysis.

[b] Sample treated by carbonate fusion.

[c] Sample treated by oxygen bomb combustion using Eschka mixture (Na_2CO_3–MgO, 1:2) as absorbing solution.

Electrochemical dialysis

Further refinement to dialysis methods can be achieved by coupling electric fields with membrane processes. For example, the transfer of ions through a membrane can be stimulated by application of an electric field across the membrane; this process is known as electrodialysis. The apparatus shown in Fig. 3 can be easily modified to include platinum gauze electrodes on either side of the membrane. This approach has been applied to the electrodialysis of metal ions using a cation-exchange membrane, $NaNO_3$ as the receiver and a 5 V/cm (peak-to-peak) sine wave potential at

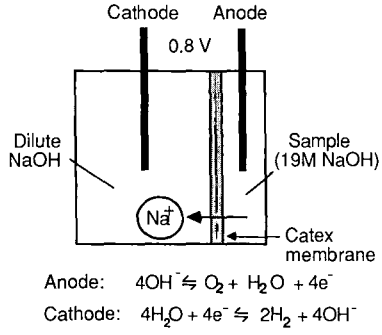

Fig. 5. Apparatus for electrodialysis of highly caustic samples[23].

TABLE III

TYPICAL PACKINGS FOR DISPOSABLE CARTRIDGE COLUMNS

Silica	C_{18}
Alumina (acidic, basic, neutral)	Anion exchange
Cation exchange (H^+ or metal form)	Polystyrenedivinylbenzene
Activated carbon	Polyvinylpyrrolidine
Chelating resins	Amino-bonded silica

1 MHz frequency[25]. The rate of transfer through the membrane was increased by up to 2.7 times as a result of application of the potential. Electrodialysis has been reported as a sample treatment method for differential pulse polarography and has not yet been applied to IC.

A two-part electrolysis cell (Fig. 5) in which the anode and cathode compartments are separated by a cation-exchange membrane has been suggested as a method for treatment of highly caustic sample prior to IC analysis[23]. The sample is placed in the anode compartment, whilst a larger volume of dilute NaOH is used to fill the cathode compartment. During electrolysis, OH^- reacts at the anode to produce O_2 and H_2O, whereas water reacts at the cathode to produce H_2 and OH^-. Thus the concentration of OH^- in the anode compartment decreases, whilst that in the cathode compartment increases. Transfer of OH^- through the membrane cannot occur, so sodium ions move from the anode compartment into the cathode compartment. The

TABLE IV

SOME EXAMPLES OF SAMPLE CLEANUP WITH CARTRIDGE COLUMNS

Matrix	*Solute ions*	*Stationary phase*	*Ref.*
Plant extract	NO_2^-, NO_3^-, SO_4^{2-}	C_{18}	26
Urine	Thiosulphate	C_{18}	27
Urine	Oxalate	C_{18}	28
Soil extract	SO_4^{2-}	C_{18}	29
Cheese	Na^+, NH_4^+, K^+	C_{18}	30
Kraft liquor	S^{2-}, $S_2O_3^{2-}$	C_{18}	31
Plasma	NO_2^-, NO_3^-	C_{18}	32
Serum	I^-	C_{18}	33
Plant extract	Cl^-, NO_3^-, SO_4^{2-}	Silica	34
Surfactants	Alkylbenzene sulfonates	Silica	35
High chloride	Anions	Cation exchange (Ag^+)	36
NaOH	Anions	Cation exchange (H^+)	8
River water	HCO_3^-, Cl^-, NO_3^-, SO_4^{2-}	Cation exchange (H^+)	37
Leachate	As(III), As(V)	Cation exchange	38
Brine	Sodium	Cation exchange (H^+)	39
Air samples	Anions	Charcoal	40
Digests	Metal oxo-anions	Anion exchange	41
Natural waters	Anions	Amino	42
Brine	Br^-, NO_3^-, SO_4^{2-}	Alumina	43
Serum	SO_4^{2-}	Polymer	44
Surfactants	Anions	Polymer	39
Aromatics	Anions	Polymer	39

net result of this process is that the concentration of NaOH in the sample is progressively lowered. Use of an electrolysis current of 0.15 A for 3 h lowered the NaOH concentration in the sample from 19 *M* to 0.3 *M*. The latter concentration was suitable for direct injection into a suppressed IC system.

Chemical modification of the sample with disposable cartridge columns

One of the most versatile and convenient means available for sample cleanup is the use of commercially available disposable cartridge columns. These devices offer rapid sample treatment and can usually be employed in tandem with disposable filters so that filtration and sample cleanup can be performed in a single operation. Some of the common stationary phases available commercially as cartridge column packings are listed in Table III.

Modes of operation

Cartridge columns can be employed in one of two ways. The first method is the selective removal of the solute ions from the sample matrix and in this approach, the solvent used to elute the sample through the cartridge should provide chromatographic conditions giving very strong retention of the solute ions. That is, the capacity factors for these solutes should be as large as possible. The alternative operational mode for cartridge columns is to retain selectively matrix components under conditions where the solute ions are unretained. That is, their capacity factors approach zero.

Keeping in mind that we wish the solute to be either well-retained or not retained at all, then several possibilities emerge from the stationary phases listed in Table III. Stationary phases which show some ion-exchange ability (such as silica, alumina, anion and cation exchangers, and amino phases), and stationary phases which show chelation ability should be suitable for the selective retention of ionic solutes from a matrix composed largely of neutral, organic species. Alternatively, hydrophobic stationary phases such as octadecylsilane and the polymeric phases should be useful for the removal of neutral organic components while showing little retention of ionic solutes. A further potential application of cartridge columns is their use for adjusting the pH of a sample in the same manner as that described earlier for ion-exchange resins used in the batch mode. Most of the abovementioned possibilities have been realised in practice and Table IV lists some examples of successful applications.

Practical aspects

Several practical aspects should receive attention when using cartridge columns, namely column pretreatment, flow-rate, method of sample application, and sample pH. First, the columns almost invariably require pretreatment in order to remove very fine particles of the packing material, to elute any contaminants, or to condition the stationary phase in order to improve the efficiency of sample binding. Significant levels of inorganic contaminants are commonly encountered in cartridge columns[1], generally as a result of residual reagents from the manufacturing process. Hydrophobic stationary phases usually require pretreatment with an organic solvent such as methanol in order to wet the stationary phase surface, so that effective binding of hydrophobic solutes is achieved from aqueous sample solutions.

The flow-rate of sample or flushing solution through the precolumn should be

TABLE V

SOME EXAMPLES OF PRE-COLUMN REACTIONS IN IC

Analyte	*Additive*	*Effect*	*Ref.*
Anions	Methanol	$[CrO_4^{2-}]$ is reduced by reaction with methanol, producing formate	46
Ascorbic acid	Boric acid	Prevents oxidation of ascorbic acid (borate converted to H_3BO_3 in suppressor)	47
Boron	Chromotropic acid	H_3BO_3–chromotropic acid complex formed	48
Boron	Hydrofluoric acid	Boron converted to BF_4^-, and quantitated in this form	49
Br^-	2-Iodosobenzoic acid + acetanilide	4-Bromoacetanilide formed is used as a measure of Br^-	50
Cations	EDTA	Complexes some metal ions	51
HCN	Water	$CN^- + H_2O \leftrightharpoons NH_3 + HCOO^-$, with $HCOO^-$ used as an indirect measure of CN^-	52
CN^-	Hypochlorite	$CN^- + OCl^- \leftrightharpoons OCN^- + Cl^-$, with cyanate used as an indirect measure of CN^-	53
CN^-	Iodine	$I_2 + HCN \leftrightharpoons H^+ + I^- + ICN$, with iodide used as a measure of CN^-	54
H_2O_2	Sulfite	$H_2O_2 + SO_3^{2-} \leftrightharpoons H_2O + SO_4^{2-}$, with SO_4^{2-} used as an indirect measure of H_2O_2	55
S^{2-}	N,N-Dimethylphenylenediamine	Reacts with H_2S to form methylene blue, which is used to quantitate S^{2-}	56
SiO_3^{2-}	Boric acid	Fluoride converted to BF_4^- to eliminate interference of F^- on silica analysis	57
SO_3^{2-}	Formaldehyde	SO_3^{2-} converted to hydroxymethane sulfonate	58
Urea	Urease	NH_4^+ produced is used to quantitate urea	59

kept as low as practicable so that mass transfer effects are minimised. Most column cartridges are designed for use with disposable syringes and the low packing density of the stationary phase permits very high flow-rates (*e.g.* 50 ml/min) to be easily achieved. Experience with analytical chromatographic columns suggests that such a high flow-rate is unlikely to produce the degree of selective separation required, so it is advisable to use flow-rates less than 10 ml/min.

The third important practical consideration is the manner in which the sample is applied to and eluted from the cartridge column. It is possible to apply a known volume of the sample to the head of the column and to elute the sample band through the column with a suitable eluent. However, this method is inadvisable in practice because of the difficulty in applying an accurate volume of sample using the syringes compatible with the cartridge column, and is recommended only when the sample volume is small or the concentration of the sample is high enough to quickly saturate the cartridge. It is generally more appropriate to pass sample continuously through the column, discarding the first two or three column volumes and then collecting sufficient effluent for analysis.

Finally, the sample pH has an important bearing on the selection of a suitable stationary phase. Apart from the obvious consideration that some stationary phases are intolerant of acidic or alkaline solutions, the sample pH is often a very useful indicator of the ionic strength. In cases where the ionic strength in unacceptably high, it may be necessary to use a second cartridge column, or an alternative cleanup procedure, to remove some of the ionic components from the sample.

In conclusion, it should also be noted that cartridge columns packed with hydrophobic stationary phases can also be used to retain ionic solutes (rather than neutral, organic solutes) if they are first conditioned with an ion-interaction reagent. The success of this approach is dependent on retention of the ion-interaction reagent on the stationary phase during sample elution, thus it is desirable that relatively hydrophobic ion-interaction reagents be used and the sample volume be limited. Tetramethylammonium hydroxide and pentanesulphonic acid have been employed as ion-interaction reagents for the removal of anionic and cationic surfactants, respectively, using a cartridge column packed with a polymeric divinylbenzene stationary phase[39].

Chemical modification of the sample by pre-column reaction

For some samples, cleanup can be best achieved using an appropriate chemical reaction to eliminate a matrix component. Alternatively, it may be necessary to derivatize a solute in order to enhance its detectability or to convert it into a form suitable for separation. Much has been written on the principles of chemical derivatization of organic solutes[45] and the same principles apply here to inorganic solutes. Table V lists some reactions which have been employed as sample treatment methods for IC, or as mobile phase reactions designed to modify the nature of the solute in an IC determination.

CONCLUSIONS

Powerful sample cleanup procedures are available for IC and permit the analysis of very complex samples, such as those with extremes of pH or having high ionic strength. Membrane techniques are especially applicable because of their ability to transfer selectively a desired ion either into or out of the sample. These techniques will undoubtedly find increasing usage in the future as commercial devices become available for sample dialysis.

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CHROMATOGRAPHIC DETERMINATION OF WATER USING SPECTROPHOTOMETRIC DETECTION

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SUMMARY

Water in various analytical samples is determined by ion-exclusion chromatography with spectrophotometric detection based on a shift in the equilibrium between cinnamaldehyde and cinnamaldehyde dimethylacetal in methanol. The shift in equilibrium is proportional to the amount of water present and occurs only in the presence of an acid catalyst. The mechanism of this unique detection system is studied in detail. The conditions for chromatographic separation and detection are optimized so that a determination of water can be completed in only 1 to 2 min. Water has been determined in a variety of samples to demonstrate the versatility of this new method.

INTRODUCTION

Although a number of approaches have been used for the determination of water in various analytical samples, the Karl Fisher titration method continues to dominate the field[1]. Even with improvements in reagents and instrumentation, this method requires a relatively large sample and is subject to a number of serious interferences. Stevens *et al.*[2] recently published a liquid chromatographic method for determination of water using a methanol eluent and a conductivity detector. Their method is fast and convenient, but the sensitivity varies widely in different ranges of water concentration. Fortier and Fritz[3] proposed a new spectrophotometric detection system for water separated by liquid chromatography. This is based on the effect of water on the equilibrium between cinnamaldehyde and cinnamaldehyde dimethylacetal in the methanol–acetonitrile eluent. Their system employed a cation-exchange column in the Li^+ form for separation, followed by a catalytic column containing a cation-exchange resin in the H^+ form.

In the present study the method of Fortier and Fritz[3] has been improved so that only a single chromatographic column is needed and a much shorter retention of water is possible. The mechanism of the detection system is now explained in detail, and the factors affecting the initial "injection" peak are elucidated.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Milton Roy Model 396/2396 mini

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pump, a Model 7010 Rheodyne injector equipped with sample loops sized from 5 to 100 μl depending on the sample water content, a Model LP-21 Scientific Systems Lo-pulse pulse-dampener, a stainless-steel 15 cm × 2.1 mm column packed with Bio-Rad Aminex Q-150S resin in the H^+ form, a Kratos Spectroflow 783 absorbance detector, and a Curken strip-chart recorder. The Aminex Q-150S column was packed on a Shandon single-piston packing pump, using upward slurry packing method. The packing solvent was reagent-grade methanol. A Hamilton PRP-X300 ion-exclusion column (15 cm × 4.6 mm) and a Supelco LC-Diol column (25 cm × 4.6 mm) were also tested.

Reagents

trans-Cinnamaldehyde, 99% (Aldrich), was used without purification. Reagent-grade methanol and HPLC-grade acetonitrile (both from Fisher Scientific) were dried over activated 3A molecular sieves (Aldrich). The dried methanol was then refluxed over CaH_2 for 5 days and distilled before use. All other chemicals were reagent grade and were used without purification. The water standards (1.00 mg H_2O/ml and 5.00 mg H_2O/ml) and anhydrous methanol (Karl Fisher-grade) were purchased from Fisher Scientific.

Eluent and standard samples

Eluent was prepared simply by dissolving carefully weighed amount of cinnamaldehyde to anhydrous methanol. Standard samples were prepared by adding accurately measured amounts of water to portions of dried methanol or acetonitrile. For maximum sensitivity and reproducibility, the eluent and all standard samples were prepared under the protection of dried nitrogen. Once prepared, the eluent was protected from atmospheric moisture by bubbling the dried nitrogen through the eluent reservoir and out through a drying tube filled with anhydrous calcium sulfate (Drierite). All sample solutions were contained in vials equipped with Mininert valves (Supelco) prior to removal from the glove bag. Water-saturated organic samples were obtained by shaking the organic solvents with water for 24 h.

Chromatographic conditions

A flow-rate of 1 ml/min was employed throughout the entire experimental work. A detection wavelength of 300 nm was used. The eluent was usually 1.0 m*M* cinnamaldehyde in methanol. For systematic studies, however, 0.5 m*M*, 2.0 m*M* and 5.0 m*M* eluents were also used.

RESULTS AND DISCUSSION

Column

Experiments were performed using methanol containing 1.0 m*M* cinnamaldehyde as the eluent with various types of cation-exchange columns. The water peak was detected spectrophotometrically at 300 nm. A single column filled with cation-exchange resin was found to be satisfactory for most samples. It is not necessary to use the combination of Li^+-form and H^+-form cation-exchangers described previously[3]. Only a small fraction of the water in a sample is used up in shifting the cinnamaldehyde dimethylacetal–cinnamaldehyde equilibrium to the right:

$$\text{acetal} + \text{water} \overset{H^+}{\rightleftarrows} \text{aldehyde} \qquad (1)$$

Most of the water remains unreacted and emerges from the column as a distinct peak with a longer retention time than the bulk of the analytical sample. The concentration of aldehyde formed in reaction 1 is proportional to the water concentration. The detection wavelength is selected to measure the aldehyde without interference from the larger amount of acetal that is present.

Several resins were tried as packing for the separation column. Aminex Q-150S (H^+) was found to work very well. It is a gel-type resin, which appears to be a desirable property for chromatographic separation of water from other substances. Hamilton PRP-X300 is said to be a good column for ion-exclusion chromatography, but it gave no separation at all for water under the conditions we used. Perhaps this is because the Hamilton resin is macroporous and not a gel. A silica-base diol column also gave no separation of water. A slight displacement of the water peak (longer retention) was due to a short Aminex Q-150S (H^+) post-column used as a catalyst.

Aminex Q-150S (H^+) columns of varying dimensions were tried. A 15 cm × 2.1 mm column, used in conjunction with a 50-μl sample loop worked the best. Columns of wider diameter gave lower detection sensitivity.

Detection system

The cinnamaldehyde added to the "dry" methanol used to prepare the eluent has the potential of reacting with the methanol to form the cinnamaldehyde dimethylacetal plus water. However, this reaction does not occur to any extent until an acid catalyst is present. This may be the H^+-form cation-exchange resin in the column, or a soluble acid can be added to the eluent. After contact with an acid catalyst, most of the cinnamaldehyde is converted to the acetal.

Equilibrium constant

The equilibrium constant for the following reaction was measured by adding varying concentrations of water to the eluent (in the presence of trace H^+) and measuring the concentrations of acetal and aldehyde spectrophotometrically:

$$\underset{\text{(b)}}{\text{water} + \text{cinnamaldehyde acetal}} \overset{H^+}{\rightleftarrows} \underset{\text{(a)}}{2\ \text{methanol} + \text{cinnamaldehyde}} \qquad (2)$$

First, the absorbance of the eluent (A_a^0) was measured before addition of an acid catalyst when all of the cinnamaldehyde is present as (a). Then the absorbance (A_b^0) is measured after acid catalysis when all of the aldehyde has been converted to (b). From Beer's law

$$A_a^0 = \varepsilon_a\, l\, C^0 \qquad (3)$$

$$A_b^0 = \varepsilon_b\, l\, C^0 \qquad (4)$$

where ε is the extinction coefficient, l is the pass length of the detection cell, and C^0 is

the initial concentration of cinnamaldehyde in the eluent. These measurements were made at 280 nm, where both (a) and (b) absorb appreciably.

Next, varying amounts of water were added to the eluent in the presence of an acid catalyst and the total absorbance (A_{tot}) was measured at 280 nm. From Beer's law:

$$A_{tot} = A_a + A_b = \varepsilon_a l[a] + \varepsilon_b l[b] \tag{5}$$

$$= \varepsilon_a l[a] + \varepsilon_b l(C^0 - [a]) \tag{6}$$

$$= (\varepsilon_a l - \varepsilon_b l)[a] + A_b^0 \tag{7}$$

where [a], [b] and A_a and A_b are the concentrations and absorbances at equilibrium. Combining these equations

$$[a] = \frac{A_{tot} - A_b^0}{\varepsilon_a l - \varepsilon_b l} \quad \text{and} \quad [b] = C^0 - [a] = \frac{A_a^0 - A_{tot}}{\varepsilon_a l - \varepsilon_b l} \tag{8}$$

The equilibrium constant for reaction 2 is:

$$K = \frac{[a]}{[b]\,[H_2O]} = \frac{A_{tot} - A_b^0}{(A_a^0 - A_{tot})\,[H_2O]} \tag{9}$$

A value of $(5.3 \pm 0.4) \cdot 10^{-4}$ mM^{-1} was found for the equilibrium constant, K.

Equation for chromatographic detection

Rearrangement of eqn. 9 gives

$$A_{tot} = \frac{A_a^0\, K[H_2O] + A_b^0}{K[H_2O] + 1} \tag{10}$$

so as long as $[H_2O]$ is small, the denominator is approximately equal to 1 and eqn. 10 is essentially linear. However, the detector response (A_{det}) depends on the difference in absorbance of the eluent and sample, so eqn. 10 can be written

$$A_{det} = \Delta A_{tot} = A_a^0\, K \left([H_2O]_{sample} - [H_2O]_{eluent} \right) \tag{11}$$

Introducing E as a factor of column and elution efficiency and using an eluent of low but constant water concentration, the following linear equation is obtained for detector response:

$$A_{det} = A_a^0\, K\, E\, [H_2O]_{sample} - \text{constant} \tag{12}$$

Detection wavelength

An earlier paper[3] recommended 310 nm for detection of water. However, the sensitivity was found to be much better at 300 nm. A detection wavelengh of 290 nm was tried, but the background absorbance was too high.

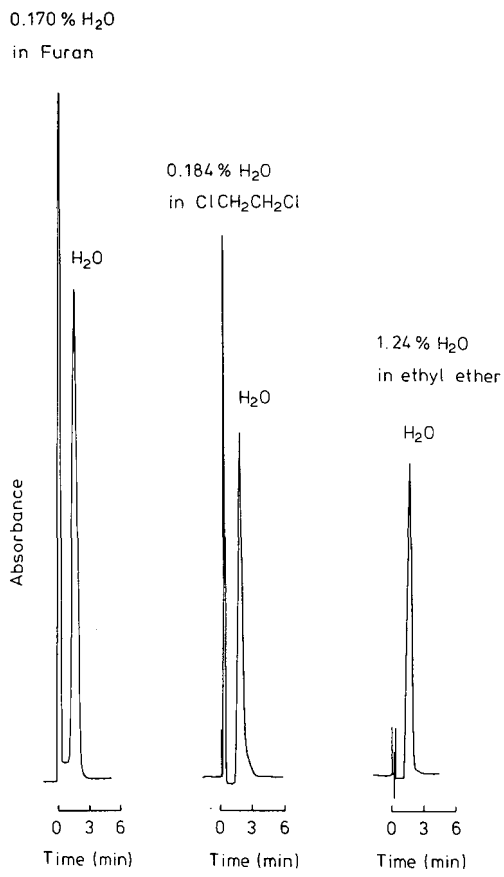

Fig. 1. Determination of water in various samples. Conditions: 15 cm × 2.1 mm column; 50-μl sample loop; eluent, 1.0 m*M* cinnamaldehyde in methanol; flow-rate, 1.0 ml/min.

Scope

Typical chromatograms for the determinaton of small amounts of water in organic liquids are shown in Fig. 1. In each case there is an injection peak that occurs at the column dead time. This is followed by the water peak which has a retention time of 1.0 to 2.0 min, depending on the chromatographic conditions.

Successful separations of water in various organic samples were obtained. These included aromatic hydrocarbons, chlorinated compounds, alcohols, furans, esters and ethers. Aldehydes, methyl ketones and dimethylsulfoxide gave very broad injection peaks that obscured the water peak. Aldehydes and ketones can undergo an acid-catalyzed reaction with methanol to form water plus acetals and ketals, respectively. Fig. 2 shows good chromatograms for water in acetylcystine and ascorbic acid. These are reducing compounds and cannot be analyzed for water by the Karl Fisher method.

Injection peaks

The source and magnitude of injection peaks was investigated. This was done by injecting samples of four organic liquids, each containing a small amount of water, into

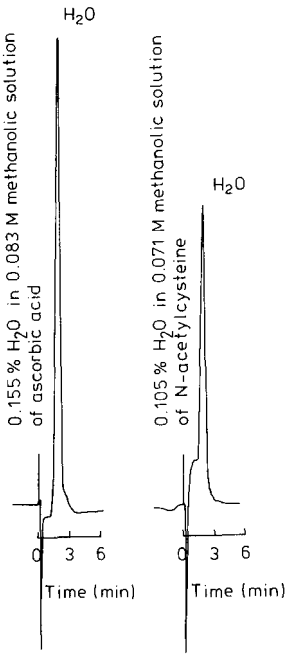

Fig. 2. Determination of water in reducing samples. Conditions as in Fig. 1.

a series of eluents containing (1) methanol only, (2) methanol plus 1 m*M* cinnamaldehyde, and (3) methanol containing 5 m*M* cinnamaldehyde. The results are summarized in Table I.

The results obtained with methanol only show that absorbance of the sample matrix can contribute to the injection peak. In this regard it should be recalled that the UV–VIS detector is very sensitive. Additional contributions to injection peaks are noted as increasing concentrations of cinnamaldehyde are added to the methanol eluent. These contributions can be explained by assuming that cinnamaldehyde can partition into the resin gel from the eluent. Then injection of a sample (which contains

TABLE I
SUMMARY OF INJECTION PEAKS

Sample	*Injection peak*		
	Methanol only	*1 mM Aldehyde*	*5 mM Aldehyde*
Methanol	None	Negative gap	Larger negative gap
Acetonitrile	Positive	Positive, negative gap	Larger positive, larger negative gap
Toluene	Large positive	Very large positive, small negative gap	Very large positive, negative gap
Hexane	Positive	Larger positive, small negative gap	Almost no positive large negative gap

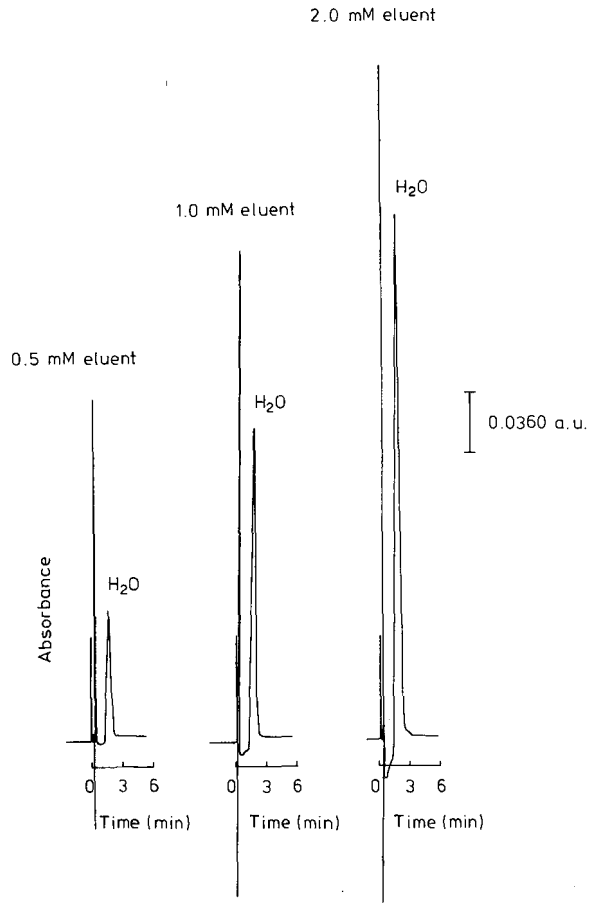

Fig. 3. Effect of cinnamaldehyde concentration in eluent on peak height. Other conditions as in Fig. 1.

no cinnamaldehyde) causes some of the aldehyde to come from the gel back into the liquid stream and thereby contribute to the injection peak. After the sample zone has passed, some aldehyde goes back into the resin gel from the eluent, causing a negative gap in the chromatogram.

Effect of cinnamaldehyde concentration

Eqn. 11 predicts that increasing concentrations of cinnamaldehyde in the eluent should increase the detector signal for samples containing a fixed concentration of water. This is indeed the case, as is shown by the chromatograms in Fig. 3. A plot of peak height against cinnamaldehyde concentration in the eluent is linear for eluent concentration points of 0.5, 1.0, 2.0 and 5.0 mM cinnamaldehyde. The efficiency, E (eqn. 12), was calculated to be 0.18.

Quantitation

Standards were prepared by adding carefully measured amounts of water to portions of dry methanol. After chromatographic separation, linear plots of peak

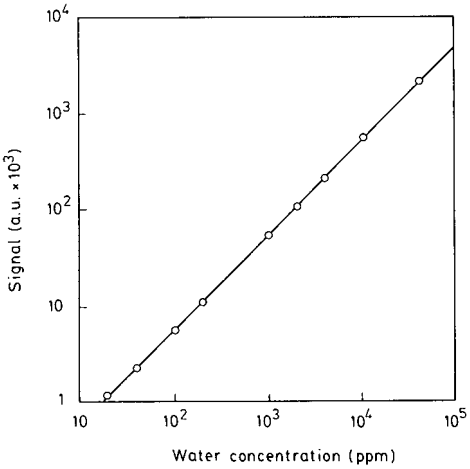

Fig. 4. Calibration curve in the low to medium range of water content. Conditions as in Fig. 1.

height against water concentration were obtained with excellent correlation coefficients for linear regression. However, such a calibration curve only shows peak height as a function of *added* water and does not account for the water already in the sample matrix and in the eluent itself.

A calibration curve of peak height *vs.* the total water in the standards was prepared with the aid of a standard (Fisher Scientific) certified to contain 1.00 ± 0.02 mg water per ml of sample. The resulting calibration plot has the same slope as that with added water, but the intercept is different. Manipulation of these two plots showed that the methanol used to prepare the standards contained 20 ppm water. The methanol eluent was calculated to contain 18 ppm water.

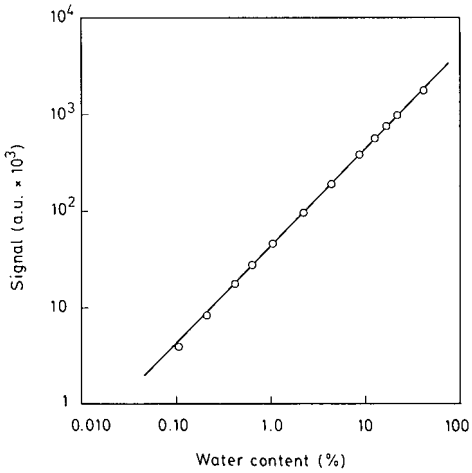

Fig. 5. Calibration curve in the high range of water content. A 5-μl sample loop was used. Other conditions as in Fig. 1.

Fig. 4 shows an excellent calibration plot for water that shows excellent linearity ($r = 0.99999$) over three orders of magnitude in water concentration. Fig. 5 shows a linear calibration curve for samples containing a high percentage of water.

Quantitative results

Samples of several organic liquids were carefully saturated with water by equilibration in a thermostat at 23°C. The water content of the organic phase was then determined by chromatographic analysis performed in triplicate. These results are summarized and compared with literature values in Table II. Some interpolation is required as the literature values are reported for slightly different temperatures than that used for the chromatographic determinations. Nevertheless, the chromatographic results are mostly in good agreement with the literature values.

TABLE II
SUMMARY OF WATER SOLUBILITY IN VARIOUS ORGANICS

Organic compound	*Solubility of water (%, w/w)*	
	Found (23°C)	*Reported*
Benzene	0.0563 ± 0.0005	0.053 (20°C)[4] 0.066 (30°C)[4]
Furan	0.170 ± 0.001	0.141 ± 0.005 (20°C)[5]
Methylene chloride	0.154 ± 0.002	0.14 (20°C)[6] 0.167 (25°C)[7]
Chloroform	0.088 ± 0.001	0.114 ± 0.004 (15°C)[8]
1,2-Dichloroethane	0.184 ± 0.001	0.17 (20°C)[6] 0.187 (25°C)[7]
Diethyl ether	1.24 ± 0.01	1.2 (20°C)[9] 1.26 ± 0.02 (RT)[8]
Carbon tetrachloride	0.022 ± 0.001	0.035 ± 0.003 (15°C)[8] 0.0075 ± 0.0005 (20°C)[5]

Response factor

A response factor (*RF*) can be defined as follows

$$RF = \frac{\text{signal in absorbance units at 300 nm}}{0.1\%\ H_2O \text{ in sample}}$$

A *RF* of 0.052 has been achieved with 1.0 m*M* cinnamaldehyde in the eluent and a 50-μl sample loop.

Detection limit

The detection limit depends on the water content of the eluent as well as the *RF*. The lowest detection limit achieved experimentally was 45 ppm of water.

CONCLUSIONS

Water can be determined very rapidly in a wide variety of samples by ion-exclusion chromatography using only a single separation column. Detection sensitivity is excellent over a broad concentration range using spectrophotometric detection at 300 nm with the acid-catalyzed cinnamaldehyde-acetal equilibrium system. Samples containing aldehydes or methyl ketones appear to require use of the two-column chromatographic system described earlier.

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DETERMINATION OF MONO-, BI- AND TRIVALENT CATIONS USING NON-SUPPRESSED ION CHROMATOGRAPHY

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SUMMARY

The ion-chromatographic separation of mono-, bi- and trivalent cations is considered both from a mechanistic point of view and with regard to the influence of the mobile phase. These methods are compared with atomic absorption spectrometry. Application to the separation of the Cs-137 and Sr-90 radionuclides is discussed.

INTRODUCTION

Non-suppressed ion chromatography of heavy metal cations has rapidly gained importance in recent years[1,2]. This method allows the separation and subsequent determination of a large number of bivalent transition metals in liquid samples in one analysis step. Even when dealing with environmental or biological samples, tedious sample clean-up procedures may be omitted in most instances[3–5]. Several eluent systems have been developed for use in non-suppressed cation chromatography. Owing to the large difference in affinity to the stationary phase, a clear distinction is made between eluents best suited for the determination of mono- and bivalent cations[6]. Monovalent cations such as alkali metals and ammonium are easily eluted with dilute solutions of nitric acid[7]. Separations of alkaline earth and transition metals require the use of stronger driving ions, such as ethylenediamine (EDA), and are further facilitated by the addition of complexing agents to the mobile phase[8,9]. The need for two eluent systems for a complete assay of mono- and bivalent cations leads to double the analysis time and expenditure. However, so far only a few studies on the simultaneous ion-chromatographic determination of metal cations of different valencies have been published[10,11].

The main reactions taking place during the ion chromatography of bivalent metal ions with the eluent system ethylenediamine (EDA)–citric acid (L) at acidic pH on a stationary phase R–metal (M) are shown by the following chemical equilibria:

$$R\text{–}M + EDAH_2^{2+} + L^{2-} \rightleftharpoons R\text{–}EDAH_2 + M^2 + L^{2-} \rightleftharpoons R\text{–}EDAH_2 + ML$$

This shows the influence of ethylendiamine concentration and complex formation on the affinity of metal ions towards the stationary phase. In the absence of citric acid, transition metal ions are bound firmly to *e.g.* the sulphonic acid groups and elution by

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the ethylenediammonium cation is governed only by the EDA concentration. Without a complexing agent, the elution of transition metals would generally be very slow. If citric acid is added to the eluent, its anionic carboxylate groups compete for the metal ion, thus favouring release from the stationary phase by ethylenediamine, eventually resulting in a decrease in retention time. The more stable the metal ion/citrate complex, the more rapidly it will be eluted. This stability depends on the nature of the metal ion and on the degree of protonation of the citrate ion[12].

The aim of this study was to establish a mechanism that can be used to explain and to alter selectively the affinity of mono- and bivalent cations to the stationary phase, and to selecting eluents of appropriate ionic strength and pH to fit the conditions for the separation of bi- and trivalent cations. Also, the ethylenediamine and citric acid eluent composition that is generally used for transition metal analysis was modified and the separation mechanism was studied over a wide pH range.

EXPERIMENTAL

Apparatus

The ion-chromatographic system consisted of a Model 1330 pump (Bio-Rad Labs., Richmond, CA, U.S.A.), an Altex Model 210 injection valve with a 100-μl loop, a conductivity detector Model HPCM (Bio-Rad Labs.) and a CR-2A integration system (Shimadzu, Kyoto, Japan). For fractionation of radioactive samples a Model 7000 Ultorac fraction collector (LKB, Bromma, Sweden) was used.

Atomic absorption spectrometry (AAS)

A Perkin-Elmer (Norwalk, CT, U.S.A.) Model 2280 atomic adsorption spectrometer with an air–acetylene flame was used. The operating parameters were set according to the manufacturer's instructions. Lead was determined at 283.3 nm and chromium at 357.9 nm. A slit width of 0.7 nm was used for both elements.

Materials and reagents

The stationary phase used for the separation of mono- and bivalent cations was a sulphonated porous 5-μm silica-based ion exchanger (TSK-IC cation SW, 50 × 4.6 mm I.D. column, Bio-Rad Labs.). For the separation of bi- and trivalent cations a weak cation exchanger consisting of 7-μm non-porous polymethacrylate beads with polyethyleneimine covalently coupled to the surface and exhaustively succinylated (HRLC-MA7C, 50 × 7.8 mm I.D. column, Bio-Rad Labs.) was used.

The mobile phases were prepared by dissolving analytical-reagent grade citric acid monohydrate (Merck, Darmstadt, F.R.G.) and ethylenediamine (Fluka, Buchs, Switzerland) in deionized water. The pH was adjusted to the desired value either by addition of hydrochloric acid or potassium hydroxide or by adding further amounts of citric acid or ethylenediamine[13].

Metal ion solutions were prepared from analytical-reagent grade chlorides or nitrates (Sigma, St. Louis, MO, U.S.A., and Fluka). Cs-137 (185 MBq, in 1 *M* hydrochloric acid) and Sr-90 (37 MBq, in 1 *M* nitric acid), used for the separation of radioactive nuclides, were purchased from Amersham International (Amersham, U.K.).

RESULTS AND DISCUSSION

Separation of mono- and bivalent cations

In order to separate mono- and bivalent cations, a silica-based porous stationary phase with sulphonic acid functional groups was investigated. Fig. 1 shows the influence of pH on the retention times of mono- and bivalent ions. The elution of monovalent cations is only slightly affected by the hydrogen ion concentration, potassium exhibiting a minor increase in retention with rising pH. Most alkaline earth and transition metal ions react in the same way, their elution becoming faster with decreasing hydrogen ion concentration.

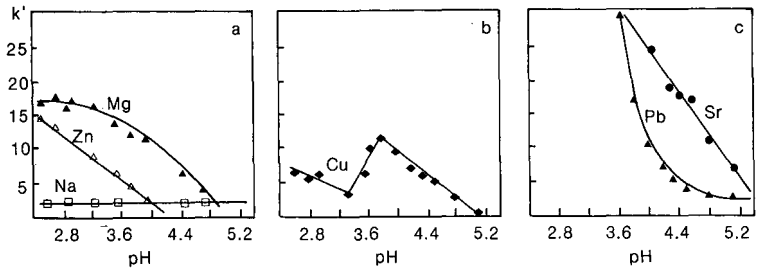

Fig. 1. Dependence of k' on the pH of the mobile phase system ethylenediamine–citric acid. Stationary phase, TSK IC cation SW; mobile phase, 3.5 mmol/l ethylenediamine–10 mmol/l citric acid; pH adjustment by addition of citric acid, ethylenediamine, HCl or KOH; flow-rate, 1 ml/min.

Some cations deviate from this behavious and exhibit different elution patterns, which have to be considered when choosing optimum conditions for the separation of mono- and bivalent ions. Copper(II) shows a sharp decrease in retention time between pH 3.8 and 3.3, followed by an increase at lower pH (Fig. 1b). At pH 3.3, which is the pH of the mixture of 3.5 mmol/l EDA and 10.0 mmol/l citric acid, which is also the eluent proposed by the column manufacturer, copper and potassium tend to coelute. The slope of Pb^{2+} is steeper than those of the other ions and shows an exponential form, indicating that lead responds more strongly than any other ion to an increase in hydrogen ion concentration. Lead is adsorbed on the column at pH < 3.6, but may be released quickly if the pH is raised. The same applies to Sr^{2+} (Fig. 1c). This fact can be utilized for on-line preconcentration. The different elution patterns exhibited by the ions mentioned may be explained by the formation of citrate complexes of various structures. Therefore, a knowledge of both the dissociation of citric acid and of the complex stabilities of the metal ions is necessary.

Fig. 2 gives a general idea of the ion species present in an aqueous solution of citric acid, depending on pH. In the pH range covered in this study (2.50–5.20), all four species exist at varying levels. The distribution maxima for $(citrate)^{2-}$ and $(citrate)^{-}$ lie at pH 5.2 and 3.6, respectively. In Table I, the stability constants of complexes of certain metals with the different citrate ions are given[14]. In general, the stability increases with deprotonation of the ligand. The order of stability of citrate complexes of the metal ions is $Sr^{2+} < Mg^{2+} < Zn^{2+} < Cu^{2+}$, and the reverse order would

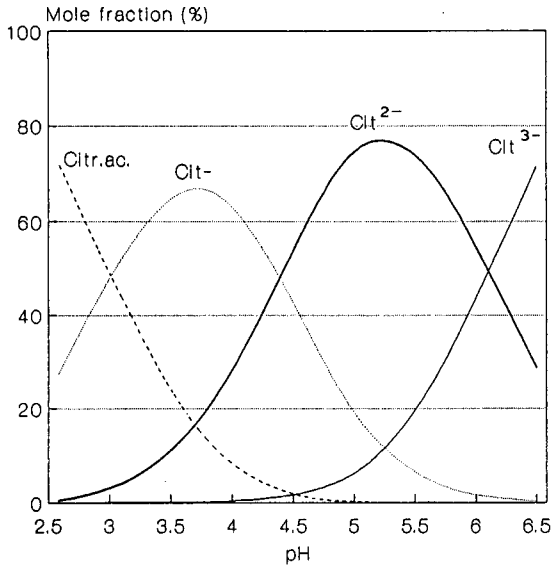

Fig. 2. Dissociation of citric acid.

correspond to their elution. Pb^{2+} forms very stable dicitrate complexes. For Sr^{2+} only complexes with the non-protonated ligand are reported.

The retention graphs of metal ions forming protonated citrate complexes (Zn^{2+}, Mg^{2+} and Cu^{2+}) appear as almost parallel lines (Fig. 1), with slopes of 4.26, 4.61 and 4.61, respectively. Mg^{2+}, however, deviates from the straight line at pH < 3, which is generally observed with ions that form low-stability complexes. This effect is attributed to the relatively high degree of protonation of the ligand, which lowers the complex stability. In this pH region, complexation reactions compete with ion-exchange mechanisms. The retention behaviour of copper may be explained by several factors attributed to unique reactions with citric acid: Cu^{2+} forms very stable complexes with $(citrate)^-$, the species that is predominant in this pH region. Additionally, stable complexes are formed with the species $(citrate)^{2-}$. Pb^{2+} does not fit into this scheme, as its stability constants suggest retention values close to those of the copper ion. Nevertheless, evidence can be found in Table I for the formation of very stable Pb^{2+}–dicitrate complexes. This unique feature explains the exponential decrease in the capacity factors of lead at pH > 3.6.

TABLE I

STABILITY CONSTANTS (log *K*) OF CITRIC ACID–METAL COMPLEXES[14]

Ion	$(Cit^{3-})M$	$(Cit^{2-})M$	$(Cit^{-})M$	$(Cit^{3-})_2M$	$(Cit^{3-})_3M$	$(Cit^{3-})_2M_2$
Zn^{2+}	4.98	2.98	1.25	5.90	–	–
Mg^{2+}	3.37	1.60	0.84	–	–	–
Cu^{2+}	5.90	3.42	2.26	–	–	8.03
Sr^{2+}	3.05	–	–	–	–	–
Pb^{2+}	4.08	2.97	1.51	6.08	6.97	–

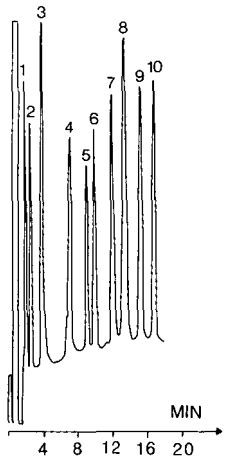

Fig. 3. Separation of alkali, alkaline earth and transition metal ions. Stationary phase, TSK IC cation SW; mobile phase, 3.5 mmol/l ethylenediamine–10.0 mmol/l citric acid (pH = 2.8, adjusted with HCl); flow-rate, 1.0 ml/min; detection, conductivity; temperature, 30°C. Peaks: 1 = Na^+ (5 ppm); 2 = K^+ (50 ppm); 3 = Cu^{2+} (10 ppm); 4 = Ni^{2+} (10 ppm); 5 = Co^{2+} (10 ppm); 6 = Zn^{2+} (10 ppm); 7 = Fe^{2+} (10 ppm); 8 = Mn^{2+} (20 ppm); 9 = Cd^{2+} (10 ppm); 10 = Ca^{2+} (15 ppm).

Sr^{2+}, on the other hand has been shown not to form protonated citrate complexes. $(Citrate)^{3-}$ ions are present in solution only at a pH > 4.0. Therefore, the elution of Sr^{2+} starts at this relatively high pH level.

Fig. 3 shows a chromatogram of alkali, alkaline earth and transition metal ions under conditions optimized for the separation of mono- and bivalent ions. At a pH of 2.80 the copper peak is well resolved from the potassium peak.

If the pH is increased to 4.2, metal ions such as Sr^{2+} and Pb^{2+} can be eluted and determined together with monovalent ions (Fig. 4). This fact was applied in radiochromatographic trace separations of Cs-137 and Sr-90. The eluted fractions

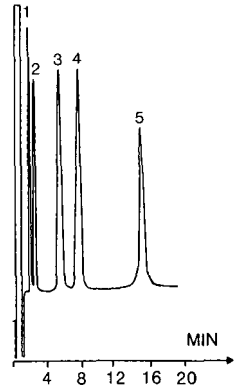

Fig. 4. Separation of mono- and bivalent ions. Chromatographic conditions as in Fig. 3; pH of the mobile phase, 4.2. Peaks: 1 = Na^+; 2 = Cs^+; 3 = Pb^{2+}; 4 = Mg^{2+}; 5 = Sr^{2+}.

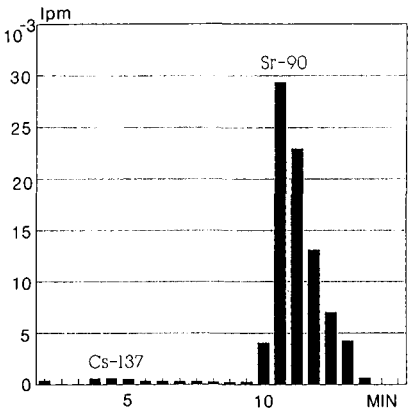

Fig. 5. Ion chromatographic trace analysis of radionuclides, impulses per minute (lpm) *versus* time. Sample: Cs-137 and Sr-90 in water. Stationary phase, TSK IC cation SW; mobile phase, 3.5 mmol/l ethylenediamine–10.0 mmol/l citric acid (pH 4.80, adjusted with NaOH); flow-rate, 0.7 ml/min; detection, β-liquid scintillation counting.

were collected and analysed using a liquid scintillation β-counter (Fig. 5). The peaks are well resolved from each other. This method seems promising for the simple and rapid determination of these fission products in either radioactive wastewater or biological samples, especially after on-line preconcentration.

Separation of bi- and trivalent cations

The stationary phase chosen for the separation of bi- and trivalent ions was a polymeric, non-porous weakly acidic cation exchanger. A nitric acid eluent was diluted step-by-step, and the elution behavior of bi- and trivalent cations was monitored and is depicted in Fig. 6. With nitric acid concentrations of 0.25 mmol/l and

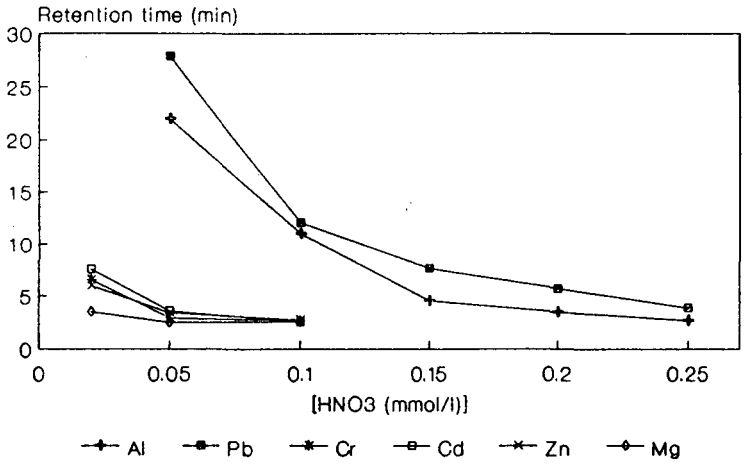

Fig. 6. Influence of nitric acid concentration on retention times of bi- and trivalent metals. Stationary phase, MA7C (50 × 7.8 mm I.D. column); mobile phase, 0.02–0.25 mmol/l nitric acid; detection, conductivity (R = 100); temperature, 30°C; sample concentration, 1.0 ppm each.

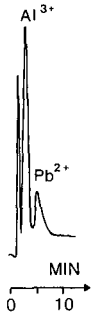

Fig. 7. Separation of bi- and trivalent cations. Sample: Pb^{2+} (0.5 ppm) and Al^{3+} (0.5 ppm). Stationary phase, MA7C (50 × 7.8 mm, I.D. column); mobile phase, 0.15 mmol/l nitric acid; flow-rate, 0.5 ml/min; detection, conductivity (R = 100).

lower, corresponding to pH 3.53 and below, Al^{3+} and Pb^{2+} were retained and exhibited an increase in retention time when the eluent concentration was lowered, whereas other trivalent metals, such as Cr^{3+} and Fe^{3+}, eluted in the dead volume. With 0.05 mmol/l nitric acid (pH 4.24) as the mobile phase, several ions, such as Cd^{2+}, Cr^{3+}, Zn^{2+} and Mg^{2+}, interacted with the stationary phase. This trend continued when the eluent concentration was decreased to 0.02 mmol/l (pH 4.47). Attempts to improve the resolution by further dilution of the mobile phase were unsuccessful owing to an unstable baseline.

In ion exchange, the degree of ionic interaction between ions and the stationary phase depends largely on their valency, trivalent ions showing the highest affinity. In this instance the elution order does not fit into this scheme, indicating that additional mechanisms contribute to the elution. The most probable reaction capable of inhibiting ionic interactions with the stationary phase is the formation of hydroxide complexes. For example, Al^{3+} is reported[14] to show signs of hydrolysis at pH values as low as pH 2. The order of stability of hydroxide complexes is $Mg^{2+} < Cd^{2+} < Zn^{2+} < Pb^{2+} < Cr^{2+} < Al^{3+}$, which explains the fact that aluminium is eluted before lead under these conditions.

Fig. 7 shows the separation of a standard solution of 0.5 ppm of Al^{3+} and Pb^{2+} using 0.15 mmol/l nitric acid as the eluent.

A comparison of this method with flame AAS for the determination of Pb^{2+} in aqueous solutions is shown in Fig. 8. The values obtained by AAS measurements were

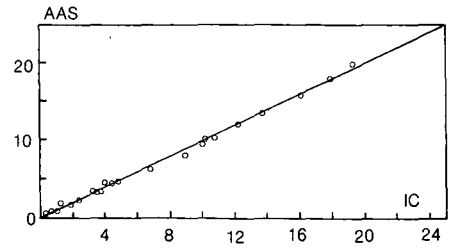

Fig. 8. Comparison of analytical data (in ppm) obtained by ion chromatography and flame AAS for Pb^{2+}. Ion chromatography: stationary phase, MA7C (50 × 7.8 mm I.D. column); mobile phase, 0.20 mmol/l nitric acid; flow-rate, 0.5 ml/min; detection, conductivity (R = 100); temperature, 30°C. AAS: flame, air–acetylene; Wavelength, 283.3 nm; slit width, 0.7 nm.

plotted against the corresponding results obtained by the reported ion-chromatographic method. Correlation in the low concentration range shows that the ion-chromatographic method allowed the detection of Pb^{2+} concentrations as low as 0.1 ppm.

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PRECISION AND ACCURACY OF ION CHROMATOGRAPHY IN DRY DEPOSITION MEASUREMENTS

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SUMMARY

A combination of ion chromatography (IC) and a so-called wet denuder system for the measurement of the deposition velocities of components such as HCl, HNO_3 and SO_2 was tested. For this type of measurement, a precision of better than 5% is required when analysing the absorption solutions of the wet denuder systems. The accuracy and precision of an IC system constructed from commercially available components was tested in the concentration range 10–5000 $\mu g\ l^{-1}$ for chloride, nitrate and sulphate. The output of conductivity, UV and ion-selective electrode detectors was linearized. Setting the calibration accuracy at 5%, a precision of 5% was obtained for sulphate and nitrate at a concentration of 30 $\mu g\ l^{-1}$, and for chloride at 50 $\mu g\ l^{-1}$. A precision of 1% was attained at concentrations of 60 and 400 $\mu g\ l^{-1}$ for sulphate and nitrate, respectively. Accuracies of 5% and 2% were achieved at concentrations of 100 and 200 $\mu g\ l^{-1}$ for sulphate, nitrate and chloride, respectively. Setting the calibration accuracy at 2%, a precision of 5% was achieved at a concentration of 20 $\mu g\ l^{-1}$ for sulphate, nitrate and chloride and a precision of 1% at concentrations of 60, 400 and 500 $\mu g\ l^{-1}$ for sulphate, nitrate and chloride, respectively. An accuracy of 5% was obtained at concentrations of 30, 100 and 100 $\mu g\ l^{-1}$ for sulphate, nitrate and chloride, respectively. At a concentration of 200 $\mu g\ l^{-1}$ of these components the accuracy was 2% or better.

INTRODUCTION

In general, the requirements regarding precision and accuracy for environmental measurements are modest. In view of other uncertainties, an accuracy and precision of the order of 5–10% relative are generally considered to be acceptable for measurements of trace components in the range 10–2000 $\mu g\ l^{-1}$. Problems regarding sampling and sample integrity will generally cause errors of at least the same order of magnitude.

A notable exception exists in the field of acid deposition research. Both wet and dry deposition are responsible for the effects of air pollutants. The measurement of wet deposition presents surmountable problems in the view of the available knowledge and methodology. However, the situation for dry deposition measurements is very different and either very fast or very precise methods are required to measure dry deposition fluxes.

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For many components, gradient measurements are used to derive dry deposition fluxes. Gradients of the air concentrations of compounds, temperature, wind speed and humidity are measured over an altitude of, *e.g.*, 10 m. From these gradients the turbulence of the atmosphere is derived and the deposition velocities and fluxes can be calculated. The concentration gradients are dependent on atmospheric conditions and the deposition velocities of the components. Generally, differences of the order of 5% (for compounds such as submicron aerosols with a deposition velocity of the order of 0.1–0.5 cm s^{-1}) to 25% (for compounds such as HNO_3 or NH_3 with deposition velocities of 1–3 cm s^{-1}) are measured between altitudes of 1 and 10 m[1,2]. Consequently, these gradient measurements must have a precision of at least 5% relative, but preferably 1%, to be useful. As differences in air concentrations are measured, accuracy is less important than precision.

Denuder techniques offer the best possibilities for measuring dry deposition velocities of important acid-deposition-related compounds such as HNO_3, NH_3 and HCl. A denuder is a tube coated with a reagent. Gases, owing to their large diffusion velocities compared with aerosols, can reach the walls of the tube and be absorbed by the coating. Aerosols that pass the denuder can be collected by a filter pack mounted in series with the denuder. If the denuder has an annular form and air is passed through the narrow section between two concentric tubes, larger sampling flows can be applied. Consequently, the sampling time of annular denuders is of the order of 10–30 min whereas simple denuders generally have sampling times of the order of hours.

ECN has developed a so-called wet denuder system (Fig. 1)[3]. The denuder is rotated along its axis and about 15 ml of a solution are pumped into the annulus and cover the walls. Gases are absorbed in this solution, depending on the properties of the gases and the absorbing solution. After a sampling time of typically 40 min, the solution is pumped out of the denuder and analysed in the laboratory. Ambient concentrations of compounds such as HNO_3, HNO_2, NH_3, SO_2, HCl and H_2O_2 can be measured selectively. The precision of the method is illustrated by parallel measurements of HNO_3 concentrations in ambient air by two wet denuder systems (Fig. 2). The standard deviation, calculated from 45 pairs of measurements, is 5.5% relative. The same order of precision was observed in parallel measurements of NH_3 and SO_2. The precision of the analytical method is 5% relative and could contribute the major part of the 5% standard deviation observed in measurements with wet denuder systems.

In view of the concentrations of important pollutants in the atmosphere, gradient measurements by means of a wet denuder system require precise measurements of Cl^-, NO_3^-, NO_2^- and SO_4^{2-} in the absorption solution of the wet denuder systems at concentrations of 50–2000 $\mu g\ l^{-1}$. The ion chromatographic system must be capable of handling a large number of analyses, as 1 week of gradient measurements at three heights results in at least 500 samples.

A computerized ion chromatographic system has been developed at ECN, that is able to carry out the required number of analyses with a precision of at least 5% relative[4]. It was decided to optimize this system for precision and accuracy, in order to minimize the analytical errors with the aim of extending the possibilities for application of the wet denuder systems to the measurement of deposition fluxes of components such as HNO_3, SO_2, HCl, NH_3 and HNO_2.

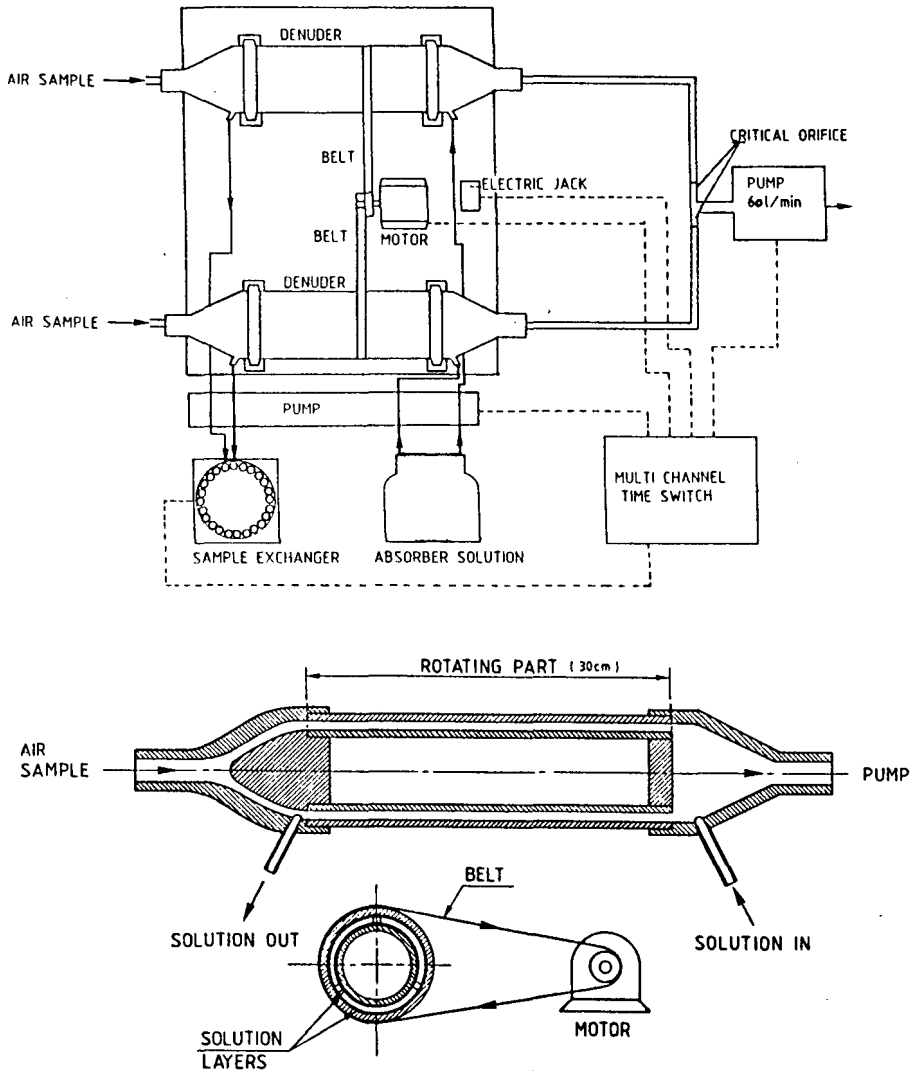

Fig. 1. Wet annular denuder system.

ION CHROMATOGRAPHIC SYSTEM

The IC system originally consisted of a Gilson sample changer, Dionex injection valves and columns, a Dionex membrane suppressor, a Waters Assoc. conductivity detector, a Shimadzu UV detector and a chloride ion-selective electrode detection system[4]. The detectors, sample changer and valves were interfaced to a Tulip microcomputer by means of a Keithley interface (Fig. 3). This was originally equipped with 12-bit analog–digital converters, which provided insufficient resolution in view of the very high signal-to-noise ratio of the Waters Assoc. conductivity detec-

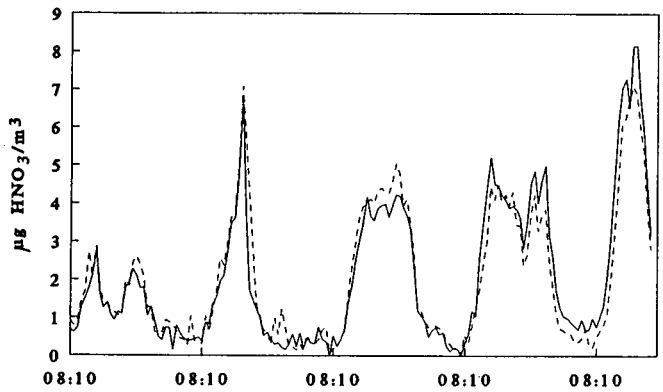

Fig. 2. Results for HNO_3 air concentrations sampled by two wet denuder systems in Rome during the period September 19th–23nd, 1988. Solid line, HNO_3 denuder 1; broken line, denuder 2.

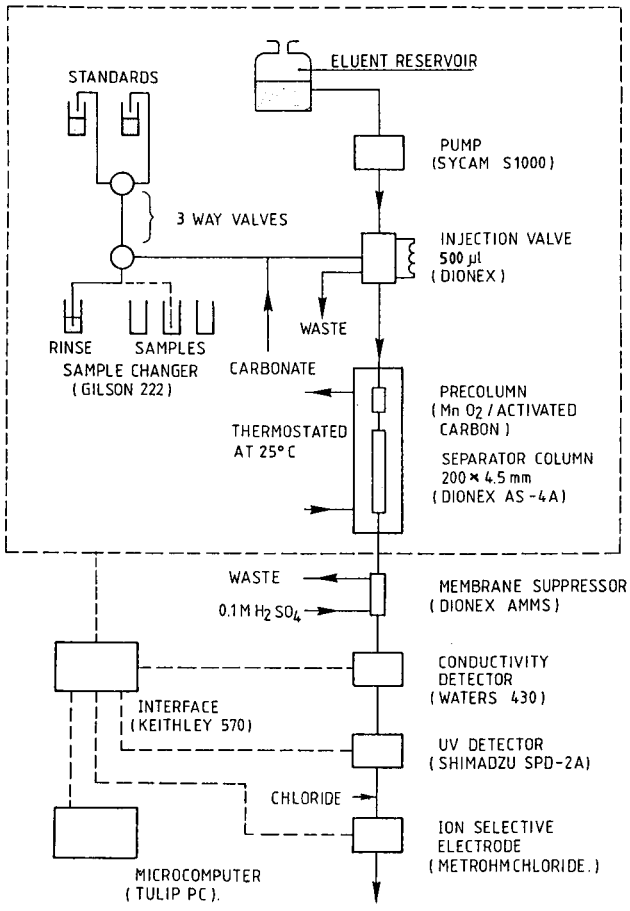

Fig. 3. Ion chromatographic system.

tor, and was replaced with a scanning digital voltmeter (Keithley) with a resolution of 5.5 digits. Enlargement of the sample loop from 100 μl[4] to 500 μl resulted in detection limits of 10 $\mu g\ l^{-1}$ or less for sulphate, nitrate and chloride.

Linearization of the signals of the conductivity detector, UV detector and ion-selective electrodes was performed as described previously[4]. Calibration was carried out by measuring two standard solutions containing 500 and 3000 $\mu g\ l^{-1}$ of sulphate, nitrate and chloride followed by linear regression of the results. Calibration was performed twice and accepted if a preset accuracy was attained. If this was not the case, the calibration procedure was repeated. After ten samples a standard was measured and the system was automatically recalibrated if the results of the standard exceeded preset boundaries.

RESULTS

Initially, precision and accuracy were investigated under "worst possible conditions". Calibration accuracy was set at a standard value of 5%. Samples in the concentration range 10–5000 $\mu g\ l^{-1}$ were analysed in random sequence. Ten samples were measured of each concentration in such a way that the results were obtained by means of different calibrations.

In Figs. 4–6, precision and accuracy are given for sulphate, nitrate and chloride as obtained from ten-fold analysis of standards in the range 10–5000 $\mu g\ l^{-1}$. The results are plotted double logarithmically. The 5 and 1% limits are indicated by straight lines. A second-order curve fit is shown to give a better indication of the overall results. Accuracy is of the order of 5% at concentrations of about 100 $\mu g\ l^{-1}$ for all components. An accuracy of 2% is possible for concentrations of more than 200 $\mu g\ l^{-1}$ of sulphate, nitrate and chloride.

A precision level of 5% is reached for sulphate and nitrate at a concentration of 30 $\mu g\ l^{-1}$ and for chloride at 50 $\mu g\ l^{-1}$. At this level, analytical errors will result in a sizable contribution to the overall error, as pointed out above. A precision of 1% for sulphate and nitrate measurements can be reached at levels of 60 and 400 $\mu g\ l^{-1}$,

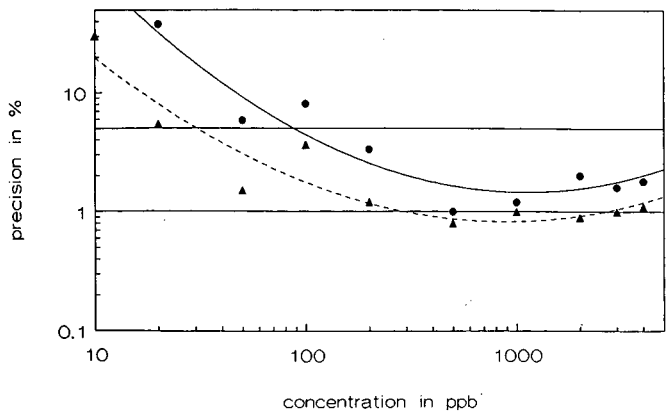

Fig. 4. (▲) Precision and (●) accuracy in percentages as a function of the sulphate concentration measured by the ion chromatographic system set at a calibration accuracy of 5%; straight lines indicate 1% and 5% precision limits.

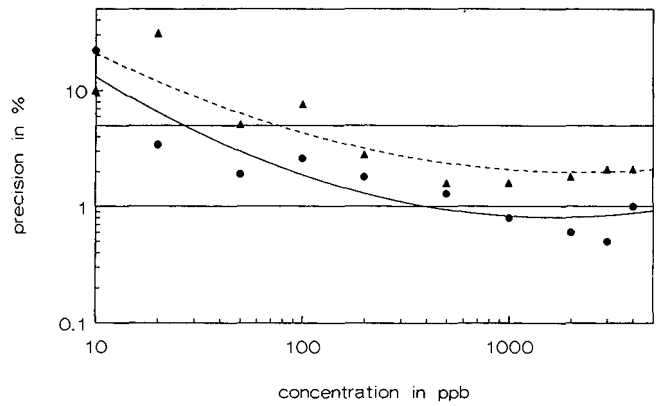

Fig. 5. (●) Precision and (▲) accuracy as a function of the nitrate concentration measured by the ion chromatographic system set at a calibration accuracy of 5%; straight lines indicate 1% and 5% precision limits.

respectively. The precision of chloride measurements is typically 2% or worse. It is expected that these precision levels will not contribute significantly to the overall error.

Generally, a sampling time of 1 h or less is employed in gradient measurements. The volume of the absorption solution of the wet denuder systems is typically 15 ml. The sampling flow of the wet denuder system is 30 l min^{-1}. The air concentrations of SO_2, HNO_3 and HCl, corresponding to the concentrations in the absorption solution where the levels of 1 and 5% precision are obtained, are given in Table I.

HNO_3 and HCl have very high deposition velocities (of the order of 2–3 cm s^{-1}) so steep gradients are generally observed and measurements are possible at the 5% precision level, corresponding to ambient levels of about 0.2 $\mu g\ m^{-3}$. This means that gradient measurements of the deposition of HNO_3 and to a lesser extent of HCl can be carried out under most circumstances, as the mean concentrations of these

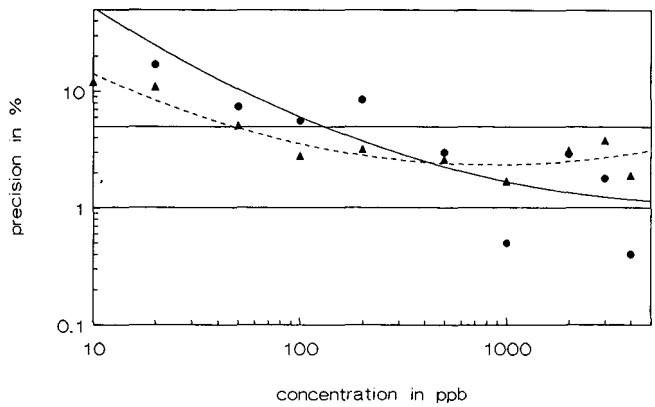

Fig. 6. (▲) Precision and (●) accuracy as a function of the chloride concentration measured by the ion chromatographic system set at a calibration accuracy of 5%; straight lines indicate 1% and 5% precision limits.

TABLE I

CONCENTRATIONS IN THE ABSORPTION SOLUTIONS OF THE WET DENUDER WITH 5% AND 1% PRECISION LIMITS AND THE CORRESPONDING AIR CONCENTRATIONS

Ion chromatographic system set at a calibration accuracy of 5%.

Species	*5% limit* ($\mu g\ l^{-1}$)	*Air concentration* ($\mu g\ m^{-3}$)	*1% limit* ($\mu g\ l^{-1}$)	*Air concentration* ($\mu g\ m^{-3}$)
SO_2	30	0.2	400	2
HNO_3	30	0.2	400	2
HCl	50	0.5	–	–

components in The Netherlands are 1.1 and 1 $\mu g\ m^{-3}$, respectively. Precise measurements at the 1% level are possible for SO_2 and HNO_3 at a level of 2 $\mu g\ m^{-3}$. This is not often observed for HNO_3 in The Netherlands, but the deposition flux of SO_2 can be measured precisely as the yearly average concentration of SO_2 in The Netherlands is of the order of 15 $\mu g\ m^{-3}$.

Accuracy and precision were also characterized for the following circumstances: limits for calibration and recalibration were set at 2%; calibration and recalibration take about twice as long as calibrations at the 5% level (eight standards are generally analysed instead of four); samples were grouped together for each concentration, which is a realistic situation as about the same concentration will be measured in gradient measurements at different altitudes; and samples of each concentration were analysed with the same calibration, a condition that in practice can be easily arranged for this type of measurements.

In Figs. 7–9, precision and accuracy are plotted for sulphate, nitrate and chloride as achieved by the analysis of six standards for each concentration in the range 10–5000 $\mu g\ l^{-1}$ under the above conditions. The 5% accuracy limit was reached at concentrations of 30, 100 and 100 $\mu g\ l^{-1}$ for sulphate, nitrate and chloride. At concentrations of 200–300 $\mu g\ l^{-1}$, an accuracy of 2% relative or better was obtainable.

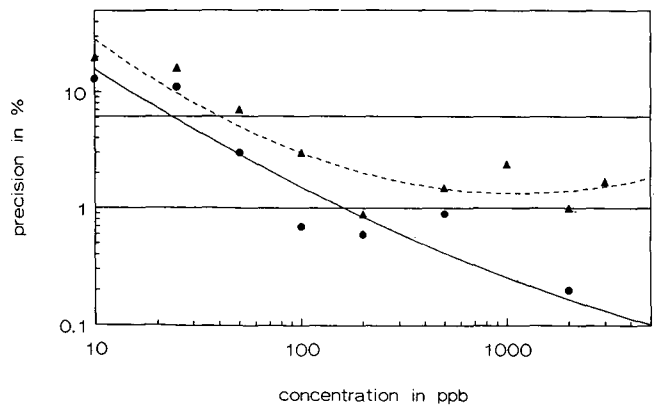

Fig. 7. (●) Precision and (▲) accuracy as a function of the sulphate concentration measured by the ion chromatographic system set at a calibration accuracy of 2%; straight lines indicate 1% and 5% precision limits.

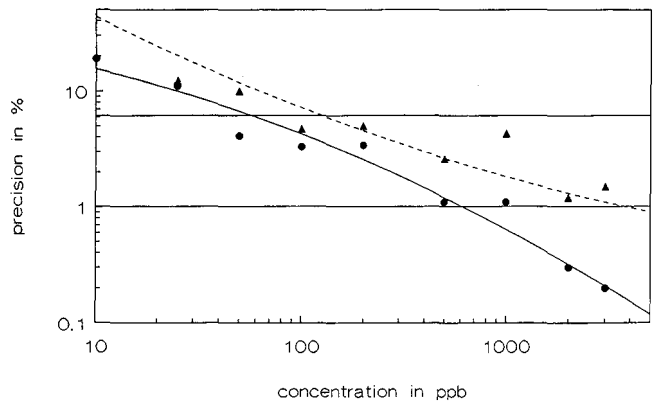

Fig. 8. (●) Precision and (▲) accuracy as a function of the nitrate concentration measured by the ion chromatographic system set at a calibration accuracy of 2%; straight lines indicate 1% and 5% precision limits.

The precision level of 5% was reached at a concentration of about 20 $\mu g\ l^{-1}$ for all three components. The 1% level was reached at 60, 400 and 500 $\mu g\ l^{-1}$ for sulphate, nitrate and chloride, respectively. The ambient concentrations, which correspond to the concentrations in the absorption solutions for which a precision of 5 and 1% can be claimed, were calculated as indicated before and given in Table II.

Deposition measurements are possible for HNO_3 and HCl, in view of their high deposition velocity, at ambient concentrations of 0.1 $\mu g\ m^{-3}$, which means that these measurements can be performed under nearly all ambient conditions. Precise measurements are possible for SO_2 at a level of 0.3 $\mu g\ m^{-3}$, a concentration that can be found in absolute background areas only. Precise deposition flux measurements for HCl and HNO_3 will only occasionally be feasible.

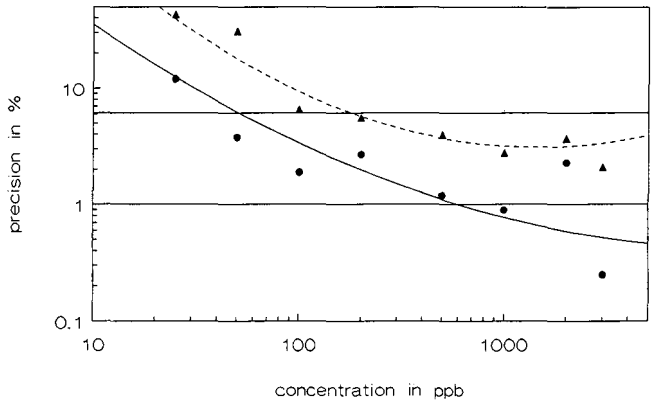

Fig. 9. (●) Precision and (▲) accuracy as a function of the chloride concentration measured by the ion chromatographic system set at a calibration accuracy of 2%; straight lines indicate 1% and 5% precision limits.

TABLE II

CONCENTRATIONS IN THE ABSORPTION SOLUTIONS OF THE WET DENUDER WITH 5% AND 1% PRECISION LIMITS AND THE CORRESPONDING AIR CONCENTRATIONS

Ion chromatographic system set at a calibration accuracy of 2%.

Species	*5% limit* ($\mu g\ l^{-1}$)	*Air concentration* ($\mu g\ m^{-3}$)	*1% limit* ($\mu g\ l^{-1}$)	*Air concentration* ($\mu g\ m^{-3}$)
SO_2	20	0.1	60	0.3
HNO_3	20	0.1	400	2
HCl	20	0.1	500	2.5

CONCLUSIONS

It is possible to optimize an ion chromatographic system in such a way that sufficient precision is obtained for this method to be applied to the measurements of gradients by means of wet denuder systems. The application to the measurement of the deposition fluxes of components such as HNO_3, HCl and SO_2 will hopefully result in more precise estimates of the deposition fluxes of acid-deposition-related components, as the present uncertainty is of the order of 40% or more.

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NEW DEVELOPMENTS IN ION CHROMATOGRAPHIC METHODS FOR ATMOSPHERIC ANALYSIS

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SUMMARY

Examples are given of the determination of inorganic anions, cations and organic acids in atmospheric samples. With amperometric detection, 2.7 ppb of bromide can be determined in the presence of 8 ppm of nitrate. With a column-switching technique, Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} can be determined within 8 min in a 50-μl sample using indirect fluorescence detection and a cerium(III) solution as eluent. A method of indirect amperometric detection after post-column derivatization with EDTA was developed for the determination of divalent transition metals, giving detection limits in the upper picogram range. With an enrichment technique involving solid-phase extraction, 0.28 ppb of monochloroacetate can be determined in rainwater samples.

INTRODUCTION

The complexity of methods used in atmospheric analysis is due to the heterogeneous composition of air. This requires careful sampling procedures in order to avoid any disturbance of the equilibrium when sampling and problems due to contamination. If a high resolution with respect to time is necessary and, for example, cloud drops are to be analysed, then even the analysis of matrix species is difficult because only a few microlitres are available for a single ion whereas several inorganic cations, anions and organic compounds may need to be analysed.

As is demonstrated in Fig. 1, the three different possible phases in the atmosphere can be easily reduced to one type of analysis in the aqueous phase. The problems with the different phases concern on the one hand the sampling procedure and on the other the requirements for enrichment.

In Table I, the inorganic anions, cations and organic acids that play an important role in atmospheric analysis and that can be analysed by using ion chromatography are listed. The reason for using ion chromatography is different for the three groups of species. For inorganic anions, the simultaneous determination of all important anions is much more advantageous than different spectrophotometric methods for each individual anion. In addition, there are fewer interferences and the detection limits are lower using ion chromatography. With inorganic cations, spectroscopic

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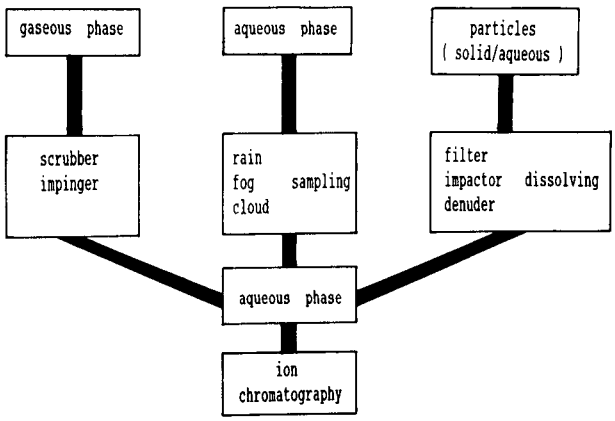


Fig. 1. Analytical pretreatment for atmospheric samples prior to ion chromatography. Problems: sampling, enrichment, contamination, storage (oxidation, bacterial degradation).

methods have lower detection limits, but the simultaneous determination of a number of elements in a small volume (*e.g.*, 10 μl) is only possible using a chromatographic method. In addition, the determination of NH_4^+, which is important in air analysis, can be performed simultaneously with that of the alkali metal ions. The determination of organic acids is possible by derivatization and gas chromatography[1] or by

TABLE I

MEAN CONCENTRATIONS OF INORGANIC ANIONS, INORGANIC CATIONS AND ORGANIC ACIDS IN THE ATMOSPHERE

Type	*Species*	*Aerosol (nmol/m³)*	*Rain (μmol/l)*	*Fog (μmol/l)*	*Cloud (μmol/l)*
Inorganic anions	Chloride	4.9	34	426	40
	Nitrate	15.4	145	700	170
	Sulphate	38.9	90	385	77
	Fluoride			130	
	Bromide	0.1	0.1		
	Nitrite		1.8	16	
	Sulphite		0.5	83	
Inorganic cations	Sodium	5.1	31	130	24
	Ammonium	82.3	150	1970	185
	Potassium	2.1	30	200	39
	Magnesium	1.5	9	30	13
	Calcium	3.7	23	160	40
	Manganese		0.2	0.7	
	Iron		0.9	7.2	
Organic acids	Formate	1400[a]; 1.10	18.5	56	6.0
	Acetate	1800[a]; 0.90	13.0	58	8.3
	Methanesulphonate	0.25	0.12		0.8
	Pyruvate	60[a]; 0.16	0.35	3.6	0.7
	Monochloroacetate	0.30	0.02		
	Dichloroacetate	0.03	0.01		

[a] Gas phase (molar mixing ratios, ppt $\times 10^{12}$).

derivatization and reversed-phase chromatography[2]. The drawbacks of these methods are that atmospheric samples are usually in the aqueous phase and that derivatization is easier in the organic phase. The determination of organic acids using ion-exchange or ion-exclusion chromatography can be carried out directly with acceptable detection limits.

EXPERIMENTAL

Sampling techniques

Sampling of rain water was carried out with a funnel made of stainless steel (area 1 m^2) combined with a fraction collector (Sepafrac MFC-111 with a Sepacon TCU-211 time control unit; Labomatic, Sinsheim, F.R.G.).

Fog samples were taken with a fan collector. Fog droplets impact on PTFE strings (diameter 0.3 mm). The air flow-rate caused by the fan was 1080 m^3/h. Cloud water samples were taken with a collector developed by Mohnen[3]. The collecting unit consists of ten slit PTFE rods (length 230 mm, slit width 1.5 mm, slit depth 2.5 mm, diameter 11 mm).

The material used for the sample bottles was polypropylene because of the low blank values for all the species analysed. All samples were filtered with Millex-GV filters (pore size 0.22 μm) before analysis.

Apparatus

Inorganic anions. The ions determined were Cl^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, NO_2^-, $(COO)_2^{2-}$ and Br^-. An IC-2000i system (Dionex, Idstein, F.R.G.), including an HPIC-AG4 precolumn and an HPIC-AS4 analytical column, was used. The eluent was 1.2 m*M* Na_2CO_3–1.5 m*M* $NaHCO_3$ at a flow rate of 2 ml/min. For conductivity detection a micromembrane suppressor (AMMS-1, 12.5 m*M* sulphuric acid, 2.5 ml/min) was used. Amperometric detection of bromide was performed with a potentiostat (Dionex) with a silver working electrode and an Ag/AgCl reference electrode at +200 mV.

Inorganic cations. Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} were determined with indirect fluorescence detection. The HPLC system included a Varian (Darmstadt, F.R.G.) Model 2510 pump, Rheodyne Model 7125 injection valve with a 50-μl sample loop and a Spectroflow 980 fluorescence detector (Applied Biosystems, Weiterstadt, F.R.G.). The excitation wavelength was set at 254 nm and an emission filter with a cut-off at 345 nm was used. The system included a GC 210 ion guard (ICT, Frankfurt/M, F.R.G.), an ION 210 separation column (ICT) for Na^+, NH_4^+ and K^+ and an HPIC-CS1 column (Dionex) for Mg^{2+} and Ca^{2+}. The eluent was a 28 μM cerium(III)nitrate solution at a flow-rate of 1 ml/min.

Transition metals. The metal ions determined were Cu^{2+}, Zn^{2+}, Mn^{2+} and Ni^{2+}. The HPLC system consisted of a Varian Model 2510 pump, a Rheodyne Model 7125 injection valve with a 20-μl sample loop and a Model 400 amperometric detector (Biotronik). Separation was effected on a Nucleosil SA (5 μm) column (250 × 4.6 mm I.D.) with 0.14 m*M* tartaric acid solution (pH 3, adjusted with sodium hydroxide) as eluent (1 ml/min). Post-column derivatization was carried out with 0.2 μM EDTA.

Organic acids. For the determination of organic acids (formate, acetate, pyruvate, methanesulphonate and monochloroacetate), the same ion chromatographic

system as for the detection of inorganic anions was used (conductivity detection) except that the eluent was 0.5 m*M* sodium hydrogencarbonate solution at 2 ml/min.

Materials

Standard solutions of the inorganic anions were prepared from their sodium salts (Suprapur; Merck, Darmstadt, F.R.G.). Standard solutions of the inorganic cations were prepared from their nitrates (analytical-reagent grade; Merck). Standard solutions of the organic acids (formate, acetate, pyruvate, methanesulphonate) were prepared from their sodium salts (analytical-reagent grade; Merck). Standard solutions of the dicarboxylic acids were prepared from their sodium salts or from the free acids. Standards solutions of monochloroacetate and dichloroacetate were prepared from the free acids (analytical-reagent grade; Fluka, Buchs, Switzerland; *ca.* 99%, Aldrich, Steinheim, F.R.G.).

All chemicals used for the eluents were of analytical-reagent grade (Merck). Deionized water from a Milli-Q-System (Millipore, Eschborn, F.R.G.) was used. All solutions were degassed under vacuum.

RESULTS AND DISCUSSION

Determination of anions

The main anionic constituents of atmospheric samples are SO_4^{2-}, NO_3^- and Cl^-, the concentrations of other ions being relatively low. The determination of components of low concentration requires either an increase in selectivity or an enrichment procedure.

Fig. 2 shows a typical chromatogram for a rain sample with conductivity and UV detection. A number of substances with low concentrations are difficult to determine. Determination can be performed better with selective detection, *e.g.*, UV detection of NO_2^-. Another example is the determination of Br^- in air, which is difficult in the presence of high concentrations of NO_3^-. Zeissler[4] found that with conductivity detection, only NO_3^-/Br^- ratios of less than 1600 are acceptable. However, with amperometric detection, it is possible to determine 2.7 ppb[a] of Br^- in the presence of 8 ppm of NO_3^-.

In some samples of fog water, a high concentration of SO_3^{2-} was found, which interferes with the amperometric detection of Br^-. This can be avoided by oxidation with oxygen in the presence of Co^{2+} as catalyst. Fig. 3 shows the different methods of detection for Br^- in rain water.

If the detection limit is higher than the concentration to be determined, then an enrichment step is necessary. In the determination of anions, most of the enrichment is carried out on precolumns without any selectivity. Therefore, the limitation is given by the total capacity of the column.

As the sum of the concentrations of SO_4^{2-}, NO_3^- and Cl^- in rain water was found to be *ca.* 3 μg on average (with the assumption of a 100-μl sample injection) and the column capacity is 50 μg, an enrichment factor of 20 is the limit in instances where no selectivity is obtained. The only possibility is to carry out a heart-cut of the analyte

[a] Throughout this article, the American billion (10^9) and trillion (10^{12}) are meant.

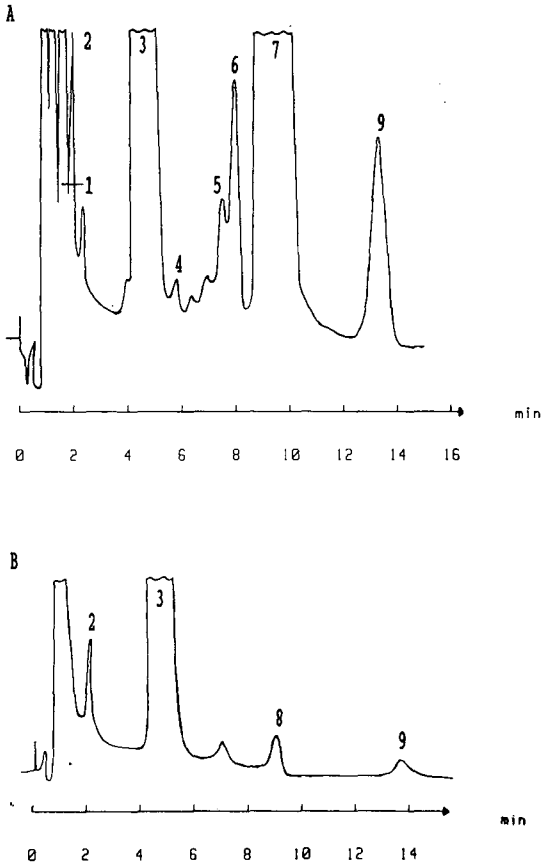


Fig. 2. Chromatograms of a rain-water sample. (A) Conductivity detection (range: 0.1 μS/cm). (B) UV detection (range: 0.005). 1 = 0.760 μg/ml Cl^-; 2 = 0.043 μg/ml NO_2^-; 3 = 6.060 μg/ml NO_3^-; 4 = 0.020 μg/ml SO_3^{2-}; 5 = 0.138 μg/ml succinate; 6 = 0.190 μg/ml malonate; 7 = 9.600 μg/ml SO_4^{2-}; 8 = 0.039 μg/ml malate; 9 = 0.218 μg/ml oxalate.

in question and to add up the fractions from several chromatographic separations. For some of the anions difficulties arise owing to their instability (SO_3^{2-}, NO_2^-) if the sample is not analysed immediately.

Determination of cations in air samples

When sampling cloud or fog water with a high time resolution, the available volumes are small[5] and a large number of substances need to be identified. Therefore, it is necessary to develop a method for the determination of several cations requiring only a few microlitres of one sample and permitting absolute detection limits at the picogram level. These requirements are usually not fulfilled by spectroscopic methods.

Ion chromatography combined with indirect fluorescence detection[6,7] allows the determination of alkali metal, ammonium and alkaline earth metal ions in the desired concentration range. Elution and simultaneous detection are carried out with

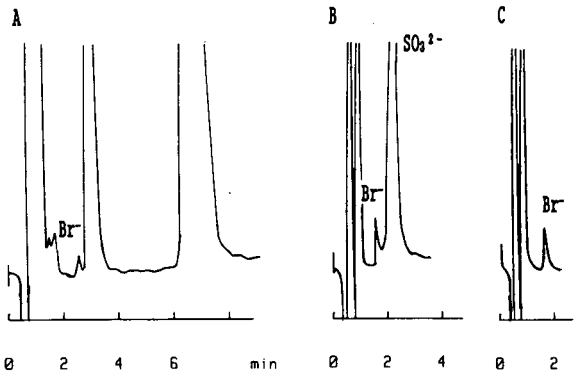
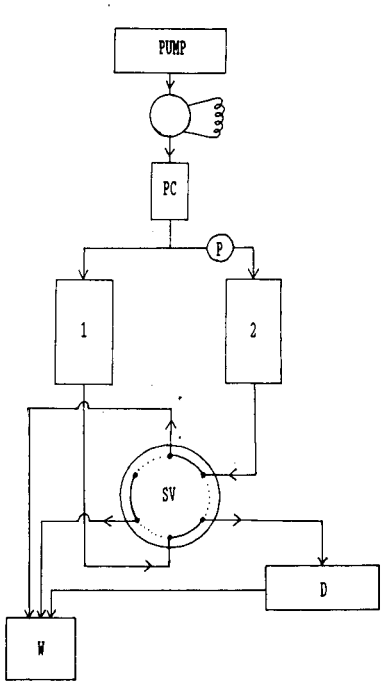


Fig. 3. Determination of bromide. (A) Conductivity detection [rain sample, 16 ng/ml Br^-; range: 0.1 μS/cm]. (B) Amperometric detection [rain sample, 13 ng/ml Br^-, before oxidation of sulphite; range: 30 nA]. (C) Amperometric detection (after oxidation of sulphite).

cerium(III) nitrate[8]. In order to perform analyses with high sensitivity, it is necessary to work with very low cerium(III) concentrations (12 mg/l). However, under these conditions, the alkaline earth metal ions cannot be eluted from the column (ION 210 metal column; ICT). The use of a CS-1 cation-exchange column (Dionex) permits the



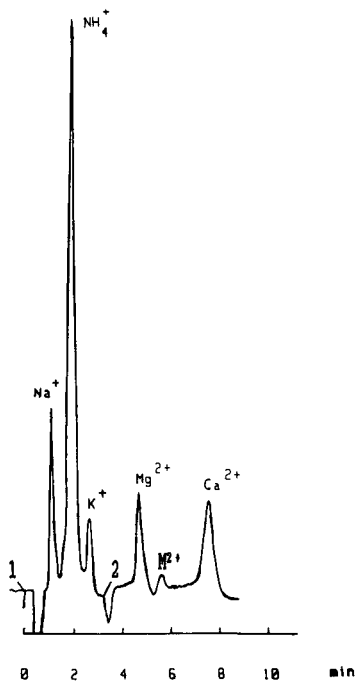
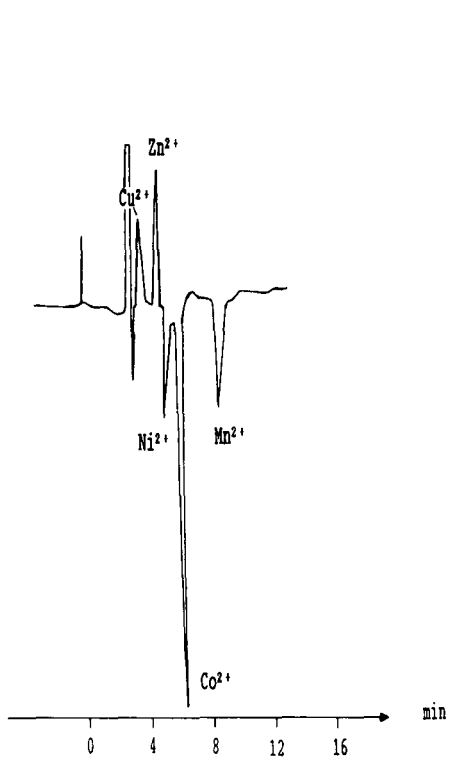


Fig. 4. Column switching. PC = Precolumn; P = pressure relief valve; 1 = ION 210 column (ICT); 2 = CS-1 column (Dionex); SV = switching valve; D = detector; W = waste.

Fig. 5. Chromatogram of a fog sample (diluted 1:50) containing 0.52 μg/ml Na^+, 1.32 μg/ml NH_4^+, 0.53 μg/ml K^+, 0.09 μg/ml Mg^{2+}, 0.26 μg/ml Ca^{2+}. M^{2+} = divalent transition metals. (1) ION 210 metal column (range: 0.01). (2) CS-1 column (range: 0.002).

separation of these ions. The detection limits for the alkali metal ions lie between 0.25 ng/ml for lithium and 10 ng/ml for cesium and for the alkaline earth metal ions between 1 ng/ml for magnesium and 100 ng/ml for barium. A system in which both columns are combined was developed that permits the determination of Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} in a 50-μl sample within 8 min (Fig. 4). Chromatography takes place simultaneously on both columns by a splitting technique. With a switching valve one can choose the effluent to be detected. After elution of the alkali metal ions from the ICT column, the valve has to be switched to detect the alkaline earth metal ions from the CS-1 column. Owing to the very different pressures of the two columns, a back-pressure is necessary to regulate the flow-rate at 1 ml/min for each column. Fig. 5 shows a chromatogram of a fog sample obtained with this column-switching technique.

In the chromatogram of the alkaline earth metal cations, the sum of the divalent transition metals can be determined between the Mg^{2+} and Ca^{2+} peaks (Fig. 5). So far we have not succeeded in separating and determining transition metals with cerium(III) as the eluent. Therefore, an ion chromatographic separation followed by



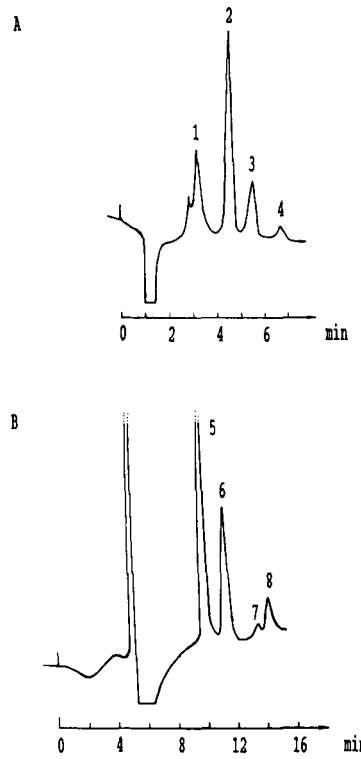


Fig. 6. Indirect amperometric detection of the transition metals Co, Zn and Cu (20 μg/ml each), Ni (35 μg/ml and Mn (0.05 μg/ml). Detection: 1100 mV Pt *vs.* Ag/AgCl. Range: 20 nA.

Fig. 7. (A) Ion-exchange chromatography (cloud sample; range 0.3 μS/cm). 1 = 0.35 μg/ml acetate; 2 = 0.23 μg/ml formate; 3 = 0.18 μg/ml pyruvate; 4 = 0.02 μg/ml methanesulphonate. (B) Ion-exclusion chromatography (rain sample; range 0.3 μS/cm). 5 = 0.72 μg/ml formic acid; 6 = 0.28 μg/ml acetic acid; 7 = 0.15 μg/ml propionic acid; 8 = carbonate.

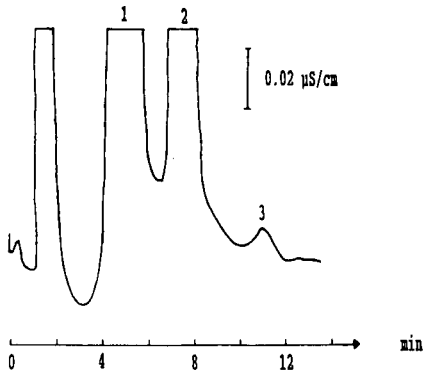


Fig. 8. Determination of monochloroacetate after preconcentration (1:50) of rain water. 1 = Acetate; 2 = formate; 3 = 0.28 ng/ml monochloroacetate.

TABLE II

RESULTS OF FIELD MEASUREMENTS (MEAN VALUES, µmol/l)

Species	*Rain (Kolmbach/Odenwald, May 27th, 1988)*	*Fog (Beedenkirchen/Odenwald, August 2nd, 1989; Kolmbach/Odenwald, November 24th, 1988)*	*Cloud (Frankfurt/Main, August 23rd, 1986; Sylt, August 23rd, 1987; Whiteface Mountain, September 30th, 1987)*
Chloride	34.0	612.0	40.0
Nitrate	145.0	805.0	170.0
Nitrite	1.8	16.0	0.9
Bromide	0.1		
Sulphite	5.0	103.0	
Sulphate	99.0	410.0	77.0
Formate	20.0	22.0	9.0
Acetate	14.0	82.0	6.4
Pyruvate		7.6	0.8
Methane-sulphonate			1.7[a] 0.2[b]
Monochloro-acetate	0.02[c]		
Dichloro-acetate	0.01[c]		
Sodium	43.0	110.0	24.0
Ammonium	148.0	1507.0	185.0
Potassium	30.0	293.0	39.0
Magnesium	9.0	31.0	13.0
Calcium	23.0	82.0	40.0

[a] Maritime.
[b] Continental.
[c] Rain sample taken in Darmstadt (1987).

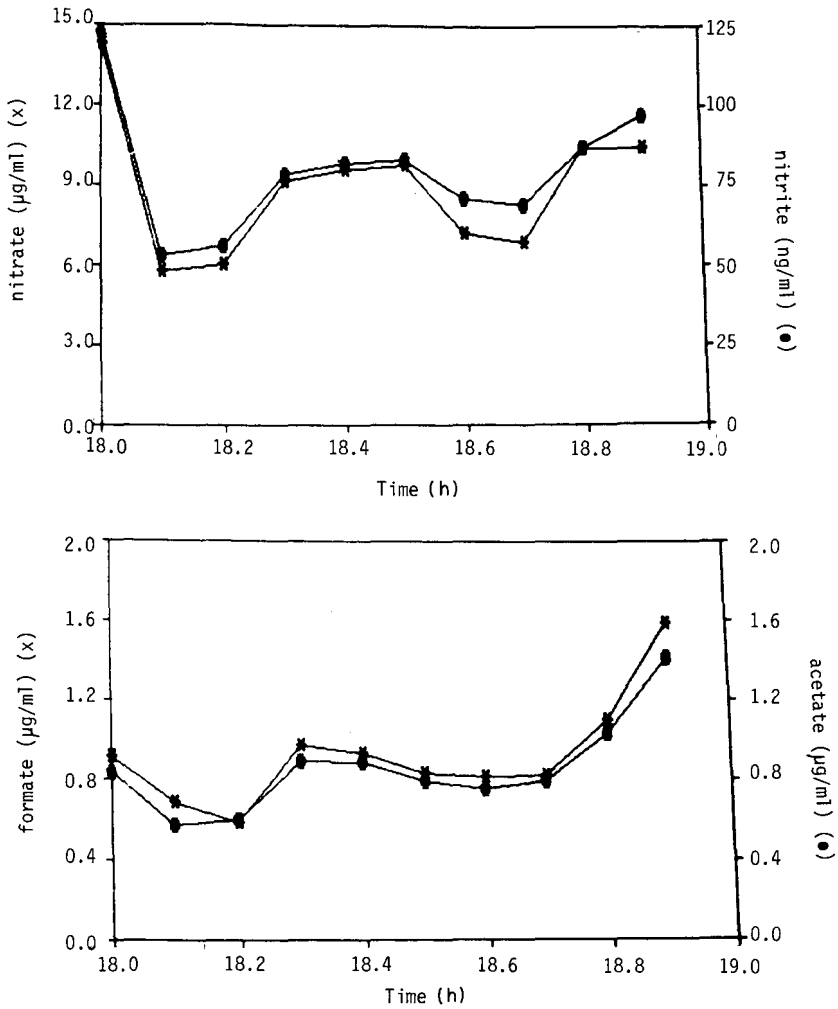


Fig. 9. Correlation of nitrate/nitrite and formate/acetate ratios during a rain sampling (Kolmbach/Odenwald, May 27th, 1988).

indirect amperometric detection was developed. The post-column complexation of the metal ions with an oxidizable organic ligand (EDTA) is measured and the change in concentration of the reagent is detected (Fig. 6). The detection limits of this method are in the upper picogram range[9].

Determination of organic acids in air samples

The main acids found in atmospheric samples are formate, acetate, dicarboxylic acids, methanesulphonic acid, pyruvic acid and monochloroacetate. In other analytical methods such as esterification and gas chromatographic separation, enrichment steps and extraction are necessary, whereas for these main components such procedures are not required when using liquid chromatography.

Chromatographic separation is possible with either ion-exchange or ion-exclusion chromatography. Using ion-exchange chromatography, the weak acids elute before the strong acids, whereas with ion-exclusion chromatography, the sequence is reversed. Fig. 7 shows typical chromatograms for cloud and rain water. The amount of methanesulphonic acid depends on the maritime contribution (sea spray) in the atmosphere.

When very low concentrations (1 ppb) of mono- or dichloroacetate have to be determined together with formate (> 1 ppm), a direct determination is impossible. Fuchs and Bächmann[10] have demonstrated how heart-cutting of monochloroacetate from many samples leads to enrichment and to a partial separation from formate, but this was a very time-consuming procedure. An enrichment procedure using solid-phase extraction was developed in which there was no appreciable selectivity in the adsorption but the enrichment factor of 50 was high enough to allow the direct determination of monochloroacetate (Fig. 8). The main difficulties arise from impurities in the solid-phase column[11].

Results of field measurements

In Table II, results for cloud, fog and rain water are summarized. The interpretation of these results will be restricted to some general comments: (1) the concentrations in the fog samples are much higher (by a factor of 10) than those in rain samples; (2) during a rain event there are dramatic changes in the concentrations of the different substances; (3) in rain and cloud water samples the concentrations of the different ions are in comparable ranges; (4) the ratios of nitrate to nitrite and formate to acetate concentrations, for example, correlate during this rain event (Fig. 9).

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Note

Determination of Pb^{II}, Cd^{II} and Bi^{III} by reversed-phase liquid chromatography of their diethyldithiocarbamate complexes with post-column ligand exchange and selective spectrophotometric detection

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Recently we reported the selective preconcentration of metal ions as their diethyldithiocarbamate[1] or diethyldithiophosphate[2] complexes, in which the selectivity was introduced via a dual pre-column system that eliminates most of the interfering compounds. As an alternative to this approach, the performance of the analytical system can be improved by using selective detection.

We have shown previously[3] that the dithiocarbamate derivative thiram (tetramethylthiuram disulphide), a notorious pesticide, can be selectively detected as its corresponding copper(II) dimethyldithiocarbamate [$Cu(DmDTC)_2$] complex, which is formed in a solid-state reactor packed with metallic copper and has a strong absorption maximum at 435 nm. During these investigations, we found that not only metallic copper and Cu^{II} ions, but also insoluble Cu^{II} salts such as copper(II) sulphide or copper(II) phosphate, react rapidly and quantitatively with thiram to form $Cu(DmDTC)_2$. This may be explained by the high complex stability of $Cu(DmDTC)_2$ ($\log \beta_2 = 21.8$)[4]. The formation of copper diethyldithiocarbamate [$Cu(DTC)_2$] was also observed, *viz.*, when copper(II) phosphate was added to a solution of metal diethyldithiocarbamate (DTC) complexes such as $Pb(DTC)_2$ or $Cd(DTC)^2$, which possess considerably lower complex stabilities than $Cu(DTC)_2$. This ligand-exchange reaction has already been used for the determination of diethyldithiocarbamate, which was preconcentrated and chromatographed as its lead(II) complex[5]. As the ligand-exchange reaction between $Pb(DTC)_2$, $Cd(DTC)_2$ or $Bi(DTC)_2$ and Cu^{II} proceeds fairly fast, we studied the use of copper(II) phosphate and, for comparison, nickel(II) phosphate as post-column complexation reagent for the selective determination of Pb^{II}, Cd^{II} and Bi^{III}. The aim of this work was to establish a method that allows the selective single-wavelength determination of these metal ions in complex matrices.

EXPERIMENTAL

Chemicals

Sodium N,N-diethyldithiocarbamate [Na(DTC)] was supplied by EGA Chemie (Steinheim, F.R.G.). All other organic chemicals were of analytical-reagent grade

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(Baker, Deventer, The Netherlands). Copper(II) nitrate, nickel(II) nitrate, lead(II) acetate, cadmium(II), acetate, bismuth(III) nitrate, potassium hydrogenphosphate and cetyltrimethylammonium bromide (cetrimide) were Baker Analyzed Reagents.

Metal diethyldithiocarbamate complexes were prepared by adding a 1 m*M* solution of the corresponding metal acetate (Pb, Cd) or nitrate (Cu, Bi) to a 2 m*M* aqueous solution of Na(DTC) in an aqueous 10 m*M* phosphate buffer (pH 6.8). All experiments were carried out with fresh solutions to avoid precipitation of the metal dithiocarbamates.

Instrumentation

The high-performance liquid chromatographic (HPLC) system (Fig. 1) consisted of a Kontron (Zürich, Switzerland) LC pump and a 200 × 2.1 mm I.D. or a 100 × 4.6 mm I.D. stainless-steel column packed with 5-μm Hypersil ODS (Shandon Southern, Runcorn, U.K.). A Hewlett-Packard (Waldbronn, F.R.G.) Model 1040 diode-array detector was used at a detection wavelength of 435 nm. Trace enrichment was carried out on a laboratory-made 4.0 × 2.1 mm I.D. precolumn, which was hand-packed with a slurry of 5-μm Hypersil ODS in methanol using a syringe. The preconcentration pump was an Altex (Berkeley, CA, U.S.A.) Model 110 pump used at a flow-rate of 1.0 ml/min. For the post-column reactor the same type of precolumns of length 2.0 or 4.0 mm and 4.6 mm I.D. was used. Acetonitrile–10 m*M* aqueous acetate buffer (pH 6.0) containing 10 m*M* cetrimide was used as the mobile phase; it was degassed ultrasonically under vacuum for 20 min.

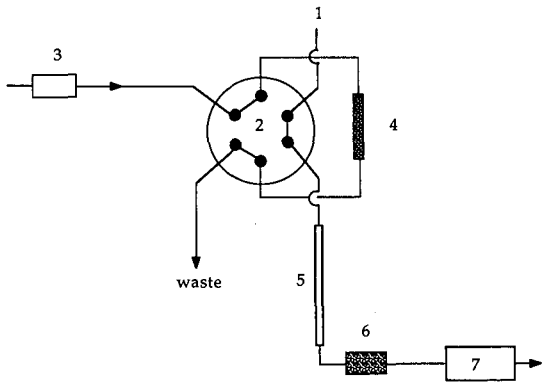


Fig. 1. Scheme of the chromatographic system. 1 = HPLC pump; 2 = six-port injection valve; 3 = preconcentration pump; 4 = C_{18} precolumn; 5 = analytical column; 6 = metal phosphate post-column reactor; 7 = detector.

Preparation of the post-column reactor

The post-column reactor was a 2.0 or 4.0 × 4.6 mm I.D. column filled with copper(II) or nickel(II) phosphate, which were prepared by mixing equal volumes of a 0.1 *M* solution of potassium hydrogenphosphate and a 0.1 *M* solution of the corresponding metal nitrates. The metal phosphate precipitate was washed twice with both doubly distilled water and methanol. After treating the metal phosphate suspension in methanol ultrasonically for 20 min, it was dried on tissue paper and pressed as densely as possible into the reactor column using a micro-spatula.

Determination of Pb^{II} and Cd^{II} in urine

After filtration over a 0.2-μm membrane filter, 500 μl of aqueous 1 *M* acetate buffer (pH 6.0) and 500 μl of aqueous 1 m*M* Na(DTC) solution were added to 9 ml of a urine sample. Injections onto the C_{18} precolumn (4.0 × 2.1 mm I.D.) were performed with a 1.0-ml loop connected to a Valco six-port injection valve. After preconcentration of the sample solution, the pre-column was flushed with 5 ml of 10 m*M* acetate buffer (pH 6.0) solution. The metal diethyldithiocarbamates were desorbed to the analytical column by means of the LC mobile phase.

RESULTS AND DISCUSSION

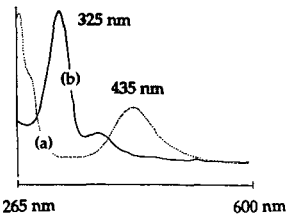
Chromatographic conditions

The metal diethyldithiocarbamates were chromatographed on C_{18}-bonded silica with acetonitrile–acetate buffer (pH 6.0) (70:30, v/v) as mobile phase. The use of methanol instead of acetonitrile led to a drastic decrease in the column efficiency. Cetrimide (10 m*M*) was added to the mobile phase in order to improve the peak shape of $Cd(DTC)_2$ (*cf.*, ref. 2). Under the stated conditions, the capacity ratios of the various metal complexes were as follows $Pb(DTC)_2$ 1.9, $Ni(DTC)_2$ 2.2, $Bi(DTC)_3$ 3.2 and $Cd(DTC)_2$ 4.1.

Parameters of the reaction–detection system

Fig. 2 shows the absorption spectra of $Cu(DTC)_2$ (λ_{max} = 435 nm, ε_{max} = 13 000)[5] and $Ni(DTC)_2$ (λ_{max} = 325 nm, ε_{max} = 38 000)[5] recorded with a photodiode-array detector. Together with peak-height measurements these spectra were used to determine the degree of conversion in the metal phosphate post-column reactors. The main parameters that influence the ligand-exchange reaction between $Pb(DTC)_2$, $Cd(DTC)_2$ or $Bi(DTC)_3$ and Cu^{II} or Ni^{II} are the reaction time and temperature, the solvent composition and the pH.

Copper(II) phosphate as post-column reagent. Fig. 3 shows the degree of conversion for $Pb(DTC)_2$, $Cd(DTC)_2$ and $Bi(DTC)_3$ at different reaction times at 20°C. Already at room temperature and at the shortest reaction time investigated (1.3 s, corresponding to a flow-rate of 1.6 ml/min), nearly complete conversion into $Cu(DTC)_2$ is obtained. This was confirmed by an evaluation of the absorption spectra for the peaks recorded after injection of $Pb(DTC)_2$ and $Cd(DTC)_2$, which were iden-



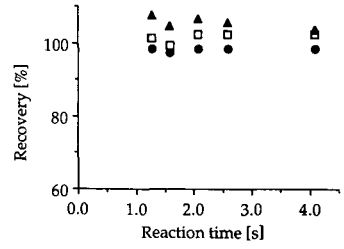


Fig. 2. Absorption spectra of (a) $Cu(DTC)_2$ and (b) $Ni(DTC)_2$.

Fig. 3. Conversion of (▲) $Pb(DTC)_2$, (□) $Cd(DTC)_2$, and (●) $Bi(DTC)_3$ at 20°C as a function of the residence time using a copper(II) phosphate reactor.

tical with the spectrum of $Cu(DTC)_2$. An increase in the reaction temperature to 40°C did not result in an improved performance.

Nickel(II) phosphate as post-column reagent. $Ni(DTC)_2$ is considerably less stable than $Cu(DTC)_2$; therefore, one can expect that, with Ni^{II}, the ligand-exchange reaction with the various metal diethyldithiocarbamates will be less efficient than with Cu^{II}. Fig. 4a, b and c show the degree of conversion for $Bi(DTC)_3$ and $Cd(DTC)_2$ and $Pb(DTC)_2$, respectively, with nickel(II) phosphate at 20, 40 and 60°C. At room temperature and at the shortest reaction time investigated (about 1 s), the degree of conversion is considerably lower than in the copper(II) phosphate reactor. $Cd(DTC)_2$ and $Pb(DTC)_2$ react significantly faster than $Bi(DTC)_3$, and they are almost completely converted at room temperature if the reaction time is at least 2 s. Maximum conversion of $Bi(DTC)_3$ is only obtained at a reaction temperature of 60°C and reaction times longer than 1.2 s; this may be due to the fact that $Bi(DTC)_3$ is more stable than $Cd(DTC)_2$ and $Pb(DTC)_2$.

Effect of the mobile phase composition. The ligand-exchange reaction is not affected by the addition of common LC organic modifiers such as acetonitrile or methanol to the mobile phase. Further, no effect of these solvents on the lifetime of the metal phosphate reactor was observed. However, in order to prevent dissolution of the metal phosphates, the pH of the mobile phase has to be higher than 5.

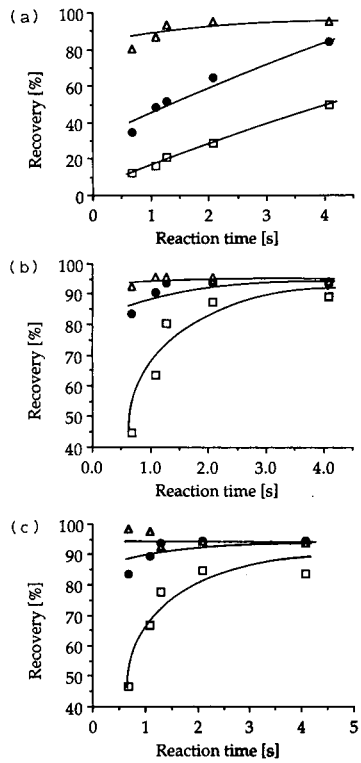


Fig. 4. Conversion of (a) $Bi(DTC)_3$, (b) $Cd(DTC)_2$ and (c) $Pb(DTC)_2$ at (□) 20, (●) 40 and (△) 60°C as a function of the residence time using a nickel(II) phosphate reactor.

External peak broadening. The peak broadening caused by the metal phosphate reactor was tested by injecting $Cu(DTC)_2$ with and without the post-column reactor installed. It was found that the 2.0 and 4.0 × 4.6 mm I.D. reactors caused an additional peak broadening of σ = 1.0–1.5 s, which is small compared with the peak broadening of the total chromatographic system (without reactor) of σ = 5.1 s. The asymmetry factors (measured at 10% of the peak height) increased from 1.2 to 1.3 (reactor length 2.0 mm) and 1.4 (reactor length 4.0 mm). Both additional peak broadening and asymmetry factors were reproducible within 10% between different reactor packings.

Analytical data

The detection limits (signal-to-noise ratio = 3:1) for Pb^{II} and Cd^{II} were 7 ng using the copper phosphate reactor and 3 ng using the nickel phosphate reactor. The sensitivity of the system with the nickel phosphate reactor is higher because of the higher ε_{max} of $Ni(DTC)_2$. The relative standard deviation for direct-loop injections of 25 ng was less than 2.5% (n = 8). The detector response was linear over almost three orders of magnitude (r = 0.999; upper limit for the copper phosphate reactor, 2000 ng). The lifetime of the metal phosphate reactors was determined only by the injected mass of compounds which react with the corresponding metal. No effects of contaminants from biological samples on the reactor lifetime were observed.

Possible interferences from disulfiram and Ni^{II}

If metal diethyldithiocarbamates such as $Pb(DTC)_2$ are detected by UV absorbance measurement at 254 nm, quantitation is often difficult owing to interferences caused by disulfiram (tetraethylthiuram disulphide) and/or $Ni(DTC)_2$ (see Fig. 5).

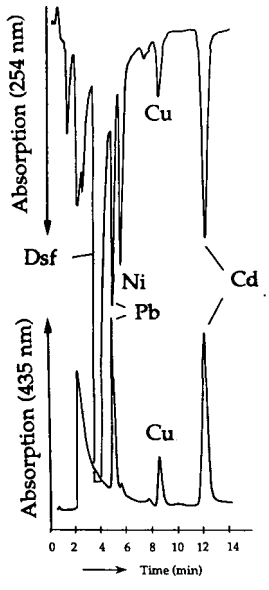


Fig. 5. Chromatogram of $Ni(DTC)_2$, $Cd(DTC)_2$, $Pb(DTC)_2$ and $Cu(DTC)_2$ with detection at (top) 254 nm and (bottom) 435 nm using the copper(II) phosphate reactor. Dsf = Disulfiram. For conditions, see Experimental.

Disulfiram is easily formed by air oxidation of diethyldithiocarbamate[7,8] and is therefore always present in samples after reaction with Na(DTC). Further, an excess of Na(DTC) can react with Ni^{II} present in the stainless-steel parts of the LC system; hence an $Ni(DTC)_2$ peak is usually found, even if no Ni^{II} was present in the sample. Both compounds, however, were found to react slowly with copper(II) phosphate. We observed that, in the presence of Cu(II), the reaction time for the quantitative conversion of disulfiram into $Cu(DTC)_2$ at 50°C is about 3 min. Under the reaction conditions chosen for the post-column ligand-exchange of $Cd(DTC)_2$ and $Pb(DTC)_2$ (room temperature, flow-rate 1.2 ml/min), disulfiram and nickel(II) therefore do not interfere with the determination of Pb^{II} (see Fig. 5).

Determination of Cd^{II} and Pb^{II} in urine

In order to demonstrate the selectivity of the detection method, urine was spiked with 200 ppb[a] of Cd^{II} and 100 ppb of Pb^{II}. After pH adjustment and the addition of Na(DTC), 1 ml of the sample was preconcentrated on a precolumn packed with C_{18}-bonded silica. Using visible detection at 435 nm after post-column ligand exchange with copper(II) phosphate, Pb^{II} and Cd^{II} can be determined with an analysis time of about 15 min, and interferences from other urine components are absent (see Fig. 6). The detection limit for the determination of these metals in urine was 10 ppb (1 ml preconcentration).

The use of the nickel(II) phosphate reactor for urine analysis was less favourable; many interferences were observed at the relatively low detection wavelength (325 nm).

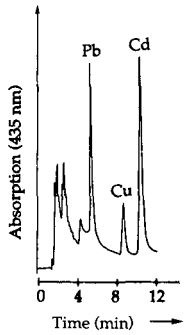


Fig. 6. Chromatogram of urine spiked with 200 ppb of Cd^{II} and 100 ppb of Pb^{II} using 1 ml preconcentration. For other conditions, see Experimental.

CONCLUSIONS

A simple detection method has been developed for the LC determination of Pb^{II}, Cd^{II} and Bd^{III}. After ligand exchange with copper(II) phosphate using a solid-state post-column reactor, both metals can be detected as $Cu(DTC)_2$ at 435 nm. Owing to the selectivity of the high detection wavelength, only a minimum of sample

[a] Throughout this article, the American billion (10^9) is meant.

pretreatment is required if Pb^{II} and Cd^{II} have to be determined in biological and other complex samples. This result compares very favourably with that of the analysis of a urine sample by LC with UV detection at 254 nm (see Fig. 2 in ref. 4), where a broad band is observed during the first 10 min of the LC run, which makes the quantification of low amounts of metal ions impossible.

The post-column conversion of $Pb(DTC)_2$ and $Cd(DTC)_2$ into $Cu(DTC)_2$ proceeds rapidly and quantitatively at conventional LC flow-rates (*ca.* 1 ml/min) and room temperature. Because of the low solubility of copper(II) phosphate in the LC eluent, the post-column reactor can be used continuously for at least 1 week. It is therefore highly suitable for (automated) routine analysis. In addition, the elimination of interferences from the oxidation products of the ligand and undesired metal dithiocarbamates such as $Ni(DTC)_2$ or $Co(DTC)_3$ is advantageous.

Substitution at the nitrogen atom of the dithiocarbamates generally does not significantly influence the complexation properties, the wavelength of maximum absorption or the molar absorptivity of metal dithiocarbamates[9]. Therefore, the present post-column complexation technique may also be applied to other dithiocarbamate ligands frequently used in the separation of metal ions, such as bis(hydroxyethyl)[10–12] or tetramethylenedithiocarbamates[8,13,14]. The method may also be extended to the determination of other metal ions such as Zn^{II}, Mn^{II} or Se^{II}. This would require that their DTC complexes can be chromatographed by reversed-phase LC, are less stable than $Cu(DTC)_2$ and exhibit a fast ligand-exchange reaction with Cu^{II} ions.

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DYESTUFF-COATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC RESINS FOR THE ION-EXCHANGE AND CHELATING-EXCHANGE SEPARATION OF METAL IONS

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SUMMARY

Methods are described for the formation of permanent coatings of triphenylmethane-type dyestuffs on high-performance liquid chromatographic-grade neutral polystyrene resins. Bromophenol blue gave an ion-exchange coating with separation properties similar to those of a low-capacity sulphonated resin, although the efficiency was lower. Most of the work was focused on Chrome Azurol S, which produced a chelating-exchange coating. The separation and preconcentration of both divalent and trivalent metal species was studied and found to be strongly influenced by the pH of the eluent, but little affected by the ionic strength. With stepped pH gradients, preconcentration and separation could be achieved using a single column, even in 1 *M* potassium nitrate solution.

INTRODUCTION

Most publications dealing with the determination of trace metals by high-performance liquid chromatography (HPLC) have concentrated on ion-exchange techniques (usually referred to as ion chromatography). Regardless of whether high- or low-capacity substrates are used, retention and separation are normally controlled by polyfunctional carboxylic acids which form relatively weak complexes with the metal ions.

Although ion-exchange separations of metal ions have been used in a number of important applications, as discussed in recent reviews[1,2], there are still several problems associated with this approach. One particularly important problem is column disturbance due to the ionic strength of the sample solution. Too high an ionic strength can cause a temporary drastic change in column capacity, destroying the separation. As a large number of sample treatment procedures result in highly concentrated solutions, *e.g.*, wet oxidation, further sample handling may be necessary to remove the bulk of the matrix before injection.

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An alternative approach to ion exchange is chelating exchange, in which the solid substrate contains chelating groups rather than ion-exchange groups. As the capacity factors of the metal ions depend on the values of the conditional stability constants in the stationary phase, the ionic strength will have much less effect on the chromatography. Therefore, at its simplest, retention can be controlled by varying the pH of the mobile phase containing only a non-complexing acid or salt. Auxiliary complexing agents, such as polyfunctional carboxylic acids, can of course still be used, but competing chelation and pH will then control the capacity factors of the metal ions. Chelating exchange is well known in classical column chromatography, where it has been used principally for matrix isolation. However, an increasing number of publications are appearing that describe metal separations on high-performance liquid chromatographic (HPLC)-grade or near HPLC-grade chelating stationary phases. Nearly all these publications describe substrates with chemically bonded chelating groups made by the workers themselves. Fritz and co-workers studied a variety of chelating functions on resins, as discussed in a book on ion chromatography by Fritz *et al.*[3], while Faltynski and Jezorek[4] reported the methods of synthesis and chromatographic performance of six bonded chelating functions on silica gel. Risner and Jezorek[5] described a more detailed study of 8-hydroxyquinoline (HQ)-bonded silica gel, with the conclusion that a very lightly loaded column gave the best separations. Chambaz and Haerdi[6] used a similar 8-HQ-bonded silica gel column to study the preconcentration and elution of a range of divalent metals. Iminodiacetic acid (IDA) has also been bonded to HPLC-grade material and a Japanese resin is now commercially available. Toei[7] investigated such a material for the separation and determination of calcium and magnesium in sea water.

Although a number of bonded chelating groups have shown some interesting results, a greater range needs to be investigated in order to ascertain the full potential of chelating-exchange HPLC substrates. One way of achieving this without resorting to lengthy and perhaps difficult syntheses of chemically bonded groupings is to coat a particular substrate with selected compounds. Modifying stationary phases by coating with specific compounds is a well known technique in ion chromatography, but mainly concerns the formation of dynamic ion-exchange coatings using quaternary ammonium- or alkyl sulphonate-based compounds. Few workers have investigated dyestuff coatings on HPLC-grade materials. However, Golombek and Schwedt[8] recently showed that it was possible to achieve excellent high-speed separations of common anions using a dynamic coating of the dyestuff methyl green on a neutral polystyrene-based resin.

We considered that it would be useful to try to extend this idea of dyestuff coatings to produce HPLC-grade cation-exchange and chelating-exchange substrates for the separation of metals. The literature contains many examples of dyestuff coatings, but on resins of large particle size. A recent review[9] shows that most of the publications describe chelating-exchange coatings on anion-exchange resins, with only a relatively small number involving neutral resins. The principal objective of most of this published work was to collect or preconcentrate groups of metal ions from a variety of matrices which, after elution, were then determined by a number of off-line techniques. There appears to be no published work as yet on analytical separations of groups of metal ions on dyestuff-loaded resins of small particle size.

This paper describes a preliminary investigation of the separation character-

istics of HPLC-grade neutral resins for metal cations when coated with organic dyes giving ion-exchange or chelating-exchange properties. Most of the work was focused on the ligand-exchange coatings as these showed the greatest potential for novel separations.

EXPERIMENTAL

Apparatus

A standard isocratic LC system was used, linked to a post-column reactor as described elsewhere[10] but with the preconcentration column replaced with a sample loop. Both the column pump and the post-column reaction pump were made of stainless steel, Model BT8100 (Biotronic, Maintal, F.R.G.). The column effluent and reagent streams (both kept at 1 ml min^{-1}) were mixed at a simple stainless-steel T-junction and then followed immediately by a reaction coil composed of 4 m × 0.3 mm I.D. PTFE tubing tightly wound on a 4-mm diameter glass rod. The detector was a Model BT8200 variable-wavelength UV–VIS spectrophotometer (Biotronic). The injector was a Model 7125 valve fitted with a 100-μl sample loop (Rheodyne, Cotati, CA, U.S.A.), connected to a stainless-steel column (150 × 4.6 mm I.D.), filled with Benson BPI-10 10-μm particle size neutral polystyrene-based resin. This resin was coated with an organic dyestuff as described below. The separations were carried out mostly at room temperature with some at 60°C, as detailed below.

Reagents

Analytical-reagent grade chemicals and solvents were used unless stated otherwise. All the dyes studied were obtained from Aldrich (Milwaukee, WI, U.S.A.) and the metal standard solutions were prepared from 1000 μg ml^{-1} stock solutions obtained from Merck (Darmstadt, F.R.G.).

Elution systems

A number of elution systems were used, based on acetic acid or lactic acid, or a mixture of both, adjusted to a particular pH with sodium hydroxide solution. For the chelating-exchange work, the ionic strength was adjusted with potassium nitrate. Exact details are given under Results and discussion.

Detector systems

Three post-column reaction reagents were used, depending on the metals under study.

Calmagite. This post-column reaction has been described elsewhere[10], except that the more stable Calmagite replaces Eriochrome Black T. The Calmagite was used to detect the divalent metals, except beryllium. The reagent solution consisted of 0.004% (w/v) Calmagite in *ca.* 0.3 *M* aqueous ammonia. The detector wavelength was set at 610 nm with metal peaks appearing as a decrease in absorbance.

Pyrocatechol violet (PCV). This reagent was used to detect Al, Ga, In, Bi^{III} and Fe^{III}. The post-column reaction solution consisted of 0.004% (w/v) PCV in 0.5 *M* aqueous hexamine adjusted to pH 6.0 with 2 *M* nitric acid. Higher concentrations of hexamine buffer will be required if the pH of the eluent is less than 1.5. The exact concentration of hexamine is not important, provided that the pH of the post-column

reaction is between 5.7 and 6.0. The detector wavelength was set at 580 nm, where metals were detected as an increase in absorbance.

Chrome Azurol S (CAS). This reagent was used to detect Be. It can also be used for Al, Ga and Fe^{III} but, unlike PCV, cannot detect In and Bi^{III}. (Note: this is the same dye that was used to coat the resin for the chelating-exchange work.) The post-column reagent consisted of a 0.008% solution of CAS in 0.5 *M* hexamine buffer adjusted to pH 5.6 with nitric acid. For more acidic eluents the same considerations apply as for PCV, taking into account that the optimum pH for the CAS reaction is 5.6. The detector was set at 560 nm, where metal peaks gave an increase in absorbance.

Column coating procedures

The resin was coated by pumping a 0.2% solution of the dyestuff in methanol–water (20:80), adjusted to pH 3 with acetic acid, through the column until breakthrough occurred, and then the pumping was continued for a further 10 min. The column was washed with deionized water and adjusted to pH 10.5 with ammonia solution until the column effluent was colourless. This non-leaching condition was established using the detector set at the wavelength of maximum absorbance of the dye. To ensure that the column was stripped clean of any adsorbed metals, 20 ml of 0.1 *M* nitric acid was pumped through, followed immediately by a solution of 0.1 *M* acetic acid. When the column was left for any length of time, including overnight, it was stored in 0.1 *M* acetic acid (*ca.* pH 3).

For the results presented in this paper, a coating of bromophenol blue was used for the ion-exchange work and a coating of Chrome Azurol S (CAS) for the chelating exchange study.

RESULTS AND DISCUSSION

Ion-exchange coating

To obtain a strong-acid cation-exchange surface on the resin requires a coating compound containing sulphonic acid groups. This is not a major problem as many dyestuffs, particularly acid–base indicators, have sulphonic acid groups to make them water soluble. However, to investigate the coating of a large number of dyes on the HPLC-grade resin would be time consuming and costly. Because of this, it was decided to screen a number of dyes on a neutral resin of large particle size packed in glass columns, to obtain some idea of the stability of the coating under a range of conditions. The resin chosen was Amberlite XAD-4 (Rohm and Haas) which, although a macroporous type, subsequently proved to be a useful guide to the behaviour of a dye coating on Benson HPLC resin.

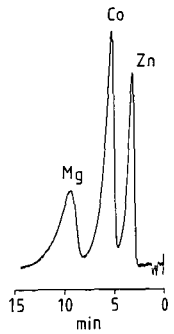
As a result of this XAD-4 screening, methyl orange and bromophenol blue were chosen for a more detailed study on the Benson column. The methyl orange coating was found to "leach" very slowly, subsequently interfering with post-column reaction detection, and so was abandoned in favour of bromophenol blue (a triphenylmethane dye). The bromophenol blue coating was particularly stable (total loading approximately 50 mg) and, once conditioned as detailed under Experimental, gave no observable leaching throughout the study. A check for leaching was carried out several times a day by switching off the post-column reaction pump and monitoring the absor-

bance of the column effluent at the wavelength maximum of the bromophenol blue dye. A 0.1 *M* lactic acid eluent was chosen to assess the separation performance of the coated column, as this was known to give good results with low-capacity ion-exchange substrates[11]. Fig. 1 shows the separation of Zn, Co and Mg using a Calmagite post-column reactor. Clearly, the column was functioning as an ion exchanger, giving similar retention times to those found on low-capacity chemically bonded phases such as those obtainable commercially. However, the separation efficiency was disappointing, made worse by significant peak tailing. Although the efficiency would undoubtedly improve if 5-μm resins were used, tailing may still be a problem. As 5-μm resins were not available, it was decided that attention should be focused on chelating-exchange coatings.

Chelating-exchange coating

The same XAD-4 screening procedure as described for ion-exchange coatings was used for this work. Ten chelating compounds were subjected to the screening process as listed in Table I. These were chosen to cover a range of chelating function, dye type and molecular size. Plainly, colour is not an important requirement of the organic compound to form a stable coating, although it is useful, as the depth of coating and the amount of leaching can easily be observed. Nevertheless, two important chelating agents, chromotropic acid and 8-hydroxyquinoline sulphonate, were included, even though they are colourless in the uncomplexed form.

From the results of the screening, Calcon, chromotropic acid, 8-hydroxyquinoline and PAR gave poor, thin coatings, Calmagite, methylthymol blue and xylenol orange gave light coatings and Chrome Azurol S, Chromoxane Cyanine R and pyrogallol red gave fairly deep coatings. Of the last three, Chrome Azurol S was chosen for further study on the Benson column as it appeared to give the deepest coating. In any event, pyrogallol red would not be suitable because it is easily oxidized in alkaline media. It is fortuitous that Chrome Azurol S gave a deep coating as it is stable to oxidation. Also, the chelating function, which is essentially a salicylic acid analogue (Fig. 2), is a weaker complexing group for many metals than the most commonly



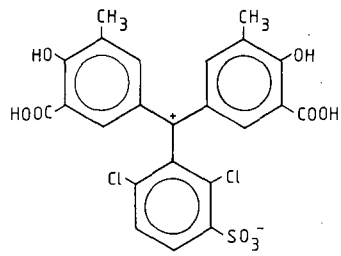


Fig. 1. Ion-exchange separation on a bromophenol blue-coated column. Sample: 100-μl injection of a mixture of cobalt, magnesium and zinc each at a concentration of 10 ppm. Chromatographic conditions: eluent, 0.1 *M* lactic acid (pH 3.8); detection, post-column reaction with Calmagite solution; wavelength, 610 nm.

Fig. 2. Structure of Chrome Azurol S.

TABLE I

LIST OF DYES SCREENED WITH AMBERLITE XAD-4

1. Calcon
2. Calmagite
3. Chrome Azurol S
4. Chromotropic acid[a]
5. Chromoxane Cyanine R
6. 8-Hydroxyquinoline[a]
7. Methylthymol blue
8. PAR
9. Pyrogallol red
10. Xylenol orange

[a] Colourless in uncomplexed form.

investigated iminodiacetic acid or 8-hydroxyquinoline functions. Therefore, it was considered that the separation properties of Chrome Azurol S (CAS) would be an interesting comparison with those of the iminodiacetic acid and 8-hydroxyquinoline bonded substrates. A Benson column was coated with CAS as detailed under Experimental and the results of the investigation are divided into two parts, divalent and trivalent metals. However, beryllium was included with the trivalent metals as it has a similar chemistry to aluminium.

Before any work could be carried out on the chelating properties of CAS, it was important to ensure that ion-exchange effects due to the presence of a sulphonate group (Fig. 2) were eliminated. This was achieved by maintaining a high ionic strength in the eluent. Thus, a concentration of at least 0.2 *M* with respect to potassium nitrate was used for all the chelating-exchange work.

Divalent metals

The results of a preliminary study of the separation properties of the CAS-coated column for a number of divalent metals are shown in Fig. 3. Fig. 3A shows a separation under simple pH control and Fig. 3B the separation of the same three

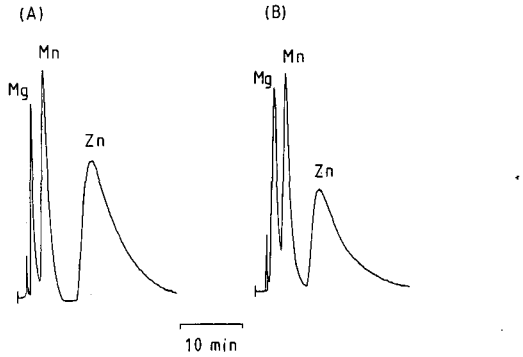


Fig. 3. Isocratic chelating-exchange separation of divalent metal ions on a CAS-coated column. Chromatographic conditions: eluent, (A) 1 *M* KNO_3 (pH 5.7); (B) 1 *M* KNO_3 containing 0.05 *M* lactic acid (pH 6.5); detection as in Fig. 1. Sample: (A) 100 µl of a mixture containing 2 ppm of Mg, 5 ppm of Mn and 40 ppm of Zn; (B) 100 µl of a mixture containing 2 ppm of Mg, 4 ppm of Mn and 20 ppm of Zn.

metals with the addition of a complexing carboxylic acid in the eluent. As expected, the elution order was the reverse of that found for an ion-exchange separation. The presence of lactic acid in the eluent speeded up the elution but did not affect the selectivity. As can be seen, the peaks were fairly broad, showing a relatively low efficiency for this column. Nevertheless, sufficient resolution was being achieved to evaluate the CAS coating.

The potential for on-column preconcentration and separation using the same column was investigated with stepped gradients. With an eluent containing 1 *M* potassium nitrate adjusted to pH 8.5, no elution of magnesium, the most weakly held cation, was observed. Even the presence of 0.05 *M* lactic acid in the eluent did not cause elution at this high pH. Injecting a mixture of dipositive metals at pH 8.5 and then stepping the eluent down to a lower pH caused the metals to elute. The peak shapes were much narrower and also better separated than if the chromatography had been carried out isocratically at the lower pH. Although gradients are known to improve performance in this way, the retention times were longer than expected considering the sharp decrease in pH. This could be explained by the fact that the CAS coating was acting as a buffer (four weak acid groups per molecule), resisting the decrease in pH, turning the step into a much more gradual change in gradient. Fig. 4 shows a four metal separation using a two-step gradient with varying sample conditions. Fig. 4A was obtained after a small-volume injection at ppm levels and Fig. 4B was obtained after a large-volume injection at ppb[a] levels. Although quantitative recoveries were not evaluated, this work clearly shows the potential for combined preconcentration and separation on a single column in solutions of high ionic strength.

Trivalent metals and beryllium

As a class, these metal species needed lower pHs for elution than the divalent metals because of the stronger complex formation with CAS. However, the same elution properties were observed under isocratic conditions, namely, broad peak shapes with a high degree of asymmetry. Table II shows the order of elution expressed as the eluent pH needed to produce a retention time of approximately 5 min. Some of the peak shapes, notably for gallium and bismuth, were very broad but were found to improve (*i.e.*, become sharper) on increasing the temperature of the column. Increasing the column temperature also increased the retention times slightly. Interestingly, increasing the column temperature did not produce sharper peaks for the divalent metals. We considered that this difference in temperature response between the divalent and trivalent metals could be due to the slower kinetics of complex formation usually found with the more highly charged metal cations. Thus, when gallium was involved reasonable separations could only be achieved at high column temperatures. Fig. 5 shows a separation of Al, In and Ga at 60°C.

The effect of auxiliary complexing ligands in the eluent was also studied. It was found that halide ions had a significant, but varying, influence on the retention of these metals, and could provide a useful way of changing the selectivity of the separation. For example, indium and bismuth(III) were markedly affected by the presence of low concentrations of chloride in the eluent, giving significantly shorter reten-

[a] Throughout this article, the American billion (10^9) is meant.

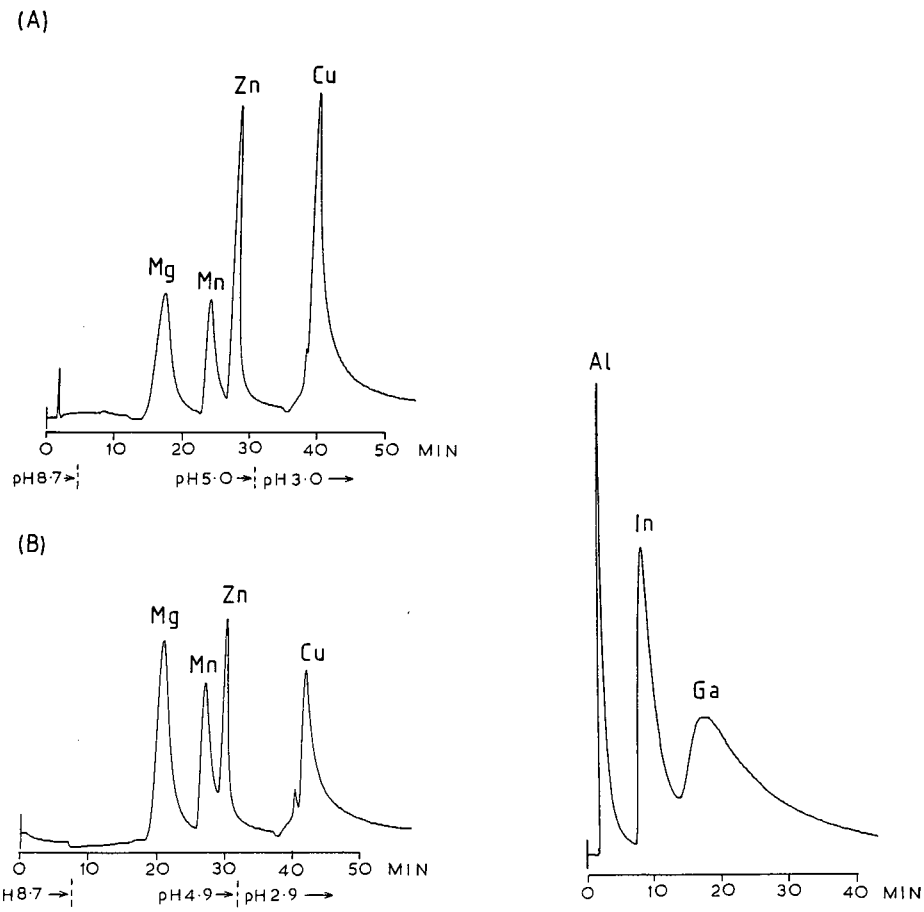


Fig. 4. Chelating-exchange preconcentration and separation of divalent metals using step gradients. Chromatographic conditions: eluent for both (A) and (B), 1 *M* KNO_3 containing 0.05 *M* lactic acid, adjusted to the appropriate pH for the step gradients as indicated in the diagrams; detection as in Fig. 1. Sample conditions: (A) injection of 100 μl of a mixture containing 5 ppm of Mg, 5 ppm of Mn, 20 ppm of Zn and 20 ppm of Cu; (B) injection of 7 ml of a mixture containing 10 ppb of Mg, 10 ppb of Mn, 20 ppb of Zn and 20 ppb of Cu.

Fig. 5. Chelating-exchange separation of some trivalent metal ions under isocratic conditions. Chromatographic conditions: eluent, 1 *M* KNO_3 (pH 2.25); column temperature, 60°C; detection, post-column reaction with PCV solution; wavelength, 580 nm. Sample: 100-μl injection of a mixture of 1.5 ppm of Al, 20 ppm of In and 15 ppm of Ga.

tion times, whereas the other elements listed in Table II were unaffected. Fig. 6 shows that the very poor peak for bismuth at room temperature is considerably sharpened by the addition of chloride to the eluent.

The influence of stepped pH gradients after preconcentration was studied, and produced similar results to the work on divalent metals. However, elevated column temperatures and large pH jumps were necessary to obtain reasonably sharp peaks. The preconcentration and elution of aluminium was studied in more detail. Alumini-

TABLE II

pH VALUES OF ELUENT PRODUCING A RETENTION TIME OF *CA.* 5 min

Element	*pH*	
	25°C	*60°C*
Be	3.2	3.0
Al	3.0	2.5
In	2.3	1.9
Ga	2.3	1.7
Fe^{III}	1.5	1.5
Bi^{III}	1.1	1.1

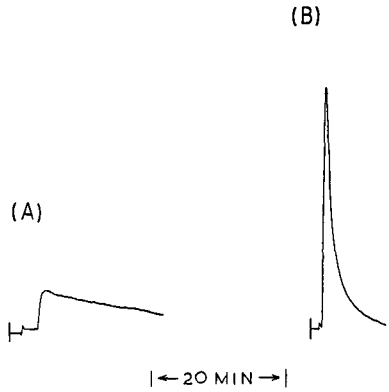


Fig. 6. Effect of chloride on the elution of bismuth(III). Chromatographic conditions: eluent, (A) 0.4 *M* KNO_3 (pH 1.05); (B) as (A) but containing also 0.05 *M* chloride; detection as in Fig. 5. Sample, 100-μl injections of 100 ppm of Bi^{III}.

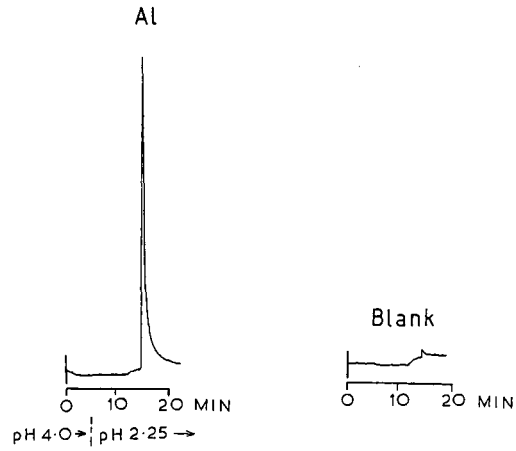


Fig. 7. Preconcentration and elution of aluminium using step gradients. Chromatographic conditions: eluent, 1 *M* KNO_3, adjusted to the appropriate pH for the step gradient as indicated in the diagram; detection as in Fig. 5. Sample: (left) 5-ml injection of a 50-ppb Al standard at pH 4; (right) blank, 5-ml injection of deionized water.

um was found to be fully retained at pH 4, at which pH all the divalent metals except beryllium and copper were unretained. A step gradient to pH 2.25 then eluted aluminium as a sharp peak (Fig. 7). Under these conditions, indium and gallium gave much longer retention times and iron(III) and bismuth(III) were strongly retained. Using the same conditions as detailed in Fig. 7, an aluminium calibration was attempted in the range 4–16 ppb. A good linear plot was obtained with a correlation coefficient of 0.9999 and a slope of 0.0038 absorbance per ppb.

CONCLUSION

The two triphenylmethane dyes chosen gave essentially permanent coatings on the Benson resin with a loading of *ca.* 30 mg per gram. The CAS chelating-exchange coating showed the greatest potential for novel separation possibilities, where the relative insensitivity to ionic strength will be useful for the analysis of samples with high salt concentrations. The ability of the CAS column to handle large-volume injections should also allow preconcentration and separation to be carried out without resorting to the addition of a second column.

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ON-LINE PRECONCENTRATION AND ELUTION OF TRACE METALS BY ION CHROMATOGRAPHY

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SUMMARY

On-line preconcentration of transition metal ions using 8-quinolinol-bonded chelating silica is described. The precolumn was coupled to an ion-pairing chromatographic column and simultaneous determination was achieved using a diode-array UV detector, 0.1 *M* potassium cyanide (pH 8.5) being used for eluting metals from both the precolumn and the analytical column. Of the metals tested, Cu, Ni, Co and Fe(II) yielded well resolved absorption bands whereas Fe(III) and Mn(II) yielded ill-characterized absorption bands. The working concentration range for Cu and Ni was tested and shown to be linear in the range 10^{-8}–$5 \cdot 10^{-6}$ *M*. Highly reproducible results were obtained at these concentrations. The sensitivity was found to be limited by background signals and the reason for this background is discussed in detail.

INTRODUCTION

The determination of trace metals at the concentration levels found in natural waters often requires a preconcentration step. Preconcentration of trace metals is most often done off-line, prior to their analysis by a chosen method. Ion chromatography has been widely used for the separation and determination of metal ions. For automation purposes, on-line preconcentration followed by ion chromatography would be necessary. On-line preconcentration has the advantage that no dilution or contamination of the preconcentrated samples is introduced.

Recently, on-line preconcentration followed by ion chromatographic techniques have been developed[1–6]. In none of these studies were chelating silicas used for preconcentration purposes. The interesting feature of chelating silicas is their specificity towards transition metals. To our knowledge, chelating silicas have not been used for on-line preconcentration owing to the difficulty in finding a common eluent that could be used for both desorption from the chelating precolumn and for performing separations on a cationic or reversed-phase column.

The eluent usually employed on the precolumn is 0.1 or 1 *M* hydrochloric or nitric acid. The metals desorbed from the chelating precolumn were determined by techniques such as atomic absorption spectrometry[7–9] or inductively coupled plasma mass spectrometry[10]. Jezorek and co-workers[11–13] attempted to separate metals

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from several chelating analytical columns using these eluents, but the chromatographic peaks were not very well resolved.

In this work we investigated the use of chelating silicas coupled on-line to an ion chromatograph for the simultaneous preconcentration and separation of trace metals. For this purpose, 8-quinolinol-bonded silicas was used in the precolumn. This chelating silica is well known to preconcentrate many transition metal ions[7–10]. Instead of hydrochloric acid, a strong complexant was used to desorb the trace metals. Cyanide ions are known to form very strong complexes with transition metal ions, as can be seen from Table I. The stability constants of cyanide are much higher than those of the 8-quinolinol-silica and hence it was chosen as the eluent. The following processes occur during preconcentration and desorption:

Preconcentration:

$$\underset{\text{(8-quinolinol-silica gel)}}{\text{QSG-H}} + M^{2+} \rightleftharpoons \underset{\text{(chelate)}}{(\text{QSG-M})^{+}} + H^{+}$$

Desorption:

$$(\text{QSG-M})^{+} + x\text{CN}^{-} \rightleftharpoons \text{QSG}^{-} + \underset{\text{(cyano complex)}}{\text{M(CN)}_x^{(x-2)-}}$$

The cyanide complexes formed are then separated using an ion-Pairing C_{18} silica column and detected by UV spectrophotometry. A slightly modified Hilton and Haddad[15] chromatographic procedure was adopted for the purpose of separation and determination of the metals.

EXPERIMENTAL

Apparatus

The chromatographic equipment (Fig. 1) consisted of two Knauer metal-free electric valves, a Dionex QIC preconcentration pump and a Knauer 64 elution pump.

TABLE I

STABILITY CONSTANTS OF METAL–CYANO AND METAL–8-QUINOLINOL COMPLEXES[14]

Metal	*8-Quinolinol*		*Cyanide:* β_4
	β_1	β_2[a]	
Fe(II)	8	(22)[b]	24[c]
Fe(III)	15	(38)[b]	31[c]
Co	9	(17)	20[c]
Ni	10	(19)	22
Cu	13	(24)	25
Zn	9	(17)	17
Cd	8	(14)	18
Pb	9	(17)	10

[a] Values in solution.
[b] β_3.
[c] β_6.

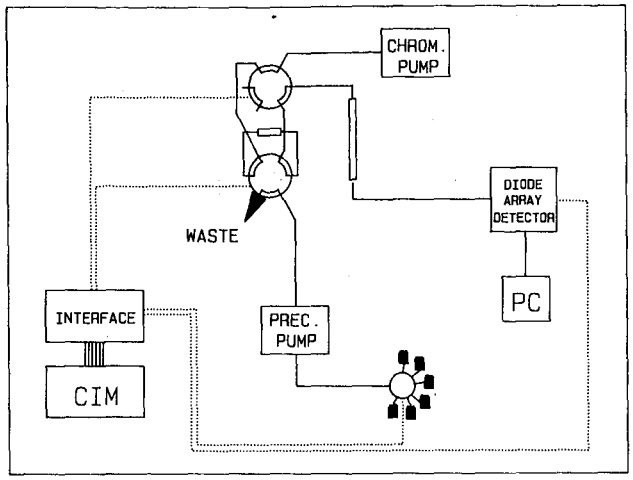

Fig. 1. Chromatographic equipment. PC = Personal computer; CIM = Control interface module.

Only the last pump was made of metal. The precolumn (30 × 2.5 mm I.D.) (Dionex) was packed manually with 8-quinolinol-silica. The 8-quinolinol-silica was placed in the precolumn with a microspatula and the column was tapped to ensure that the solid was well packed. An SGE glass-lined analytical column was packed with ODS-2 silica (5 μm). One Rheodyne six-way low-pressure valve was used with the preconcentration pump for switching between sample and washing solutions. Plastic tubing and connections were used. A Hewlett-Packard 1040 A diode-array UV–visible detector was used. The whole system was automated using a CIM-300 (control interface module) from Autochrom coupled to an interface specially designed in our laboratory.

Reagents

Preconcentration. Unless stated otherwise, all chemicals were of analytical-reagent grade from Merck or Fluka. All solutions were made with freshly prepared doubly distilled water. The 8-quinolinol-silica was synthesized in our laboratory using the procedure described by Fulcher *et al.*[16] Its loading capacity was 360 μmol/g. Macherey, Nagel & Co. Polygosyl silica 60 (40–63 μm) was used as the starting material for the synthesis.

Eluent. A solution of 0.1 *M* potassium-cyanide (Biochemica Microselect, Fluka) in 13–18% acetonitrile (Far-UV, Romil) in water was used as the eluent. Ion pairing was achieved using $1.5 \cdot 10^{-3}$–$5 \cdot 10^{-3}$ *M* tetrabutylammonium reagent (Low-UV PIC A, Waters Assoc.). The pH was adjusted to 8.5 with hydrochloric acid, (Suprapur, Merck).

Procedure

A 20-ml volume of solution containing the metal ions was pumped into the chelating silica precolumn, where the metals were preconcentrated, then 6 ml of water were pumped into the precolumn for rinsing. The metals that were retained were desorbed by using the above eluent. For this purpose, the chromatographic pump was used (0.7 ml/min, P = 115 bar). During the desorption step, the precolumn and

the analytical column were linked. The metals, after separation on the analytical column, were detected by means of a diode-array detector. The precolumn was disconnected from the analytical column 2 min after passing the eluent. While separation was proceeding, the precolumn cleanup was carried out by washing it with 10 ml of 0.1 *M* potassium cyanide solution (pH 8.5) followed by 10 ml of water.

RESULTS AND DISCUSSION

UV–visible characteristics of metal–cyano complexes

Metal–cyano complexes show well resolved absorption bands in the UV spectrum, *e.g.*, λ_{Cu} = 238 nm, λ_{Ni} = 270 nm, λ_{Co} = 310 nm. The UV–visible diode-array detector allows the simultaneous determination of these complexes at their absorption maxima (λ_{max}) and therefore high sensitivities can be obtained. However, not all metal–cyano complexes are well defined. For instance, the complexes of heavy metals such as Zn, Hg, Cd and Pb do not show UV–visible absorption whereas Fe(III) and Mn(II) show ill-characterized absorption bands. Fe(III) and Mn(II) can nevertheless be determined at 220 and perhaps at 210 nm, but at the latter wavelength the reproducibility of the results may be affected by interferences from cyanide or acetonitrile background absorptions. Even though Fe(II) shows a wellresolved peak, it has a lower molar absorption coefficient and hence the method is not very sensitive for the determination of Fe(II).

Desorption

The cyanide concentration and pH were varied to establish the optimum working conditions. The best results were found using 0.1 *M* potassium cyanide solution at pH 8.0–8.5. At lower concentrations of cyanide the rate of desorption was too slow. Under the optimum conditions, desorption was found to be very efficient even at high metal concentrations, *e.g.*, more then 99% with 1 ml of CN^- (see Fig. 2). The peak tailing in Fig. 2 is due to the decomposition of the 8-quinolinol-silica and not to the presence of the copper complex.

Fifty cycles can be achieved using this procedure with a loss of only 20% of the loading capacity of the precolumn. It should be pointed out that under the working conditions, the precolumn is used much below its maximum loading capacity and hence the results will be unaffected for a very long period.

Chromatographic separation

The method described by Hilton and Haddad[15] was found to be reasonable for the separation of Cu, Co and Ni but it was impossible to separate Fe and Mn. We therefore modified their experimental conditions slightly because of the high concentration of cyanide used here. A lower proportion of acetonitrile (18%) and a lower concentration of tetrabutylammonium ($1.5 \cdot 10^{-3}$ *M*) were used. A typical chromatographic separation is shown in Fig. 3.

It should be pointed out that the analytical column undergoes slow decomposition and the experimental conditions have to be changed accordingly, *e.g.*, over a period of 2 months the following modifications were necessary: a reduction in acetonitrile concentration from 18 to 13% and an increase in tetrabutylammonium ion concentration from $1.5 \cdot 10^{-3}$ to $3 \cdot 10^{-3}$ *M*.

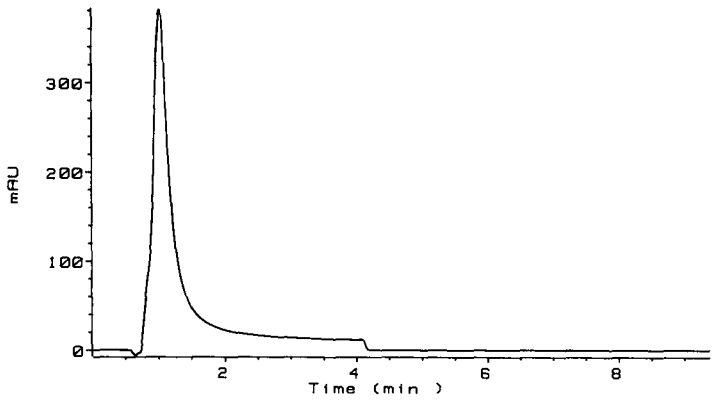

Fig. 2. Desorption of preconcentrated copper on 8-quinolinol-bonded silica. Preconcentration: 20 ml of 10^{-6} *M* Cu^{2+}. Eluent: 0.1 *M* potassium cyanide (pH 8.5) without analytical column.

On-line preconcentration

We chose Cu and Ni ions for on-line preconcentration. These metals have been preconcentrated previously on materials other than chelating silica[1–6]. The reasons for selecting Cu and Ni are that well defined peaks are obtained and high sensitivities can be achieved owing to their high molar absorption coefficients. Co was omitted because at low concentrations the very high solvent peak masks the Co peak. The Cu peak was found to be masked by a degradation peak from 8-quinolinol-silica and it was necessary to modify the experimental conditions slightly to avoid interferences from this peak. Under the modified conditions ($5 \cdot 10^{-3}$ *M* tetrabutylammonium, 13% acetonitrile), well defined peaks were obtained (Fig. 4).

Sensitivity. The calibration graphs in Fig. 5 show that excellent linearity is obtained in the concentration range $5 \cdot 10^{-6} - 10^{-8} M$, indicating that the method is highly sensitive for these metal ions. The method could not be extended to lower

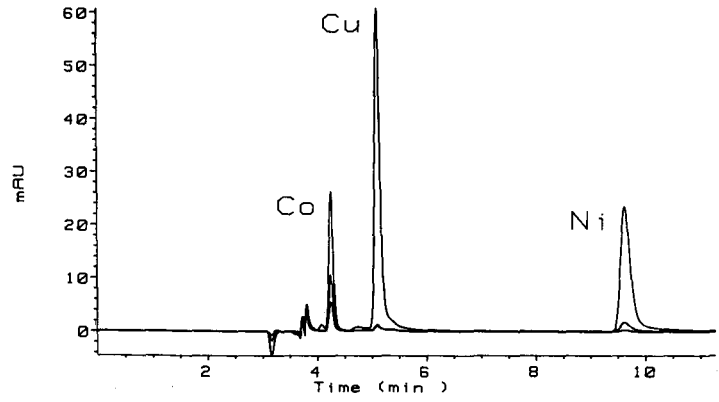

Fig. 3. Chromatographic separation of metal–cyano complexes. Injection: 20 µl of samples containing $2 \cdot 10^{-5}$ *M* of metal ions. Eluent: 0.1 *M* potassium cyanide (pH 8)–$1.5 \cdot 10^{-3}$ *M* tetrabutylammonium reagent in 18% (v/v) acetonitrile–water, flow-rate 0.7 ml/min. Detection: 310 nm for Co, 238 nm for Cu and 270 nm for Ni.

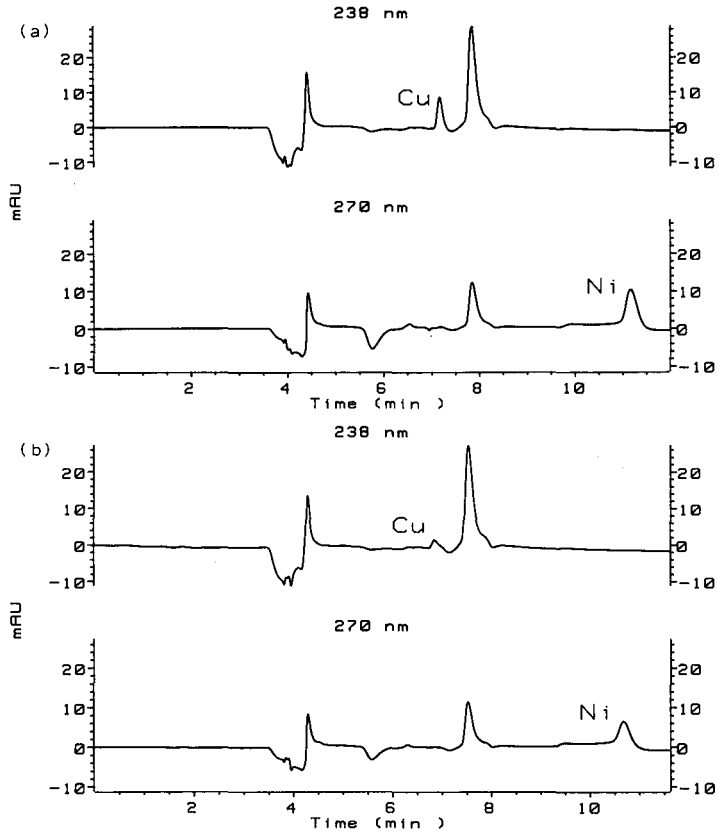

Fig. 4. Chromatographic separation of preconcentrated samples. (a) 20 ml of 10^{-8} *M* Ni^{2+} and Cu^{2+}; (b) blank, 20 ml of doubly distilled water. Eluent: 0.1 *M* potassium cyanide (pH 8.5)–$5 \cdot 10^{-3}$ *M* tetrabutylammonium reagent in 13% (v/v) acetonitrile–water, flow-rate 0.7 ml/min.

sensitivity owing to the high background absorption (about 0.006 absorbance units, which correspond to a concentration of metal of $5 \cdot 10^{-9}$ *M*; see Fig. 4). The high background signals may be due to inability to remove ultra-trace amounts of metals from doubly distilled water. It may also be due to incomplete desorption of the metal ions. This is supported by the fact that the background increased after eluting high concentrations of metals ($5 \cdot 10^{-6}$ *M*). This finding is not unique to cyanide ions but also occurs with other eluents, *e.g.*, hydrochloric acid[17].

Reproducibility. The reproducibility of the method was tested by making replicate measurements using 10^{-7} *M* Ni(II) on different days. At constant cyanide concentration, reproducible results are obtained (standard deviation 5.4% for desorption times of 2 min). It should be pointed out that a desorption time of less than 2 min is insufficient to achieve complete transfer. It is interesting that slight variations in the acetonitrile of tetrabutylammonium ion concentrations do not affect the reproducibility of the results.

Similar studies with Cu posed problems owing to the degradation of the 8-quinolinol-silica, as mentioned earlier.

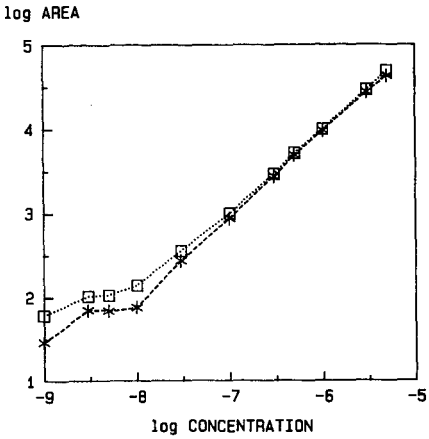

Fig. 5. Calibration graphs for (∗) Cu and (□) Ni. Conditions as in Fig. 4.

CONCLUSION

The results indicate that it is possible to use the method described here to determine concentrations of Cu and Ni at levels of 10^{-8} *M*. The background contamination limits the extension of the method to lower levels. Provided that the doubly distilled water is ultra-pure, the sensitivity limit can be extended if larger volumes of the sample are preconcentrated. The maximum sample volume that can be used will depend on the breakthrough volume (*ca.* 200 ml in this instance). If the simultaneous determination of metal ions is not required, then by coupling the ion chromatographic column to a classical UV detector (at a fixed wavelength) instead of a diode-array UV detector, increased sensitivities can be obtained.

This method, however, has the following disadvantages: it is not a universal method but is restricted to the determination of Cu and Ni; 8-quinolinol-bonded silica has a tendency to decompose and may mask metal peaks; it is extremely difficult to remove the last traces of metal from the preconcentration column; and the use of a diode-array detector is a prerequisite as high cyanide concentrations hamper the determination of the metal cyano complexes at 214 nm.

Further efforts will be required to find silicas that are resistant to decomposition and have fast chelating kinetics and a more universal method for determining transition metal ions.

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CHROM. 21 546

INJECTION PEAKS IN ANION CHROMATOGRAPHY

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SUMMARY

When sample solutions of salts containing eluent ions in the same concentration and pH as the mobile phase are injected into a column, they yield positive injection peaks that are quantitatively related to the sample peak areas. Injection peak areas in anion chromatography are linearly related to the cation concentration of the salts injected. Binary mixtures of salts in the presence of moderate amounts of acid or base can be quantitated by the combination of information from injection and sample peak areas.

INTRODUCTION

In single-column ion chromatography (SCIC) the first peak is always the injection peak, which is caused by the displacement of eluent ions by the injected sample. It may be either positive or negative, depending on the concentration of the injected sample to which this peak has been shown to be quantitatively related[1]. This has, however, never been fully interpreted, and many chromatograms shown in the literature[2] do not make use of the first few minutes of the chromatographic separation, although the injection peak, which occurs in that period, is potentially a rich source of information.

Several investigators have dealt with the appearance of chromatographic peaks other than sample peaks, namely injection and system peaks. They have pointed out that these are due to the fact that the eluent contains more than one component. Strahanan and Deming[4] explained them as being caused by the change that occurs in the distribution of mobile phase components following sample injection. Levin and Grushka[5] have dealt with system peaks occurring in the chromatographic separation of amino acids when using acetate buffers as eluents. Hummel and Dreyer[6] have applied gel permeation chromatography to the investigation of protein binding to small molecules. They showed that the appearance of an injection and a system peak is the result of injecting a protein sample and a ligand into a column pre-equilibrated with that ligand and in the same concentration.

Two effects contribute to the area of the injection peak, *viz.*, the displacement of

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eluent ions, caused by the adsorption of the sample ions on the ion-exchange column, and the dilution of the eluent ions, which occurs when the sample is more dilute than the eluent. The first effect tends to increase the area of the injection peak, while the second effect tends to reduce it. Negative injection peaks are observed in many chromatograms cited in the literature[3].

The second effect can be overcome by preparing the sample so that its concentration of eluent ions is the same as that of the eluent itself. The result is a positive injection peak which can be related to the concentration of the sample.

It was the aim of this work to show that the injection peak area of a salt in anion chromatography is proportional to the cationic content of that salt. By combining the information obtained from the injection peak and from the sample peak, mixtures of salts in acidic, alkaline or neutral solutions can be quantitated.

EXPERIMENTAL

The chromatographic system was built from several components. An LKB2150 high-performance liquid chromatography (HPLC) pump was used to control eluent delivery. A Wescan ICM II ion analyzer with conductivity detection was maintained at a constant temperature. Samples were introduced through a Rheodyne Model 7125 injection valve fitted with a 100-μl loop. A Shimadzu CR3A integrator was used in conjunction with a Curkin Scientific strip chart recorder. The peak retention times, areas and heights were obtained from the integrator. All separations were effected with a commercial Wescan 269-029 anion-exchange column (25 cm). The flow-rate of the eluent, $1.5 \cdot 10^{-3}$ *M* sodium phthalate (pH 4.3), was maintained at 1.0 ml/min.

Standard solutions were prepared with reagent-grade chemicals and with deionized water (Milli Q reagent grade water system). The concentration of the salt solutions injected ranged from $0.2 \cdot 10^{-3}$ to $1.0 \cdot 10^{-3}$ *M*. All solutions were prepared in $1.5 \cdot 10^{-3}$ *M* sodium phthalate, by the addition of 15.00 ml $1.5 \cdot 10^{-2}$ *M* sodium phthalate before diluting the sample to 100 ml. The injection of each solution was repeated three times, and the average value was used, the relative standard deviation being lower than 2%.

RESULTS AND DISCUSSION

The injection peak

The size of the injection peak of a salt solution containing the same concentration of the eluent as the mobile phase depends mainly on the cation content of that salt. This is illustrated in Fig. 1, which shows injection peak areas of various sodium salts *vs.* concentration. The linear correlation between the sodium concentration and the peak area is independent of the salt's anion; all points for three different sodium salts fall on one and the same straight line passing through the origin at zero salt concentration. This can be explained as follows: if a sodium salt solution is injected into a chromatographic anion-exchange column, the anion of the salt is retained on the column, displacing eluent ions. In the present case the eluent is sodium biphthalate (buffered at pH 4.30), and biphthalate ions will accordingly be displaced, the injection peak area being proportional to

$$\mathrm{pA} \propto \lambda_{Na^+}[Na^+] + \lambda_{HP^-}[HP^-] + 2\lambda_{P^{2-}}[P^{2-}] \quad (1)$$

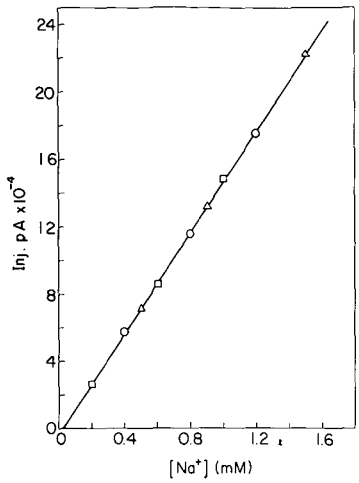


Fig. 1. Injection peak areas of various sodium salts as a function of concentration: □, NaCl; ○, NaOH; △, NaO_2CCH_3.

where pA = peak area in arbitrary units; λ_n = the equivalent conductivity of the conducting species, n, in aqueous solution; and HP^-, P^{2-} = biphthalate and phthalate anions.

The area of the injection peak is therefore dependent on the cation content of the sample and on the composition of the eluent before and after injection. It is thus dependent only on the concentration of the anion injected and independent of its type.

Quantitative relationship between the areas of the injection peak and of the sample peak

This relationship can best be illustrated by the data in Table I, which shows that there is a certain ratio between the areas of the injection peak and of the sample peak, that ratio being characteristic of the injection of sodium chloride solutions.

The two peaks obtained by injecting only one salt have different meanings.

TABLE I

INJECTION AND SAMPLE PEAK AREAS OF SODIUM CHLORIDE SOLUTIONS

Eluent: $1.5 \cdot 10^{-3}$ *M* NaHP; pH 4.30

Concentration of NaCl (10^3 M)	*Injection peak area*	*Peak area of Cl^-*	*Ratio between peak areas*
0.2	33 820 (1.0% R.S.D.)	16 702 (1.2% R.S.D.)	2.025
0.4	64 544 (0.70%)	34 731 (0.57%)	1.86
0.6	94 254 (0.71%)	53 410 (0.7%)	1.76
0.8	124 855 (0.41%)	72 215 (0.12%)	1.73
1.0	154 971 (0.62%)	90 625 (0.08%)	1.71

The response of the detector comprising the injection peak area (in arbitrary units) measures the difference between the conductivity of the displaced eluent ions plus that of the sample's cation, and the "background conductivity", *viz.*, that of the eluent, as follows:

$$10^{-3}K\Delta G_{\text{inj.}} = \lambda_{Na^+}[Na^+]_2 + \lambda_{HP^-}[HP^-]_2 + 2\lambda_{P^{2-}}[P^{2-}]_2 - B \quad (2)$$

where the term $B = \lambda_{Na^+}[Na^+]_1 + \lambda_{HP^-}[HP^-]_1 + 2\lambda_{P^{2-}}[P^{2-}]_1$ is the background conductivity, and $\lambda_{Na^+} = 50$, $\lambda_{HP^-} = 38.2$ and $\lambda_{P^{2-}} = 76.4$ cm^2 equiv.$^{-1}$ Ω^{-1}, K = conductivity cell constant and $\Delta G_{\text{inj.}}$ = detector response to injection.

The sample peak, for its part, measures the difference between the conductivities of the sample and of the background, respectively. This can be expressed as

$$10^{-3}K\Delta G_s = (\lambda_{Na^+} + \lambda_{Cl^-})I_S C_S - B \quad (3)$$

where G_s = sample peak conductivity, $\lambda_{Cl^-} = 76.3$ cm^2 equiv.$^{-1}$ Ω^{-1}, C_s = concentration of the sample and I_s = fractional ionization of the sample (equals unity in the present case).

Dividing eqn. 2 by eqn. 3 gives the ratio of the peak areas as compiled in Table I for the specific case in which the eluent is sodium phthalate at pH 4.30. The injection peak areas of salts other than of sodium can be calculated in the same manner, taking into account the appropriate equivalent conductivity data.

The numerical value of the injection peak conductance, calculated from eqn. 2 for $2 \cdot 10^{-4}$ *M* NaCl, is 0.0182, and for the sample peak is 0.00926. The ratio between the two conductances is 1.965, which is in good agreement with the experimental value given in Table I (2.025). As can be seen from Table I, that ratio decreases somewhat as the concentration of the injected sample is increased. This can be explained by the redistribution of phthalate species with increasing concentration of the injected sample.

Data for binary salt mixtures

Fig. 2 shows three chromatograms of the chloride salts of different cations. The chloride concentration is identical for all three salts, and their sample peaks therefore have the same areas; but the injection peaks are different. Potassium chloride, because of its high equivalent conductivity, has the largest peak area. The ratio between the injection peak areas of KCl and NaCl is 121 737/94 254 = 1.292; calculated ratio 1.283. It is thus equal to the ratio of the equivalent conductivities of KHP and NaHP multiplied by the ratio of their concentrations.

The case of $CaCl_2$ is somewhat different. The equivalent conductivity of Ca^{2+} is higher than that of Na^+ (59 *vs.* 50 cm^2 equiv.$^{-1}$ Ω^{-1}), but the peak area of Ca^{2+} is smaller than that of Na^+. The reason is that a divalent ion such as Ca^{2+} will partially interact with P^{2-} to form non-conducting calcium phthalate.

An important fact, however, is that for all three salts the correlation between the cation concentration and the injection peak area is linear, as shown in Fig. 3. Combining the information from the injection peak and sample peak areas enables the composition of binary salt mixtures to be determined, as follows.

Suppose a mixture of KCl and NaCl is injected

$$\text{Injection peak area, Inj.pA} = m[Na^+] + n[K^+] \quad (4)$$

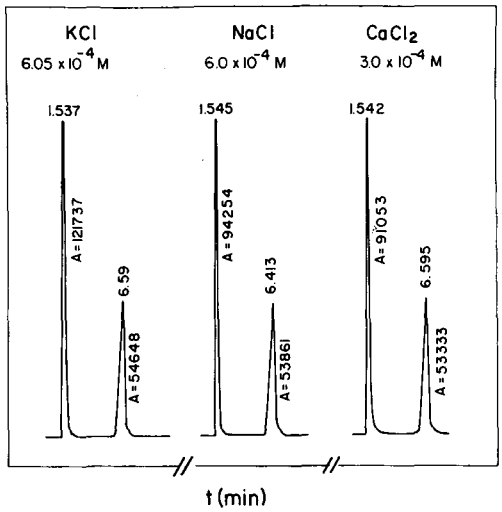


Fig. 2. Injection peaks and sample peaks of three different chloride salts.

where m and n are the respective slopes of the straight lines of the salts in Fig. 3. Then

$$\text{Sample peak area, } pA_{Cl^-} = b([Na^+] + [K^+]) \tag{5}$$

where b is the slope of the calibration line (area *vs.* concentration) of chloride ions (Fig. 5).

There are two unknowns in eqns. 4 and 5, namely $[Na^+]$ and $[K^+]$. They can be accurately determined by measuring the areas of the injection peak and the sample peak and substituting the slopes of the calibration graphs.

Thus, the constituents' concentrations can be derived from eqns. 4 and 5:

$$[K^+] = \frac{\frac{pACl^-}{b} - pAinj}{m - n}$$

$$[Na^+] = \frac{pACl^-}{b} - [K^+]$$

In the case of two salts with two different anions, two sample peaks are available for the determination of the binary mixture. It is therefore quite possible that three salts with different anions can also be determined simultaneously.

Determination of salt mixtures in the presence of an acid or a base

Acidic solutions of salt mixtures can be determined quantitatively as described before. When HCl with eluent (NaHP $1.5 \cdot 10^{-3}$ *M*), but no salt, is injected into the column, the injection peak areas are very small, as is seen in Fig. 4. This is because the

TABLE II

QUANTITATION OF A SALT MIXTURE IN THE PRESENCE OF AN ACID OR A BASE

Composition of injected sample[a]	*Inj.pA of mixture*	*Inj.pA of Na^+ from Fig. 5*	*Inj.pA K^+ from Fig. 5*	*Sum*	*Rel. error (%)*	*Sample peak Cl^-*	*Sample peak Cl^- from Fig. 5*	*Rel. error (%)*	*Sample peak NO_3^-*	*Sample peak from Fig. 5*	*Rel. error (%)*
$NaCl + KNO_3$	207 485 (0.46% R.S.D.)	89 275 (0.3%)	114 737 (0.03%)	204 010	1.7	54 468 (1.8%)	54 307 (0.51%)	0.296	34 338 (1.6%)	34 266 (0.25%)	0.21
$NaCl + KNO_3 + NaOH$	301 766 (0.2%)	89 275 × 2	114 737	293 290	2.89	53 910 (0.5%)	54 307	0.74	35 282 (1.1%)	34 266	2.96
$NaCl + KNO_3 + HCl$	208 366 (0.2%)	89 275	114 737	204 010	2.13	111 080 (0.2%)	54 307 × 2	2.27	34 471 (1.3%)	34 266	0.59

[a] The concentration of each salt, acid or base was $6 \cdot 10^{-4}$ *M*.

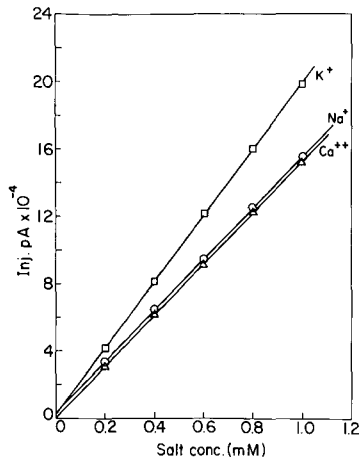
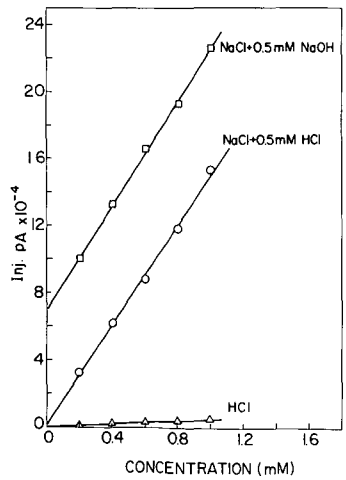


Fig. 3. Injection peaks of salts of K^+, Na^+ and Ca^{2+} as a function of concentration.

added H^+ converts some HP^- into H_2P, and the overall effect is therefore small. If salts are injected in the presence of HCl, that effect will be even smaller, because much more HP^- is displaced, and only a small part of it converted into H_2P, so that the area of the injection peak of HCl in the presence of salts is negligible. As is also seen from Fig. 4, the straight line plot of the injection peak areas of NaCl + HCl intersects with the origin and is identical with the line for NaCl alone (Fig. 1).

In alkaline solution the addition of OH^- does not appreciably affect the injection peak, because some HP^- is converted into P^{2-}, but the sodium ion increases the injection peak area. This is also illustrated in Fig. 4, where a mixture of NaOH and



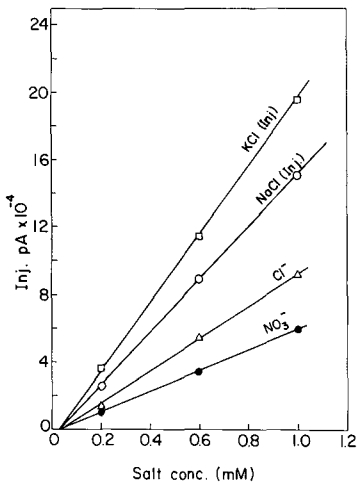


Fig. 4. Injection peak calibration graphs for NaCl in the presence of HCl and NaOH.

Fig. 5. Calibration graphs of injection peaks and sample peaks needed for the quantitation of a mixture of NaCl and KNO_3.

NaCl yields a straight line parallel to that for NaCl + HCl; but its intercept is much higher, because the added sodium ions contribute to the injection peak area. There is, of course, a limit to the acid or base content of a sample that can be tolerated.

Experimental results of the quantitation of a solution containing known concentrations of NaCl and KNO_3 are shown in Table II. Each determination was made three times, and the data for the chromatographic peaks obtained were compared with those of the calibration lines of Fig. 5. From Table II, there is good correspondence between the experimental data for the mixture and the data obtained by injecting each constituent separately. The relative error of the determinations does not exceed 3%, and is is interesting that the relative standard deviations of the injection peaks are much lower than those of the sample peaks.

CONCLUSIONS

In single-column ion chromatography the commonly used eluents are salts of weak organic acids, which provide the background conductivity of the eluent. When a salt solution is injected into a chromatographic anion-exchange column, anions of the eluent are displaced from the column, and the constituents of the eluent are redistributed. This change in the momentary composition of the eluent, together with the injected cation (of the sample), contributes to the conductivity of the injection peak.

By taking advantage of quantitative information on cations derived from the injection peak, and for anions from the sample peaks, salt mixtures may be quantitated.

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CHROM. 21 831

LANTHANIDE LUMINESCENCE QUENCHING AS A DETECTION METHOD IN ION CHROMATOGRAPHY

CHROMATE IN SURFACE AND DRINKING WATER

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SUMMARY

Dynamic quenching of Eu(III) and Tb(III) luminescence by inorganic anions as a detection method in ion chromatography was investigated. To obtain a high luminescence intensity, lanthanide(III) complexes are formed with ligands which make indirect excitation of the ions possible. Only a few anions (*e.g.*, nitrite, chromate) induce efficient dynamic luminescence quenching. Chromate is an efficient quencher of Tb–acac luminescence. Samples of tap water and surface water, spiked with chromate, were injected into a high-performance liquid chromatographic system with post-column addition of the luminescent complex. In this way, a detection limit of $1.1 \cdot 10^{-7}$ *M* (13 ppb) of chromate could be obtained.

INTRODUCTION

In addition to conductivity detection, spectroscopic methods have been introduced in ion chromatography, the best known being absorption and fluorescence detection applied in both direct and indirect modes[1]. The indirect mode is based on the displacement of chromophoric or fluorophoric eluent anions by the analyte. In our laboratory we have developed another indirect spectroscopic detection technique, *viz.*, phosphorescence detection, which is not related to displacement effects. In this method, the dynamic quenching of a phosphorescence signal is induced by the analyte. The decrease in signal monitored is independent of the concentration of the phosphorescent compound. This is an important advantage over indirect methods based on displacement effects, where low concentrations of analyte can only be observed if low concentrations of the chromophoric or fluorophoric eluent anions are used. Applications of indirect detection by dynamic quenching of phosphorescence have been described, with biacetyl[2–5] and, to a minor extent, brominated naphthalenes as phosphorophores, present as a solute in the eluent[3,4,6]. The phosphorescence could only be observed if oxygen was removed from the high-performance

[a] Author deceased.

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liquid chromatographic (HPLC) system, which was achieved by purging nitrogen through the eluent vessel and using stainless-steel capillaries in the experimental set-up.

In addition to the compounds mentioned, which display a long-lived phosphorescence in liquid solutions, there are also inorganic compounds that show long-lived luminescence in liquid solutions, *e.g.*, the lanthanide ions Eu(III) and Tb(III). An important advantage of this type of luminescence is that the intensities and associated lifetimes are hardly or not quenched by oxygen[7]. Preliminary results have led to the conclusion that a number of inorganic anions cause efficient dynamic quenching of lanthanide luminescence[8]. However, excitation of the lanthanide(III) ions is not effective because of their low absorptivities. This is a serious hindrance for its applicability in ion chromatography: the noise on a weak luminescence signal is relatively high, which implies that the attainable detection limits based on a signal decrease are unfavourable.

The purpose of this study was to improve the excitation of Eu(III) and Tb(III) by making use of complexation and indirect excitation via a ligand with high absorptivity. Further, dynamic quenching of these lanthanide complexes by inorganic anions was studied with emphasis on the compatibility of this principle with HPLC conditions. The relevance of the method was shown by investigating its applicability to the determination of chromate in surface and drinking-water samples. Many epidemiological studies have indicated that chromium(VI) is carcinogenic in humans[9]. The maximum allowable concentration in drinking water established in 1963 by the World Health Organization is 50 ppb, so that a method of analysis should give a detection limit down to about 10 ppb.

EXPERIMENTAL

Chemicals

The lanthanide salts $EuCl_3 \cdot 6H_2O$ (99.9%) and $TbCl_3 \cdot 6H_2O$ (99.9%) and 2-thenoyltrifluoroacetone were purchased from Aldrich (Milwaukee, WI, U.S.A.). Acetylacetone (>99%) was from Merck (Hohenbrunn, F.R.G.), Trizma base (reagent grade) from Sigma (St. Louis, MO, U.S.A.), tetrabutylammonium bromide from Kodak (Rochester, NY, U.S.A.) and K_2CrO_4 (99.5%) from Merck (Darmstadt, F.R.G.). Acetonitrile (Baker analyzed HPLC reagent), ethanol (Baker analyzed reagent), methanol (Baker analyzed HPLC reagent), sodium nitrite (Baker laboratory grade), $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ (Baker analyzed reagent) were obtained from Baker (Deventer, The Netherlands). Deionized water was distilled twice before use.

Instrumentation

Batch experiments were carried out with a Perkin-Elmer (Beaconsfield, U.K.) MPF 44 fluorescence spectrometer, supplied with a continuous XBO 150-W xenon lamp and two Hamamatsu type R777-01-HA photomultipliers.

The HPLC sysem consisted of a Gilson (Villers le Bel, France) 302 HPLC pump equipped with a Gilson 802c manometric module, a Valco six-port injection valve, a stainless-steel column (250 × 3.1 mm I.D. for nitrite separations, 150 × 3.1 mm I.D. for chromate separations) packed with 5-μm LiChrosorb RP-18 (Merck, Darmstadt,

F.R.G.), a Kratos (Ramsay, NY, U.S.A.) URS 051 post-column unit (pump and mixing device) and a Perkin-Elmer LS-2 filter fluorimeter. The Perkin Elmer LS-2 luminescence detector, containing a xenon discharge lamp pulsed at line frequency, was employed in the time-resolved phosphorescence mode with a delay time of 0.1 ms and a gating time of 2.0 ms. As the excitation filter a UG 11 filter (which has maximum transmission between 300 and 350 nm) was used; maximum emission wavelengths of the Eu(III) and Tb(III) complexes were 614 and 545 nm, respectively.

For clean-up of surface water, disposable octadecyl extraction columns (Baker) were used.

RESULTS AND DISCUSSION

Indirect excitation of Eu(III) and Tb(III)

To achieve the efficient indirect excitation of lanthanide ions without the need for deoxygenation, in general three conditions should be fulfilled. First, a stable complex must be formed. Second, the ligand should have a high absorptivity, preferably at wavelengths higher than 280 nm, to guarantee a high output of the xenon excitation lamp. Third, the energy transfer to the lanthanide ion should be efficient compared with other energy decay processes of the ligand; here the energy of the lowest triplet electronic level of the ligand plays a crucial role. It is known that β-diketonates, $[RC(O)CR'C(O)R'']^-$, form stable complexes with lanthanide(III) ions[10]. Several β-diketonates have high triplet levels necessary for energy transfer to the resonance level of lanthanide ions. To obtain maximum luminescence intensities of Eu(III) and Tb(III), different β-diketonates should be chosen, as the energies of the resonance levels of Eu(III) and Tb(III) are different. 2-Thenoyltrifluoroacetate (ttac) and acetylacetonate (acac) have appropriate triplet energies for the indirect excitation of Eu(III) and Tb(III), respectively[11]. The indirect excitation of Tb(III) by acac is depicted schematically in Fig. 1 and characteristics of the Eu–ttac and Tb–acac complexes are given in Table I.

In the literature, generally well defined synthesized complexes of lanthanide ions and β-diketonates have been studied. In our study, we utilized mixed solutions of ligand and lanthanide(III) salt, as in buffered solutions these complexes are formed rapidly (the choice of the buffer is described below). As the stoichiometries of the complexes in water are not known, we prefer to write Eu–ttac and Tb–acac.

Solubility, influence of pH and temperature dependence

Eu–ttac. A solution of $1 \cdot 10^{-4}$ *M* Eu(III) and $3 \cdot 10^{-4}$ *M* ttac in buffer–ethanol (80:20, v/v) showed an irreproducible fluorescence signal, probably caused by the low solubility of ttac. With a mixture of buffer–ethanol (50:50, v/v) no solubility problems were observed, so this ratio was chosen for the experiments.

A maximum luminescence signal was observed at pH 7.0. At higher pH the luminescence intensity decreased, probably owing to hydrolysis of Eu(III). At lower pH protonation of the β-diketonate may interfere with the complexation of Eu(III), thus causing a lower signal. For these reasons, a Tris buffer of pH 7.0 was used.

The luminescence intensity of Eu–ttac in a solution of buffer–ethanol (50:50, v/v) was found to be strongly dependent on the temperature, which is in line with literature data on $Eu(ttac)_3$[13]. This was a serious problem with the experimental

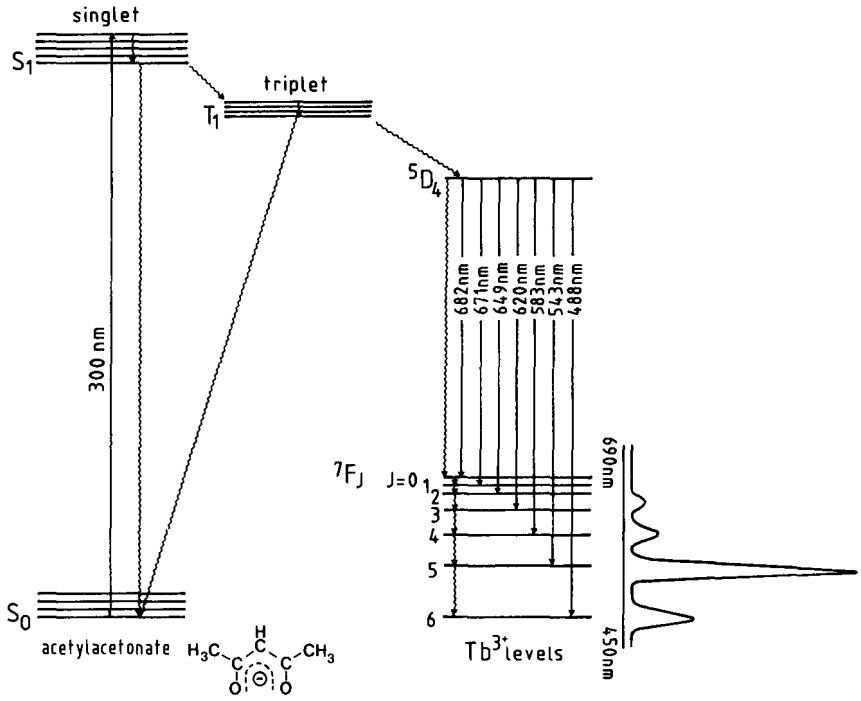

Fig. 1. Schematic representation of the indirect excitation of Tb(III) by acetylacetonate.

set-up that we used in flow experiments. The metal parts are not thermally isolated and the lamp produces so much heat that a rise in temperature of the inlet capillary connected to the flow cell of more than 10°C in a period of 4 h was observed. In the same period the signal decreased by 50%. This effect could be considerably reduced by water cooling of the flow cell compartment.

Tb–acac. With solutions of $1 \cdot 10^{-4}$ *M* Tb(III) and $3 \cdot 10^{-4}$ *M* acac, no solubility problems were observed, even in 100% water. Maximum luminescence intensity

TABLE I

SPECTROSCOPIC CHARACTERISTICS OF THE LANTHANIDE COMPLEXES Eu–ttac AND Tb–acac

Parameter	*Ion*	
	Eu(III)	*Tb(III)*
Ligand	ttac	acac
Maximum excitation wavelength (nm)	360	300
Emitting level of the ion	$^5D_0$	$^5D_4$
Energy of the emitting level[12] (cm^{-1})	17 200	20 500
Maximum emission wavelength (nm)	614	545
Typical luminescence lifetime of the complex in water–ethanol (50:50, v/v) (ms)	0.14	0.45

was observed at pH 7.0. As for Eu–ttac, at higher pH hydrolysis of Tb(III) will take place and at lower pH protonation of the β-diketonate will diminish the complexation with Tb(III).

The temperature dependence of the luminescence of Tb–acac was less dramatic than that for Eu–ttac and a stable signal could be maintained easily by using the water cooling of the flow cell compartment. Considering these results it seems that, for application in an HPLC system, Tb–acac has some advantages over Eu–ttac.

Dynamic quenching

The results of quenching experiments are the most important in drawing conclusions about the applicability of dynamic quenching of lanthanide luminescence as a detection method. Dynamic quenching of luminescence is described by the Stern–Volmer equation:

$$I_0/I = 1 + k_q\tau_0[Q] \quad (1)$$

In the absence of a quencher the luminescence intensity is I_0; in the presence of a dynamic quencher the luminiscence intensity is decreased to I; k_q is the quenching constant (l $mol^{-1}s^{-1}$), τ_0 is the luminescence lifetime (s) in the absence of a quencher and [Q] is the concentration of the quencher (M).

A high value of the product $k_q\tau_0$ indicates that a low limit of detection for the quencher Q is possible. When the product has a value of $1.0 \cdot 10^5$ l mol^{-1}, a concentration of $1.0 \cdot 10^{-7}$ M induces a signal decrease of 1.0%. Higher values of $k_q\tau_0$ result in lower detection limits. Quenching of lanthanide luminescence by various compounds has been studied before[8,14–19]; most experiments showed that k_q is often low. We have studied the dynamic quenching of the luminescence of the Eu(III) and Tb(III) complexes by anions. From the results given in Table II, it is clear that only NO_2^-, CrO_4^{2-}, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ show dynamic quenching. It appears that some anions, when present at high concentrations, cause a decrease in signal in

TABLE II

LUMINESCENCE QUENCHING OF LANTHANIDE COMPLEXES: $k_q\tau_0$ VALUES FOR DIFFERENT ANIONS

Anion	$k_q\tau_0$ (*l mol*$^{-1}$)	
	Eu–ttac[a]	*Tb–acac*[b]
NO_2^-	$1.4 \cdot 10^3$	$9.9 \cdot 10^4$
CrO_4^{2-}	$1.7 \cdot 10^3$[c]	$7.4 \cdot 10^5$
$Fe(CN)_6^{3-}$	$4.4 \cdot 10^3$	$1.3 \cdot 10^6$
$Fe(CN)_6^{4-}$	–[d]	$9.0 \cdot 10^5$
PO_4^{3-}, CO_3^{2-}, SO_4^{2-}, F^-	Signal decrease probably caused by ligand exchange	
NO_3^-, Cl^-, CN^-, SO_3^{2-}, $S_2O_3^{2-}$	No signal decrease observed[e]	

[a] $1 \cdot 10^{-4}$ M Eu(III) and $3 \cdot 10^{-4}$ M ttac in 5 mM aqueous Tris buffer (pH 7.0)–ethanol (50:50, v/v).
[b] $1 \cdot 10^{-4}$ M Tb(III) and $1 \cdot 10^{-4}$ M acac in 5 mM aqueous Tris buffer (pH 7.0).
[c] $1 \cdot 10^{-5}$ M Eu(III) and $1 \cdot 10^{-5}$ M ttac in 5 mM aqueous Tris buffer (pH 7.0)–ethanol (50:50, v/v).
[d] No linear Stern–Volmer plot.
[e] Maximum concentration of anion tested, $1 \cdot 10^{-4}$ M.

batch which is probably caused by a ligand-exchange process. When the β-diketonate is replaced with a non-donating ligand, a decrease in the Eu(III) or Tb(III) luminescence is observed. This also occurs with phosphate ions; for this reason phosphate buffers cannot be used.

As far as we know, efficient dynamic quenching of Tb(III) luminescence by chromate has not been reported before. The results in Table II indicate that quenching of Tb–acac luminescence by chromate seems very interesting.

Chromatographic experiments

Experimental set-up. Because of its relevance, we shall demonstrate the detection method for the selective determination of chromate in water samples in an HPLC system. We previously tested the chromatographic system with nitrite as a model ion. The experimental set-up for HPLC experiments is shown in Fig. 2. The Tb–acac solution is added post-column. In this way the chromatographic separation of the anions is not interfered with the presence of the Tb–acac complex.

Ion separations were carried out by ion-pair reversed-phase chromatography. Tetrabutylammonium bromide (TBABr) was used as the ion-pairing reagent. This reagent had no negative effect on the Tb–acac luminescence.

From the Stern–Volmer expression (eqn. 1), it is clear that not the signal decrease $(I_0 - I)$ but $(1/I_0 - 1/I)$ is proportional to [Q]. We used a signal converter which converts the intensity I into a signal $1/I$, so that the peak height is proportional to [Q]. A description of the signal converter has been given before[2].

Nitrite solutions. In order to test the experimental set-up, chromatographic experiments with nitrite as model compound were performed with an LC mobile phase consisting of aqueous $5.0 \cdot 10^{-3}$ *M* Tris buffer (pH 7.0)–methanol (90:10); for anion separation $5.0 \cdot 10^{-4}$ *M* TBABr was added. For the post-column solution, which had the same composition as the mobile phase, concentrations of $2.0 \cdot 10^{-4}$ *M* of Tb(III) and acac were used. Nitrite solutions were prepared in the eluent and injected (the injection volume was 100 μl). A chromatogram is shown in Fig. 3. The detection limit was $2.5 \cdot 10^{-7}$ *M* and the repeatability was 1.3% (at $5 \cdot 10^{-6}$ *M*, n = 6). Linearity was observed in the range $5 \cdot 10^{-7}$–$1 \cdot 10^{-4}$ *M*, with r = 0.9994 (n = 10).

The detection limit is five times higher than that reported by Baumann *et al.*[8] who used aqueous Tb(III) for luminescence. Although the excitation of Tb(III) has

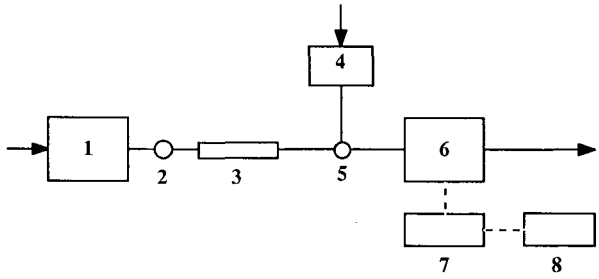

Fig. 2. Schematic diagram of the HPLC system. 1 = LC pump; 2 = injection valve; 3 = analytical column; 4 = post-column pump; 5 = mixing tee; 6 = detector; 7 = signal converter; 8 = recorder.

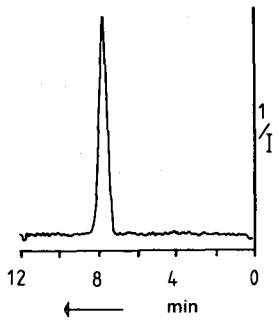

Fig. 3. Chromatogram of a solution of $5.0 \cdot 10^{-6}$ *M* nitrite in the eluent (injection volume 100 µl).

been improved, the efficiency of quenching by nitrite decreased after complexation of Tb(III).

Chromate solutions. For chromate experiments, acetonitrile was used as organic modifier in order to obtain reasonable retention times. The composition of the eluent was $5 \cdot 10^{-4}$ *M* TBABr in aqueous $5 \cdot 10^{-3}$ *M* Tris buffer (pH 7.0)–acetronitile (90:10). The post-column solution additionally contained $1 \cdot 10^{-4}$ *M* Tb(III) and $1 \cdot 10^{-4}$ *M* acac. Chromate solutions were prepared in the eluent and injected; the injection volume was 20 µl. From the chromatogram in Fig. 4, it is clear that slightly asymmetric peaks result. Tailing is also observed when direct UV absorption detection is applied and is probably caused by interaction of chromate with metal parts. The detection limit for 20-µl injections in this system was $1.1 \cdot 10^{-7}$ *M*. The calibration graph was linear from the detection limit up to $1.0 \cdot 10^{-5}$ *M* ($r = 0.9997$, $n = 7$) and the repeatability was 1% (at $3.0 \cdot 10^{-6}$ *M*, $n = 6$).

After complexation, the luminescence intensity of Tb(III) increased and quenching by chromate was still efficient enough to obtain interesting detection limits. Except for the tailing, the previously reported chromatographic problems with chromate[8] were not observed in this instance.

Chromate in surface and drinking water. Chromatographic experiments with surface and drinking water were performed with a newly packed column of the same

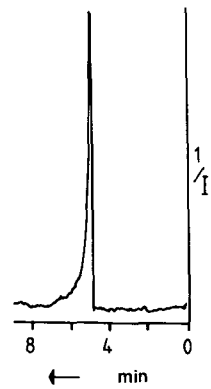

Fig. 4. Chromatogram of a solution of $2.5 \cdot 10^{-6}$ *M* chromate in the eluent (injection volume 20 µl).

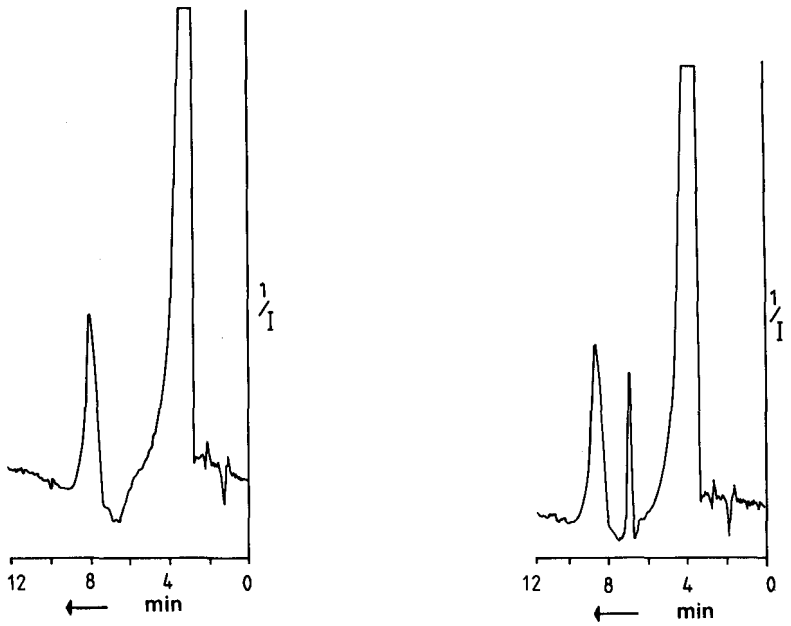

Fig. 5. Chromatogram of a blank surface water sample (left) and of a surface water sample spiked with $1.0 \cdot 10^{-6}$ *M* chromate (right) (injection volume 20 μl).

dimensions as used for the artificial sample (150 × 3.1 mm I.D.). However, the retention characteristics of this column were different from that used previously; when the mobile phase contained 12% of acetonitrile, the retention time of chromate was still longer than in the previous instance.

Tap water and surface water were spiked with chromate, the samples were filtered over a Millipore filter and the surface water samples were subjected to further clean-up by filtering over an octadecyl extraction column. In Fig. 5, chromatograms of a blank and a spiked sample of surface water are depicted. The two peaks in the blank arise from hydrogencarbonate (4 min), which is present in water at high concentrations, and sulphate (> 8 min), which is present at a concentration of *ca.* $4 \cdot 10^{-4}$ *M*. These peaks are also observed when drinking water is injected. A decrease in intensity (owing to the converter, positive peaks are registered) is caused by ligand exchange; both carbonate and sulphate may form complexes with lanthanide(III) ions. In spite of the presence of these ions, chromate can be detected fairly well. The analytical data for chromate in surface and drinking water were similar to the results for the artificial solution; a limit of detection of $1.1 \cdot 10^{-7}$ *M* (13 ppb) was found, which is comparable to those with other detection techniques[5].

CONCLUSION

The results show the potential of dynamic lanthanide luminescence quenching for detection in ion chromatography. In particular, solutions of Tb–acac can be used as a post-column flow without compatibility problems. The intensely luminescent

Tb–acac complexes are simply obtained by mixing equimolar solutions of Tb(III) and acac. Oxygen removal is not necessary. The method has a high inherent selectivity as only a few inorganic anions induce efficient dynamic quenching, although some anions induce a decrease in the luminescence intensity of the complex by ligand exchange. Dynamic luminescence quenching is an appropriate detection method for chromate in water samples. The applicability of the method to the detection of other compounds, *e.g.*, hexacyanoferrate(II) and hexacyanoferrate(III) complexes, is still under study.

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SIMULTANEOUS DETERMINATION OF Cr(III) AND Cr(VI) AT ULTRA-TRACE LEVELS USING ION CHROMATOGRAPHY WITH CHEMILUMINESCENCE DETECTION

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SUMMARY

An ion chromatography system was developed to determine both trivalent and hexavalent chromium. The separation system involved both anion and cation exchange columns connected in parallel with aqueous potassium sulphate as the mobile phase. Determination of Cr(III) and Cr(VI) was performed simultaneously without the need for sample pretreatment. Very-high-sensitivity detection was achieved by post-column reaction with luminol chemiluminescence. The calibration graphs for both Cr(III) and Cr(VI) were linear over at least three orders of magnitude down to the sub $\mu g\ l^{-1}$ range. Detection limits found for Cr(III) and Cr(VI) were 0.1 and 0.3 $\mu g\ l^{-1}$ respectively. The chromium content of a simulated fresh water certified reference material was determined giving good agreement with the certificate value.

INTRODUCTION

Chromium exists in the environment predominantly in two oxidation states Cr(III) and Cr(VI). The hexavalent state of chromium has been shown to be more toxic than the trivalent state in animal experiments[1,2] and the trivalent state is necessary for the maintenance of the normal glucose tolerance factor. There is, therefore, a need for information on speciation and ion exchange chromatography would seem the most appropriate system for study.

Several workers[3,4] have used high-performance liquid chromatography for the simultaneous determination of Cr(III) and Cr(VI). In each case a substantial amount of sample handling and pretreatment was required which is undesirable from a trace analysis viewpoint, and also considerably lengthens the analysis time.

The post-column chemiluminescence detection of Co after ion chromatography has been shown by Jones *et al.*[5] to be an attractive technique due to the extremely high sensitivities obtained, coupled with the freedom of interferences and improvement in reproducibility that results when using a chromatographic technique.

Cr(III) is known to act in a similar way to cobalt in the catalysis of the luminol CL reaction[5] but Cr(VI) does not produce any chemiluminescence. However, Cr(VI) is easily reduced to Cr(III) and so it was considered that a useful approach would be to reduce Cr(VI) post-column just before mixing with the luminol reagent.

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EXPERIMENTAL

Instrumentation

The four-pump liquid chromatography system used is shown in Fig. 1. A high-pressure titanium pump (Model 2150, LKB, Bromma, Sweden) was used for the elution of Cr(VI) and a high-pressure inert plastic pump (Dionex gradient pump, Dionex, Sunnyvale, CA, U.S.A.) for the Cr(III). The reduction system employed a high-pressure stainless-steel pump (Model 6000A, Waters Assoc., MA, U.S.A.) as did the post-column reagent pump (Knauer, Bad Homburg, F.R.G.). All connections were achieved with either titanium or PTFE tubing. A sample was loaded into two sample loops, 200 μl for Cr(III) and 100 μl for Cr(VI), using a ten-port zirconium valve (Valco, Schenkon, Switzerland) and injected simultaneously onto two separate columns connected in parallel. The separations of Cr(III) and Cr(VI) were carried out on a Dionex HPLC CG2 cation-exchange column (50 mm × 4.6 mm I.D.) and a Dionex HPLC AG4A anion-exchange column (50 mm × 4.6 mm I.D.) respectively. The eluents and reducing agent were connected and mixed by means of a cross-piece and the resulting stream was them mixed with the luminol post-column reagent with the aid of a T-piece. This resulting solution was passed through a PTFE coiled flow cell (volume 300 μl) which was mounted directly onto the photomultiplier tube of a fluorescence detector (950 Floromat, Kratos, Westwood, NJ, U.S.A.) which was operated with the lamp removed.

Reagents and standards

Analytical-reagent grade chemicals except luminol and potassium sulphite were used throughout. Solutions were prepared with high-quality deionized water from a MilliQ system (Millipore). Potassium hydroxide, 30% hydrogen peroxide, potassium sulphate and boric acid were obtained from BDH (Poole, U.K.). Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) was obtained from Sigma (Poole, U.K.).

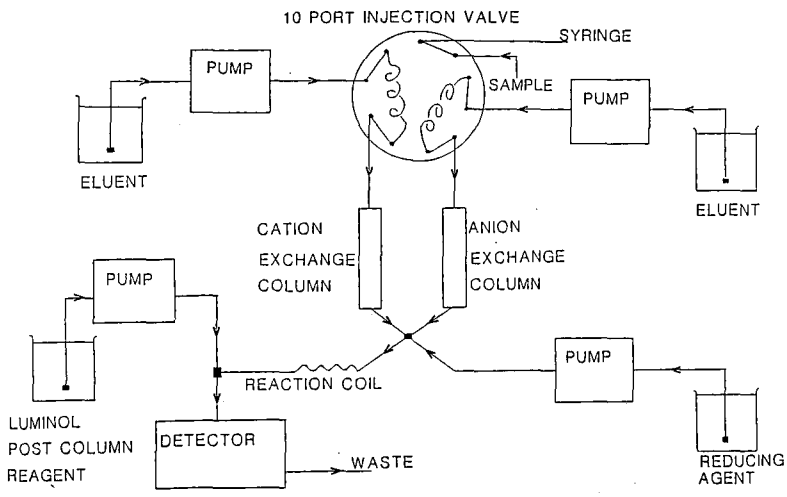

Fig. 1. The HPLC system employed for simultaneous Cr(III) and Cr(VI) determinations.

Metal standards were prepared by sequential dilution of 1000 μg ml^{-1} Spectrosol stock solution (BDH).

Eluent solutions

The eluent used for the Cr(III) separation was prepared by diluting potassium sulphate to 0.085 *M* and the solution was adjusted to pH 3.0 with nitric acid.

The eluent used for the Cr(VI) separation was also potassium sulphate pH 3.0 but at a lower concentration of 0.003 *M*.

Reduction system

The reducing agent used was potassium sulphite 0.015 *M* which was freshly prepared daily and adjusted to pH 3.0 with nitic acid.

Post-column reagent

A 1-l volume of solution was prepared by adding 0.06 g of luminol, 1 ml of 30% hydrogen peroxide and 6.0 g of boric acid to deionized water. The post-column reagent was adjusted to pH 11.5 with concentrated aqueous potassium hydroxide.

RESULTS AND DISCUSSION

Cr(III) only

Early attempts at the determination of Cr(III) using luminol chemiluminescence after separation on low capacity resins with lactate eluents gave very poor peak shapes. These poor peak shapes were presumably due to the slow kinetics of Cr(III)–chelate systems. The Cr(III)–chelate system was abandoned in favour of a potassium sulphate eluent which has been proved to be a useful elution system for Al(III) determinations[6]. As with Al(III) the strong retention of Cr(III) warranted the use of a short column.

Detector performance. Having optimized the luminol chemiluminescence system for Cr(III) detection, in terms of the best signal-to-noise ratio, an interference study was carried out to ascertain whether there was overlap of other metals on the Cr(III) signal. None of the 2^+ metals that were tested, Fe^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}, were found to interfere even at high levels [10 μg ml^{-1} metal^{2+} co-injected with 0.1 μg ml^{-1} Cr(III)]. Fe^{3+} and Al^{3+} were also tested for interference properties and were only found to have an effect at relatively high levels [40 μg ml^{-1} metal^{3+} co-injected with 0.1 μg ml^{-1} Cr(III) almost completely suppressed the Cr chemiluminescence signal].

Linear range detecton limit. Linear calibrations were obtained covering approximately three orders of magnitude from 0.5 μg l^{-1} to 1 mg l^{-1}. The calibration plot of these data showed excellent linearity (r = 0.9999). The reproducibility was acceptable at the 100 μg l^{-1} level where a relative standard deviation of 3.3% was found for twelve replicate injections. The detection limit defined as three standard deviations of the blank signal was 0.1 ng.

Cr(VI) only

Hexavalent chromium itself will not catalyse the luminol chemiluminescence reaction. Since it has been shown that Cr(III) is an efficient catalyst, Cr(VI) was reduced post-column by an aqueous stream of potassium sulphite. The Cr(III) thus

produced was then free to catalyse the luminol chemiluminescence reaction. Other reduction systems using on-line columns packed with various reducing metals such as Cd, Zn and Sn were tried but proved unsatisfactory. The main problem with these on-line reductors were that the packing materials were not of good quality and so (a) the chromatography of the Cr(III) and Cr(VI) was affected and (b) the noise level increased to a much higher level.

Detector performance. Having optimized the reduction conditions, an interference trial was set up to see if there was any suppression or enhancement from overlap of the chromate signal with other anions. Phosphate, vanadate and molybdate were all tested and found not to interfere even at relatively high concentrations (10 μg ml^{-1} anion co-injected with 0.1 μg ml^{-1} chromate).

Linear range and detection limit. A linear range of at least three orders of magnitude was established (1 μg l^{-1} to 1 mg l^{-1}) although the absolute upper limit was not determined. The calibration plot from these data showed good linearity (r = 0.999). The reproducibility was good at the 150 μg l^{-1} level where a relative standard deviation of 2.1% was obtained for seven replicate trials.

The detection limit (3σ the blank signal) was found to be 0.3 ng. It is felt by the authors that the detection limit could be improved somewhat if a higher purity reduction system were employed.

Simultaneous determination of Cr(III) and Cr(VI)

Using the ten-port injection valve with two sample loops the system was set up to load two replicate samples from the same standard solution. When switched to inject,

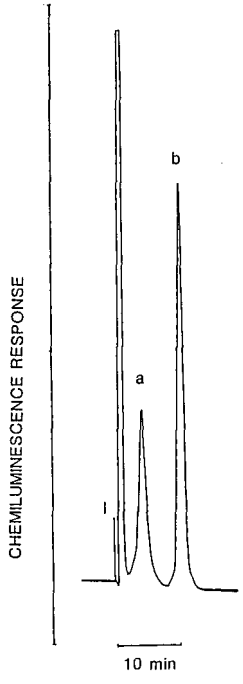

Fig. 2. A typical separation of (a) Cr(VI) and (b) Cr(III) with chemiluminescence detection.

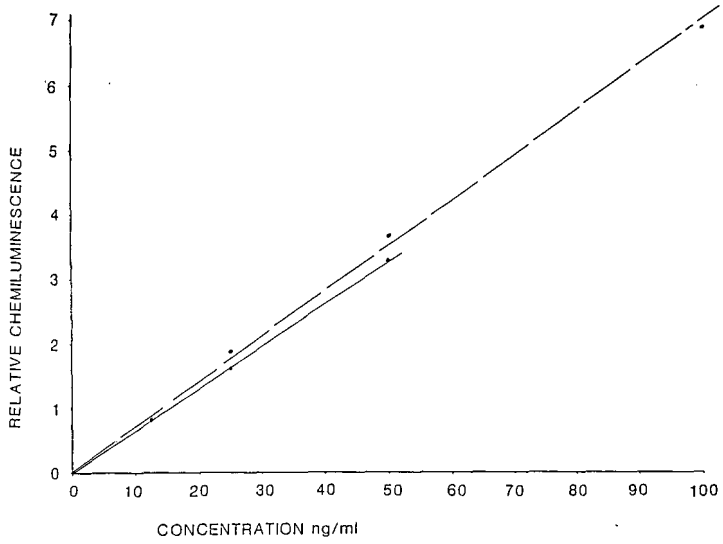

Fig. 3. Simultaneous calibration of (———) Cr(III) and (– – – – –) Cr(VI).

one sample was injected onto the cation-exchange column whilst the other was injected onto the anion-exchange column. Fig. 2 shows a typical trace of this simultaneous determination, both analytes being 150 μg l^{-1} in the standard. Of the sample that is injected onto the cation-exchange column any Cr(VI) that is present will have no attraction for the column and will elute on the solvent front. Similarly any Cr(III) that is present in the sample that is injected onto the anion exchange column will also elute on the solvent front. It can be seen from Fig. 2 that these two solvent fronts combine to produce a signal.

The conditions are such that Cr(VI) elutes from the anion-exchange column after 5 min whilst the Cr(III) is retained on the cation-exchange column for well over 10

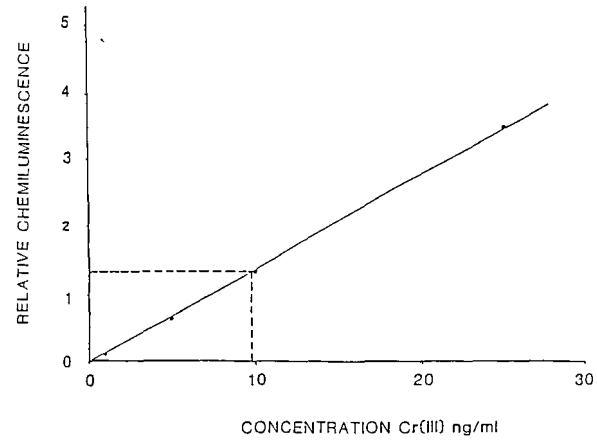

Fig. 4. The determination of Cr(III) from a certified fresh water sample IAEAW4.

min thus ensuring complete separation of the two species. Fig. 3 shows a simultaneous calibration of the two species at the lower end of the linear range.

Sample analysis

To evaluate the quantitative performance and accuracy of the system a sample of low chromium (III) content was chosen. Unfortunately no certified reference material with both Cr(III) and Cr(VI) present was available so a simulated fresh water sample IAEA/W4 with a Cr(III) content of 9.9 ng ml^{-1} was selected.

The analysis was performed by simply injecting a 200-μl sample into the system, followed by the appropriate blank solution. Certificate value Cr(III), 9.9 ng ml^{-1}; confidence level, 9.0–10.5 μg ml^{-1}; found value ($\bar{x}$) on seven trials, 9.9 ng ml^{-1}; R.S.D. on seven trials, 3.3% (see Fig. 4).

CONCLUSION

The simultaneous determination of the two chromium species without any sample pretreatment has been achieved and described. Good sensitivity is obtainable with chemiluminescence detection. Even better detection limits are possible with an improved reduction step for Cr(VI) to Cr(III). The use of a good quality on-line reductor *e.g.* a cadmium metal immobilized short column, would eliminate one of the pumps and therefore reduce the complexity of the system. However problems were found when using this type of set up and so this on-line reduction system was abandoned in favour of the less troublesome four pump aqueous reduction system.

The near absence of any interferences and the successful determination of Cr(III) in a certified reference material combined with the excellent sensitivity and linearity of the system may possibly make it an attractive method for chromium analyses in real samples.

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CHEMICALLY SUPPRESSED ANION CHROMATOGRAPHY BASED ON MACROCYCLE–CATION COMPLEXATION

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SUMMARY

A novel ion chromatographic technique is described using macrocyclic ligand–cation complexes as anion-exchange sites, yielding columns of variable capacity and chromatograms independent of sample cation. Hydrophobic macrocycles are coated onto commercially available C_{18}-derivatized silica or polystyrene columns. The aqueous eluent contains cations that bind dynamically to the macrocycle, forming positively charged exchange sites. The nature of the ion-exchange sites, and thus the column characteristics, can be altered simply by changing the eluent cation.

INTRODUCTION

Macrocyclic ligands, such as crown ethers and cryptands, are well known for their size-selective binding of metal and other cations[1,2]. This selectivity has been successfully exploited for the separation of cations by solvent-extraction, liquid-membrane and chromatographic methods for analytical and other purposes. Further analytical applications of these compounds have been reported in ion-selective electrode and spectrophotometric techniques[3,4].

Column chromatographic applications of macrocyclic ligands have focused primarily on cation separations, although other species have also been separated. Blasius and co-workers[4–6] prepared a large number of polymeric crown ethers which serve as anchor groups for chromatographic separation of alkali, alkaline earth and precious metals and also some organic compounds and water. These relatively soft polymers are not suited to high-performance liquid chromatography (HPLC). Further, Dotsevi *et al.*[7] used chiral macrocycles covalently bound to polystyrene for the chromatographic separation of the optical isomers of amino acids. More recently, Bradshaw *et al.*[8] and Dudler *et al.*[9] reported the preparation of silica gel-bound crown ethers, such as 18-crown-6, which provide excellent preparative-scale metal ion separations in acidic or neutral solution.

The HPLC separation of cations using macrocycles was pioneered by Kimura's group using silica supports. The crown ether or cryptand exchange sites were held in place in one of two ways: (1) by covalent bonding to the silica support[10–14] or (2) by dynamic coating of lipophilic macrocycles on octadecylsilanized silica (ODS)[15,16]. In the former instance, the synthetic procedure required the use of benzo-substituted

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crowns, which are less selective than the parent compounds and demonstrate relatively weak cation binding[1]. Nonetheless, good separations of alkali metals were achieved when dimeric or polymeric crowns were used. Dynamic coating of lipophilic crown ethers also yielded excellent separations of alkali and alkaline earth metal ions. However, the lipophilically substituted cryptand 2.2.2 bound alkali metals and other cations too strongly to yield a practical elution profile. Highly stable coatings were obtained even after exposure to large volumes of eluents containing 40% methanol. Macrocycles hav also been successfully employed as mobile phase modifiers in the separation of amino acids[17] and β-lactam antibiotics[18] on cation-exchange columns.

Using column chromatography, Blasius *et al.*[6] showed that anions may be separated on polymeric crown ether resins containing bound metal cations[6]. Igawa *et al.*[19], Nakajima *et al.*[11,12] and Blasius and co-workers[20–22] reported how this principle can be succesfully applied to HPLC using bis- and polymeric crown ethers covalently bonded or polymerically coated on silica. In these studies, non-ionic eluents (water or water–methanol) were used. Sample cations and anions eluted in bands as ion pairs at rates dictated by the interaction of sample cations with the neutral macrocyclic exchange sites. The retention times of sample anions depended strongly on the sample cation present.

We report here a novel method for making use of macrocyclic ligands as exchange sites in the analysis of anions by chemically suppressed ion chromatography. The column preparation is based on the principle described by Cassidy and co-workers[23–26] and by Kimura and co-workers[15,16] of dynamic coating of hydrophobic exchangers on commercially available C_{18}-derivatized silica or polystyrene columns. The aqueous eluent contains a cation that has an affinity for the macrocycle coating, causing the formation of positively charged cation–macrocycle complex exchange sites. The effect on anion retention of sample cations is minimized by an anion-exchange mechanism in the presence of an overwhelming concentration of eluent cations. Of special value in this method is the capability of altering the nature of the ion-exchange sites simply by changing the eluent cation. This capability makes it possible to customize the ion-exchange stationary phase to yield the desired separation. The use of new polystyrene-based C_{18} columns eliminates problems of support degradation that are common with silica-based columns when basic anion eluents are used.

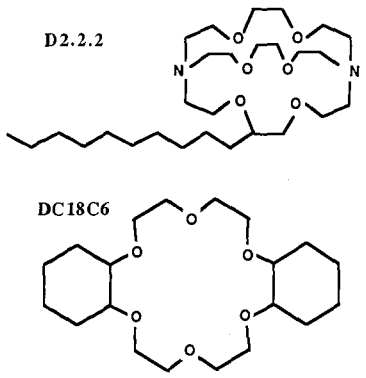
EXPERIMENTAL

Materials

Analytical-reagent grade macrocyclic ligands cryptand *n*-decyl-2.2.2 (D2.2.2), 18-crown-6 (18C6) and dicyclohexano-18-crown-6 (DC18C6) (mixture of isomers) were obtained from Parish Chemical. HPLC-grade methanol was obtained from Fisher Scientific. Eluent water was purified to 18 MΩ cm using a Milli-Q purification system (Millipore). Eluents were degassed by helium purging or sonication. All chemicals used to prepare eluent and standard solutions were of analytical-reagent grade.

Equipment and columns

All chromatograms were obtained using Dionex 2000i (isocratic) or 4000i



(gradient) ion chromatographs, which have non-metal pumps and valves. Eluent suppression for conductometric detection was provided by a Dionex Anion Micromembrane Suppressor using 12.5 m*M* sulfuric acid at 3 ml/min. Chromatograms were collected using the Spectraphysics Labnet computer system.

Four kinds of reversed-phase columns were used: Dionex MPIC NS1 (25 cm × 4.6 mm I.D.), polystyrene–divinylbenzene; Spherisorb 10-μm ODS-2 (25 cm × 4.6 mm I.D.), C_{18} on silica; Spherisorb 5-μm S5 ODS-1 (25 cm × 4.6 mm I.D.), C_{18} on silica; and Interaction ACT-1 10-μm average particle size (15 cm × 4.6 mm I.D.), C_{18} on polystyrene–divinylbenzene.

Column coating procedure

D2.2.2 was supplied as a 50 wt.-% solution in toluene; 50 μl of this solution were added to 100 ml of methanol–water (60:40, v/v) which was degassed by sonication for 10 min. After the column had been rinsed with methanol–water (60:40, v/v) solution, the D2.2.2 solution was pumped in recycle fashion through the column for 16 h at 0.5 ml/min.

RESULTS AND DISCUSSION

Macrocycles as mobile phase modifiers

Our early experiments using macrocycles in ion chromatography (IC) involved the incorporation of water-soluble crown ethers, such as 18C6 and DC18C6, into the ion chromatographic mobile phase. In this instance a Dionex MPIC (mobile phase ion chromatography) column was used. It was expected that some sample cations would complex with the crown ether in the mobile phase and thereby develop sufficient hydrophobic character to be retained on the non-polar stationary phase as in pairs with eluent anions. The eluent contained 10 m*M* nitric acid, which was chemically suppressed for conductivity detection. Indeed, the alkali metals were retained in the same order as that of their 18C6 complex stability constants, $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$, eluting as nitrate salts. Typical chromatograms using 18C6 and DC18C6 are shown in Fig. 1a and b, respectively.

A series of experiments was performed using various amounts of DC18C6 in the mobile phase. The results (Fig. 2) show a maximum capacity factor (k') for K^+ at a DC18C6 concentration of approximately 5 m*M*. Below this level there is insufficient

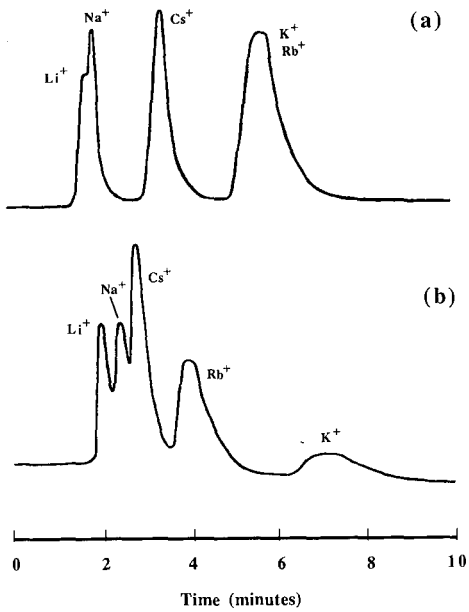


Fig. 1. MPIC of cation nitrate salts (Li^+, 132 μM; Na^+, 124 μM; Cs^+, 248 μM; Rb^+, 260 μM; K^+, 193 μM) using crown ethers in the mobile phase. Flow-rate, 1.0 ml/min. (a) 25 mM 18C6 in 500 μM HNO_3; (b) 20 mM DC18C6 in 500 μM HNO_3.

crown ether for effective retention and above it there is competition for adsorption on the stationary phase by cation-free over cation-bound macrocycle. In these experiments, before each point could be measured, it was necessary to allow sufficient time for equilibration of the reaction $[DC18C6]_S = [DC18C6]_M$ to occur, where S represents stationary phase and M mobile phase. The establishment of equilibrium was indicated

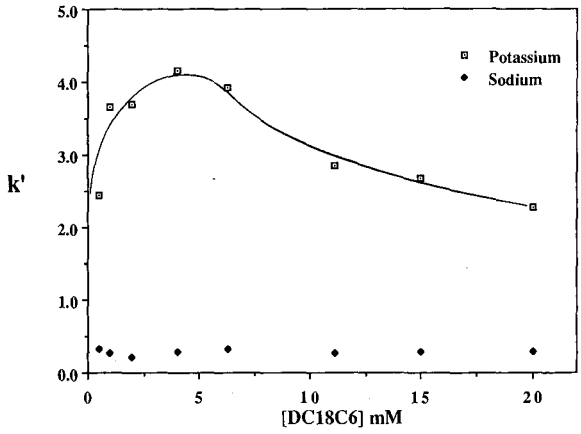


Fig. 2. Variation of capacity factor, k', of K^+ and Na^+ with [DC18C6] in MPIC system. The eluent also contans 500 μM HNO_3.

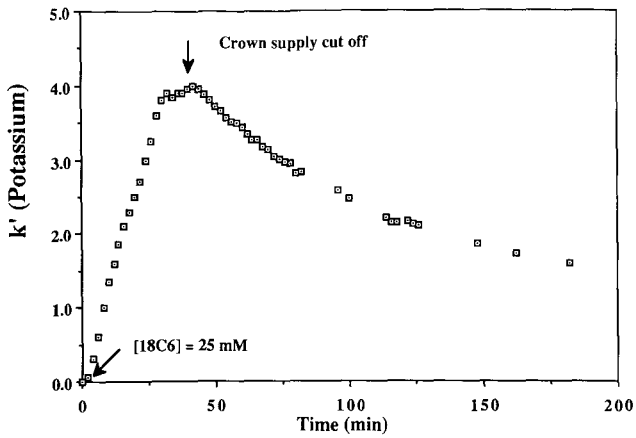


Fig. 3. Variation of k' (K^+) with time after introduction and subsequent removal of 25 mM 18C6 as eluent in MPIC system. The eluent also contains 500 μM HNO_3.

by no further change in k' as a sequence of sample injections was performed at each DC18C6 concentration.

The results of the above experiments made it clear that the MPIC column was sorbing unbound macrocycle. Fig. 3 shows that even the hydrophilic 18C6, which is soluble in water to concentrations over 2.0 M, has a considerable affinity for the reversed-phase column. When 25 mM 18C6 eluent was first pumped through the MPIC column, the capacity factor for K^+ increased gradually over a period of about 40 min, after which a steady state was reached. When the crown supply was cut off, the capacity factor decreased slowly with time. Clearly, even this hydrophilic crown was not acting in the mobile phase as expected, but was adsorbing on the non-polar stationary phase, where it served to retain the K^+ ions by a complexation–sorption mechanism. Hence, in the early portion of the curve (Fig. 2) the column is being saturated with sorbed macrocycle, and in the latter portion K^+ retention persists even though no crown is present in the mobile phase. Therefore, it appears more accurate to describe this system as involving "dynamically bound" exchange sites than ion pairing in the mobile phase.

Cation macrocycle complexes as exchange sites for anion separations

The ready immobilization of macrocycles onto non-polar stationary phases offers a novel approach to anion chromatography using chemical suppression and conductivity detection. As noted in the Introduction, others have used dynamically bound macrocycles for the HPLC determination of cations. The ODS columns on which the macrocycles were adsorbed are sensitive to base, which makes difficult the achievement of anion separation by such systems using basic eluents which are amenable to chemical suppression. However, we have found that by careful control of the eluent pH, macrocycles coated on ODS columns can be used for the chemically suppressed IC of anions. Further, the recent development of C_{18} reversed-phase columns based on polystyrene that are not susceptible to base degradation offer a new alternative for applying macrocycles to anion chromatography in this way.

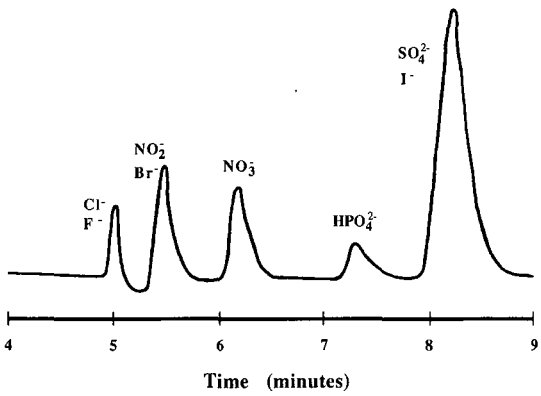


Fig. 4. Chromatogram of eight anions (F^-, 15 μM; Cl^-, 17 μM; Br^-, 29 μM; NO_2^-, 25 μM; NO_3^-, 39 μM; HPO_4^{2-}, 36 μM; I^-, 30 μM; SO_4^{2-}, 42 μM; all as K^+ salts) using an ODS column (5 μm) loaded with D2.2.2. Eluent, 5.6 mM $KHCO_3$–29.3 mM H_3BO_3 in water; flow-rate, 0.7 ml/min.

When a sample containing 50 μM KCl, KNO_3, KF, KI, K_2SO_4 or KSCN was injected onto the ODS column which had not been coated with D2.2.2, no retention was observed, but when a sample containing eight anions (F^-, Cl^-, Br^-, NO_2^-, NO_3^-, HPO_4^{2-}, I^-, SO_4^{2-}) was injected onto an ODS column that had been treated with D2.2.2, the chromatogram shown in Fig. 4 was obtained. In this instance, the eluent was an aqueous buffer (pH 7.62) consisting of 5.6 mM $KHCO_3$ and 29.3 mM H_3BO_3 at a flow-rate of 0.7 ml/min. Excellent peak resolution is observed with good Gaussian peak shapes.

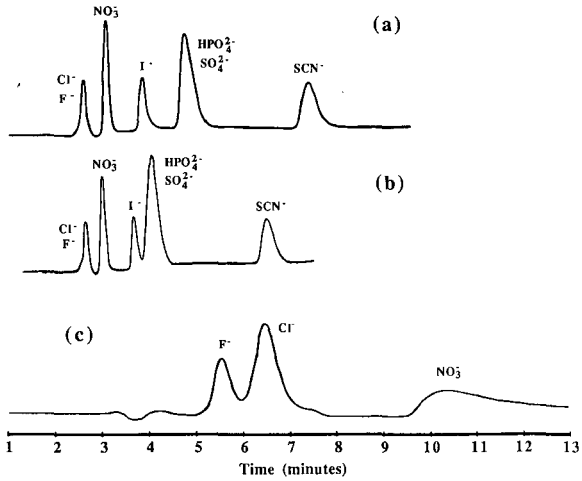


Fig. 5. Chromatograms of seven anions (F^-, 15 μM; Cl^-, 17 μM; NO_3^-, 39 μM; I^-, 30 μM; HPO_4^{2-}, 36 μM; SO_4^{2-}, 42 μM; SCN^-, 47 μM; all as K^+ salts) using an ODS column (10 μM) and eluent of pH 9.3 (5 mM $K_2B_4O_7$–5 mM H_3BO_3) at a flow-rate of 0.9 ml/min, (a) during first hour and (b) after 5 h. (c) Chromatogram of three sample anions (F^-, 34 μM; Cl^-, 39 μM; NO_3^-, 90 μM) using an ACT column loaded with D2.2.2. Same eluent as for (a) and (b), except in methanol–water (5:95, v/v) and flow-rate 0.5 ml/min.

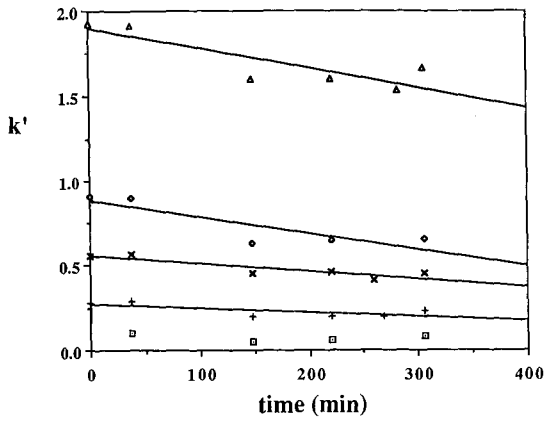


Fig. 6. Decrease in k' with time for five anions: (□) Cl^-; (+) NO_3^-; (×) I^-; (◇) SO_4^{2-}; (△) SCN^-. Experimental conditions as in Fig. 5a and b.

Column stability

The principle obstacle to using macrocycle-coated ODS columns for anion separations in this fashion is the sensitivity of these columns to base. It was expected that the chromatogram in Fig. 4 could be improved by increasing the pH of the eluent. In an attempt to extend the limits of the pH tolerance specifications of the silica column, a buffer of pH 9.3 was used, consisting of 5 m*M* H_3BO_3 and 5 m*M* $K_2B_4O_7$ in water. When a sample containing potassium salts of F^-, Cl^-, NO_3^-, I^-, SO_4^{2-} and SCN^- was injected at a flow-rate of 0.9 ml/min, the chromatogram shown in Fig. 5a was obtained. It was clear from the chromatogram in Fig. 5b, however, that after 5 h of eluent flow the capacity had decreased significantly. The change in capacity factor for the five ion peaks (F^- and Cl^- eluted together) with time is shown in Fig. 6. The

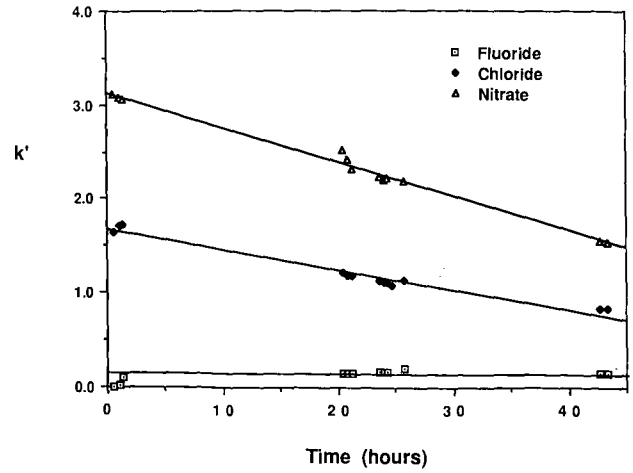


Fig. 7. Decrease in k' with time for three anions using the ACT column and methanol–water (40:60, v/v) as eluent.

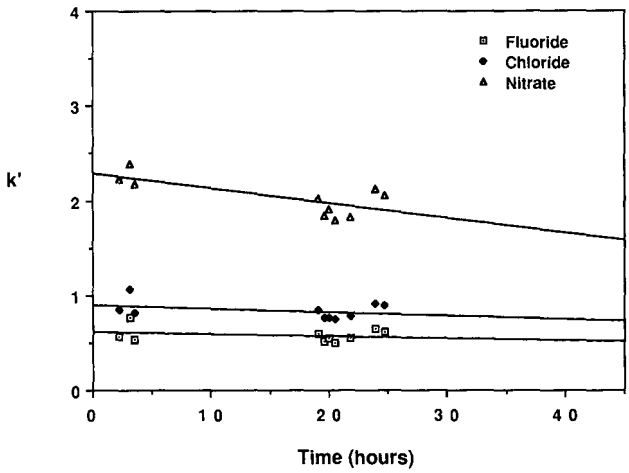


Fig. 8. Decrease in k' with time for three anions using the ACT column and methanol–water (15:85, v/v) as eluent.

sensitivity of the ODS column to base makes it difficult to determine whether or not the loss of D2.2.2, and hence capacity, was due at least in part to loss of the C_{18} coating. It is possible to use ODS columns for the application described here, but only if the eluent pH is kept below 8, as specified by the manufacturer.

The sensitivity of ODS columns to base as described above imposes serious constraints on the eluent composition for anion chromatography. A clear solution to this problem is to adopt a chemically inert polymer-based C_{18} column, such as the ACT-1. However, the gain in chemical stability with this column is partially offset by the higher cost and less uniform particle size, with a concomitant decrease in column efficiency. This column, loaded with D2.2.2, was used in conjunction with an eluent identical with that of pH 9.3 described above, except that the solvent was a methanol–water mixture. The relatively lower efficiency of this column is illustrated in Fig. 5c. Calculations based on the NO_3^- peak give an efficiency of $N \approx 600$ for the ACT column compared with $N \approx 1800$ for the 10-μm ODS column.

An eluent comparable to that described above was prepared with various amounts of methanol to determine the stability of the D2.2.2-coated ACT to loss of macrocycle. At 90% methanol, the cryptand was stripped immediately from the column. At 40% methanol, the anion capacity factors decreased sigificantly over a 44-h period (representing approximately 1.3 l of eluent flow), as illustrated in Fig. 7. At 15% methanol (Fig. 8), the decrease is less rapid (the slope of the NO_3^- line is $-0.016\ h^{-1}$, compared with $-0.037\ h^{-1}$ for the 40% methanol plot). At 5% methanol, no decrease in k' was observed even after 60 h. The long-term stability of these dynamically coated columns against loss of macrocycle at low methanol concentrations corresponds to the stability observations by Kimura *et al.*[16] and Cassidy and Elchuk[23] under dynamic conditions.

Effect of eluent cation concentration

Fig. 9. illustrates the effect on anion capacity factor of variations in the concentration of eluent K^+ ion. In this instance, a simple potassium hydroxide eluent

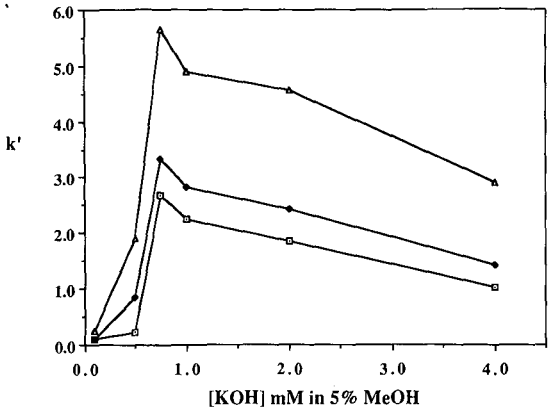


Fig. 9. Change in k' for three anions with [KOH] in methanol (MeOH)–water (5:95, v/v) as eluent using the ACT column loaded with D2.2.2. (□) F^-; (◆) Cl^-; (△) NO_3^-.

in methanol–water (5:95, v/v) was used with the ACT column. At very low concentrations of K^+, the anions were not significantly retained and were not separated from one another. This observation conforms to the proposed mechanism of retention, *i.e.*, the formation of anion-exchange sites via the complexation of eluent metal cations. As the eluent [K^+] increases, the column capacity increases quickly and anion separation begins. Capacity factors reach a maximum at a K^+ eluent concentration of approximately 0.75 m*M* and then fall gradually. The probable explanation for this behavior is that at the curve maximum, the cryptand sites are fully occupied by K^+ ions and further increases in [K^+] have no favorable effect. Beyond the maximum, the effect of increasing [OH^-] comes into play, as eluent anions compete with sample anions for the cryptand complex exchange sites.

The position of the curve maxima in Fig. 9 helps to elucidate the nature of the stationary phase in these dynamically bound systems. There are at least two possible ways in which D2.2.2 might be sorbed on the C_{18} stationary phase coating: (1) with the hydrophobic tail immersed in the C_{18} coating and the cryptand head protruding into the mobile phase; or (2) with the entire cryptand molecule buried in the coating. If the former mechanism predominates the reaction for K^+ uptake by the macrocycle should be very similar to the complexation reaction in water:

$$K^+_{aq} + 2.2.2_{aq} \rightleftharpoons (K2.2.2)^+_{aq} \quad (1)$$

This similarity arises from the fact that the hydrophobic tail on D2.2.2 has very little effect on the thermodynamics of its metal cation binding, as it attaches to an aliphatic portion of the parent molecule. Hence the equilibrium constants for its reaction with cations are similar to those of 2.2.2[1]. On the other hand, if the second sorption mechanism predominates the reaction for K^+ uptake ought to be more similar to the extraction equation.

$$K^+_{aq} + A^-_{aq} + 2.2.2_{org} \rightleftharpoons (K2.2.2)^+A^-_{org} \quad (2)$$

where "aq" represents species in the aqueous phase and "org" species in the organic phase.

We may test which of the two above descriptions best conforms to the results in Fig. 9. The equilibrium constant K for reaction (1) in water is 2.0×10^5 (ref. 1) and may be expressed as

$$K\,[K^+]_{aq} = \frac{[K2.2.2]^+_{aq}}{[2.2.2]_{aq}} \tag{3}$$

If the cryptand is predominantly in the water phase, then at the lowest concentration of potassium hydroxide (0.10 mM), simple calculation shows that 95% of the cryptand sites should be occupied by K^+ ions. The fact that very little retention or separation of anions is observed at 0.10 mM potassium hydroxide implies that the concentration of $K2.2.2^+$ complex sites cannot be that high. Indeed, eqn. 3 states that if 99% of the cryptand sites are occupied by K^+ at $[K^+] = 0.75$ mM (the position of the maximum from Fig. 9), the value of the thermodynamic binding constant must be closer to 10^4 than to $2.0 \cdot 10^5$.

Although we do not yet have a measured value for the extraction constant, K_e, for eqn. 2, we can deduce a reasonable value. We have measured the extraction constants of alkali and alkaline earth metal salts with simple inorganic anions between water and toluene or phenylhexane containing lipophilic crown ethers[27]. These K_e values are typically 1–2 orders of magnitude smaller than the corresponding aqueous equilibrium constants with the hydrophobic parent macrocycles. Therefore, K_e for eqn. 2 is likely between $2.0 \cdot 10^3$ and $2.0 \cdot 10^4$, a range that does accommodate the data in Fig. 9. We conclude that the cryptand sites are not primarily present in the aqueous mobile phase environment, but that the eluent potassium salt is extracted into the hydrophobic stationary phase environment. Given the amphiphilic nature of the resulting complex, it is reasonable to speculate that the ionic head of the complex molecule resides at the surface of the C_{18} coating.

Variation of ion-exchange sites by changing eluent cation

A novel characteristic of the anion chromatographic system described above is that the ion-exchange sites on the stationary phase may be altered simply by changing the eluent cation. Further, the percentage of adsorbed D2.2.2 molecules that contain cations may be varied by using cations with various thermodynamic affinities for the ligand, or by changing the eluent cation concentration.

Fig. 10 shows the chromatograms of six anions, Cl^-, Br^-, NO_3^-, I^-, HPO_4^{2-} and SO_4^{2-}, all as K^+ salts, using eluents that differ only in the eluent cation. The eluent in each instance was 0.1 mM $MHCO_3$ (M = Na^+, K^+ or Cs^+)–6.05 mM H_3BO_3 at a flow-rate of 0.6 ml/min. It was attemped to keep the eluent pH values and component concentrations a comparable as possible. The pH values were Cs^+ 7.55, K^+ 7.86 and Na^+ 7.65, all below the level that causes column deterioration according to the manufacturer's specifications. Conductivity detection was used following chemical suppression. All peaks were positively identified by standard addition experiments. Table I compares the capacity factors for the six anions with each of the three solvent cations. These k' values (averages of three determinations with standard deviations)

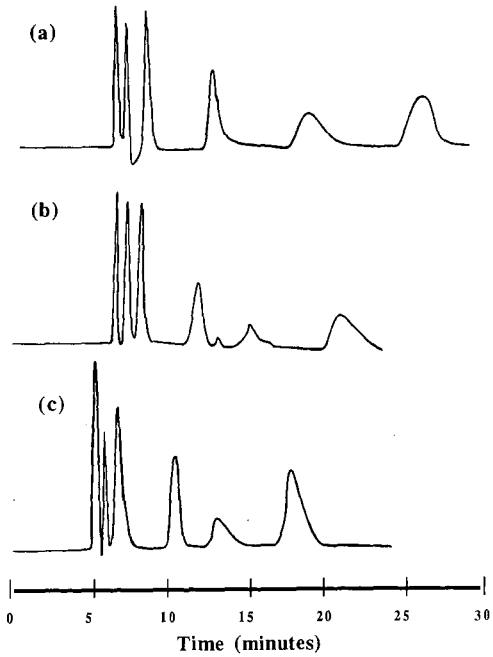


Fig. 10. Chromatogram of six anions (K^+ salts) using three eluents of the type 1.0 m*M* $MHCO_3$–6.05 m*M* H_3BO_3 at 0.6 ml/min. (a) M = K^+; (b) M = Cs^+; (c) M = Na^+. Column, ODS (5 μm) loaded with D2.2.2. Anions elute in the order Cl^-, 19 μM; Br^-, 33 μM; NO_3^-, 45 μM; I^-, 35 μM; HPO_4^{2-}, 42 μM; SO_4^{2-}, 34 μM in all three chromatograms.

consistently increase in the eluent cation series $Na^+ < Cs^+ < K^+$. Small variations in eluent pH may play some role in this trend. The K^+ eluent provides the greatest anion retention. However, the Na^+ eluent provides excellent resolution in a considerably shorter time. The detector response for the anions was the same within experimental error for all three eluent cations.

Fig. 11 demonstrates the effect of three eluent alkali metal cations on the separation of a mixture of F^-, Cl^-, NO_3^-, NO_2^-, HPO_3^{2-}, I^- and SO_4^{2-} using the ACT column. In all instances the eluent consisted of 10 m*M* MOH (M = Li, Na, or K) in

TABLE I

CAPACITY FACTORS (*k'*) FOR SIX ANALYTE ANIONS USNG THREE ELUENTS WHICH DIFFER ONLY IN THE ELUENT CATION

Eluent[a]	*Sample anion*					
	Cl^-	Br^-	NO_3^-	I^-	HPO_4^{2-}	SO_4^{2-}
Na^+	0.026 ± 0.034	0.099 ± 0.035	0.22 ± 0.04	0.74 ± 0.05	1.09 ± 0.06	1.75 ± 0.08
Cs^+	0.10 ± 0.03	0.21 ± 0.03	0.35 ± 0.03	0.93 ± 0.04	1.49 ± 0.06	2.36 ± 0.04
K^+	0.12 ± 0.01	0.24 ± 0.01	0.454 ± 0.008	1.22 ± 0.05	2.29 ± 0.05	3.44 ± 0.02

[a] See text for experimental details.

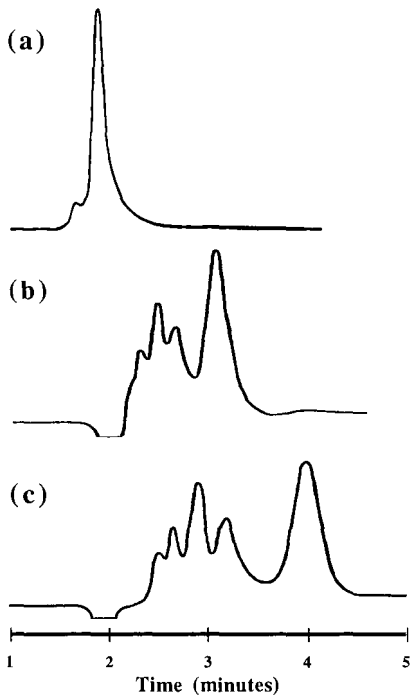


Fig. 11. Chromatograms of seven anions (F^-, Cl^-, NO_3^-, NO_2^-, HPO_4^{2-}, I^- and SO_4^{2-}, concentrations similar to those in Fig. 10; all as K^+ salts) using three eluents of the type 10 m*M* MOH in methanol–water (5:95, v/v) at 1.0 ml/min. (a) M = Li^+; (b) M = Na^+; (c) M = K^+. Column, ACT loaded with D2.2.2. The first four peaks in (c) correspond to F^-, Cl^-, NO_2^- and NO_3^-, with all other species eluting together in the last peak.

methanol–water (5:95, v/v) at a flow-rate of 1.0 ml/min. Hardly any separation is observed with the Li^+ eluent, consistent with the very weak binding of Li^+ to D2.2.2[1]. The small differences between the chromatograms obtained with Na^+ and K^+ and K^+ eluents cannot be due to differences in the relative percentages of bound D2.2.2, as virtually all of the cryptand molecules would be expected to be occupied under these circumstances with both cations. The relative complex size may be important: the Na^+ complex should be more compact. These observations are the subject of further investigations.

TABLE II

CAPACITY AND RESPONSE FACTORS FOR NO_3^- ELUTION USING K^+ ELUENT WITH VARIOUS SAMPLE CATIONS

Sample cation	*k'*	*Response factor*
Mg^{2+}	0.51 ± 0.04	1.6 ± 0.1
Rb^+	0.52 ± 0.04	1.5 ± 0.2
Na^+	0.50 ± 0.04	1.5 ± 0.1
Cs^+	0.49 ± 0.08	1.3 ± 0.4
K^+	0.54 ± 0.02	1.53 ± 0.07

Effect of sample cation

An experiment was performed to determine whether the sample cation affects the analysis of anions in these systems, as it does in other macrocycle-based systems where pure water is used as the eluent. An eluent containing 1.0 m*M* $KHCO_3$–6.05 m*M* H_3BO_3 was used with the ODS column at a flow-rate of 0.6 ml/min. Cations were chosen among those which do not have a greater affinity for D2.2.2 than does K^+. The results for NO_3^- analysis are shown in Table II. Replicate chromatograms were obtained in series to eliminate possible systematic variations due to changes in the chromatographic system conditions with time. The NO_3^- capacity factors are independent of the sample cation within experimental error, as is the detector response. This result is not unexpected, as sample cations with a D2.2.2 affinity less than that of K^+ should pass through the column with the void volume, leaving retained sample anions behind. This feature makes the present system much more amenable to application than aqueous eluent systems where cation–anion pairs elute in all their permutations.

It is possible that sample cations that have greater affinity for D2.2.2 than the eluent cation will cause variations in k'. Such cations will definitely compete with the eluent cation for D2.2.2 sites. However, the overwhelming concentration effect of the eluent cation may be employed to advantage. Further, the eluent cation may best be chosen from among those cations which bind the macrocycle most strongly. This topic is undergoing further investigation.

Column capacity

Because of its high affinity for the cryptand, Sr^{2+} is an appropriate species with which to measure the capacity of the D2.2.2-loaded column. The macrocycle-coated ACT column was equilibrated with Sr^{2+} by pumping 10 m*M* $Sr(OH)_2$ eluent for 2 h. The column was rinsed for 30 min with water, then stripped of residual Sr^{2+} with a 30-min rinse of 0.1 *M* hydrochloric acid, all at 1.0 ml/min. Inductively coupled plasma spectroscopic analysis of the acid stripping solution showed that there were 79 ± 4 μmol Sr^{2+} on the column, giving an overall capacity of approximately 30 μmol/ml of cryptand, given a total stationary phase volume of 2.5 ml. This value represents a lower limit, in that a small amount of Sr^{2+} may be lost in the rinsing step. The assumption that the loss on rinsing is small is based on kinetic data for the rate of the $(Sr2.2.2)^{2+}$ dissociation reaction[1]. When the same eperiment was performed with K^+, which forms a much more labile complex, 37 ± 7 μmol of K^+ were recovered. The capacity determined by the Sr^{2+} experiment compares favorably with that obtained by Cassidy and Elchuk[23] using dynamically coated ion exchangers.

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DETERMINATION OF ORGANIC AND INORGANIC ACIDS IN PRECIPITATION SAMPLES

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SUMMARY

This paper describes an ion chromatographic method for simultanous analysis of major organic and inorganic acids (formic, acetic, nitric, sulfuric, hydrochloric and hydrofluoric) in precipitation samples. The method can also determine several other acids commonly cited in literature on precipitation-related samples; namely, propionic, glycolic, butyric, methanesulfonic, nitrous, hydroxymethylsulfonic, oxalic, phosphoric and citric acids. The method can be adapted for routine analysis of these acids, which are resolved in less than 10 min. Three types of natural waters were used and 60 recoveries made giving a percent recovery range of 100 ± 10%.

INTRODUCTION

While inorganic acids (as Cl^-, SO_4^{2-}, NO_3^-) continue to be very important constituents in acid rain studies, organic acids are becoming more and more a prerequisite for proper accounting of atmospheric chemistry processes and precipitation ionic balances[1–5]. In fact, Grosjean *et al.*[6] very recently showed that in Los Angeles smogs, acetic and formic acids (the major components of organic acids) are present in greater quantity than nitric and hydrochloric acid combined. Likewise, Solomon *et al.*[7] reported high gas phase formic acid concentration compared to HNO_3, HCl and HF in Los Angeles area. Keene and Galloway[1] reported the two organic acids contributed 16% of free acidity in central Virginia precipitation, whereas Backman and Peden[3] reported 8% total acidity in central Illinois rainwater. The ionic balances of Ferek *et al.*[5], which did not include organic acids, consistently showed anion deficit with an 8% average. Thus organic acids particularly formic and acetic are important constituents to study.

Fluoride is an interesting element to study also because F^- in rain samples partially originate from the notorious ozone-depleting chlorofluorocarbons (CFCs) and it is required for accurate ionic balance calculations. Since atomic F has a much smaller ozone depletion potential than Cl following decomposition reactions of CFCs in the stratosphere, F does not readily enter in the ozone-depletion mechanism and is therefore more available to form HF. The latter finds its way back to earth surface, because once formed HF is a permanent "reservoir" molecule, remaining chemically unchanged until it diffuses into the troposphere and is removed by rain[8,9].

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Fluoride is commonly analysed by ion-selective electrode or by ion chromatography (using the Dionex workhorse column HPIC-AS4A with CO_3^{2-}/HCO_3^- eluent), that relies on matrix matching technique. But the technique, though adequate for eliminating the water dip interference, is an additional step of a method and it lessens the potential to simultanously analyse the anions and certain monovalent cations[10]. Furthermore, these conditions will result in F^-/acetate/formate co-elution.

While F^-, acetate and formate can be simultanously determined using HPIC-AS4-B_4O_7 conditions[11], most peaks of other anions of interest are unacceptably long and broad. Similarly, the three ions can be acceptably resolved[12] using HPIC-AS6-B_4O_7, but at the expense of the other important inorganic ions. The two organic acids and others can also be adequately determined by ion-exclusion chromatography[11,13], but again the major inorganic ions cannot be obtained in the same run.

Rocklin *et al.*[14] published a gradient elution chromatogram of 36 organic and inorganic parameters which were resolved in 30 min. A good deal of the parameters, however, are not of interest to current acid rain studies. To a routine laboratory, which supports precipitation projects requesting for major ions (acetate, formate, F^-, Cl^-, NO_3^-, SO_4^{2-}), both time and analyte constraints (<30 min, <36 parameters) must be tailored to increase cost-effectiveness.

It would be desirable for a routine laboratory to have a method capable of simultanously analysing the major organic and inorganic anions within a reasonably short time. This paper describes such a method, which in 10 min resolves the major as well as other acids commonly reported in the precipitation-related samples. Table I lists the organic acids cited in several recent publications and studied here.

EXPERIMENTAL

Chemicals

Milli-Q water (18 MΩ) was used. High-purity chemicals used were 50% NaOH, LiOH, NH_4OH, H_2SO_4, butyric acid, sodium and potassium salts: carbonate,

TABLE I

ORGANIC ACIDS CITED IN SOME RECENT LITERATURE FOR PRECIPITATION-RELATED SAMPLES

Acids	*Formula*	*Refs.*
Formic	$HCOOH$	1–4, 6, 7, 15–18
Acetic	CH_3COOH	1–4, 6, 15–18
Oxalic	$HOOCCOOH$	4, 6
Gylcolic	$HOCH_2COOH$	16
Propionic	CH_3CH_2COOH	16, 17
Lactic	$CH_3CHOHCOOH$	16
Butyric	$CH_3CH_2CH_2COOH$	16, 18
Succinic[a]	$HOOCCH_2CH_2COOH$	19
Citric	$HOOC(CH_2COOH)_2COOH$	3
Methanesulfonic	CH_3SO_3H	15
Hydroxymethylsulfonic	$HOCH_2SO_3H$	16

[a] Not cited in these samples, but is an important component of natural organic matter (see discussion).

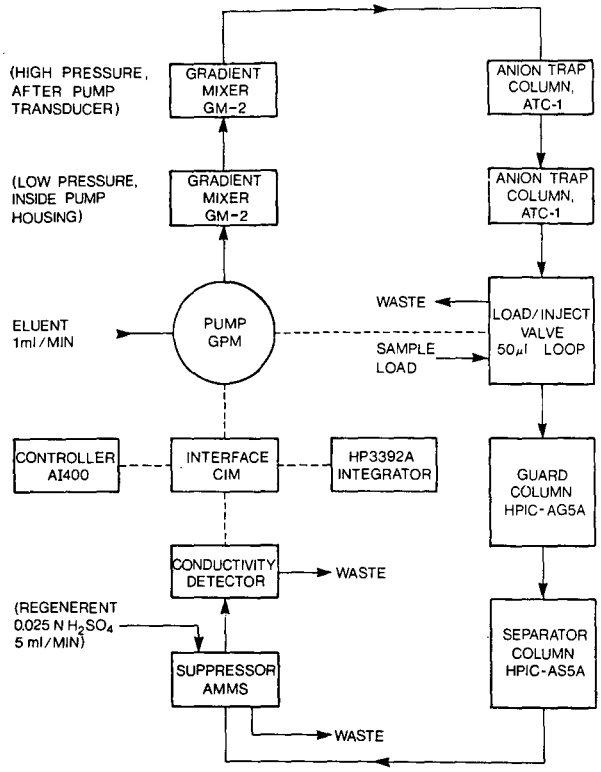

Fig. 1. System schematic (AMMS = anion micromembrane suppressor).

bicarbonate, fluoride, acetate, propionate, formate, methanesulfonate, chloride, nitrite, succinate, sulfate, oxalate, phosphate, nitrate, citrate, borate and hydroxymethylsulfonate. A stock solution of 1000 ppm (mg/l) of each acid was prepared, the organic acids being preserved with 0.2% HPLC-grade chloroform.

Equipment and operation conditions

The system comprises Dionex's gradient pump, columns, conductivity detector CDM-1, autoion 400 and is schematized in Fig. 1. The two ATC columns were used to minimize background contaminants. An eluent profile and main steps of a run are shown in Table II. To avoid CO_2 pickup by eluents, at the beginning of each week, the eluents were carefully prepared using helium-degassed water and 50% NaOH, which was pipetted from the middle of the bottle, and the He atmosphere constantly applied over the eluents until the end of the week. The eluent flow-rate is 1.0 ml/min, and the regenerent (0.025 *N* H_2SO_4) flow-rate about 5 ml/min throughout.

RESULTS AND DISCUSSION

Common acids in precipitation samples

Based on some recent publications, the common acids comprise the major ones (SO_4^{2-}, NO_3^-, F^-, Cl^-, formate and acetate) and propionate, lactate, glycolate,

butyrate, methanesulfonate, hydroxymethylsulfonate, nitrite, oxalate, phosphate, and citrate. Although succinate is not commonly reported (Table I), the acid is a controversial one vis-a-vis its inherent part of natural organic matters and has recently been identified to be one of the important acids in the make-up of humic and fulvic acids[17]; we therefore chose to include it in this study.

Optimization

Using a standard containing most of the above acids, several potential eluents including LiOH, NaOH, $Na_2B_4O_7$, NH_4OH, Na_2CO_3, $NaHCO_3$ and some combinations of them were tested on three different separation columns, HPIC-AS4A, -AS5A and -AS6A. The NaOH-AS5A combination was found to be most effective in resolving the mono-, di- and trivalent analytes of interest.

Experiments showed that 0.75 m*M* NaOH eluent as used by Rocklin *et al.* [14] was not always effective in resolving the weakly retained monovalent acids, possibly due to the difficulty in maintaining a CO_2-free eluent or a constant CO_2 content in the eluent, or the slight variability of the AMMS-suppressing capacity, resulting in variable background conductivity, μ_0. After each weekly change of eluent, μ_0 is not always the same, which can result in poor resolution of monovalents; a slight change in eluent concentration was necessary to achieve a better resolution. This can be best realized using a water eluent (E_1) in conjunction with a 10 m*M* NaOH eluent (E_2) to quickly find the effective concentration at the beginning of each week (Table II). Thus, instead of a fixed concentration of NaOH, a small range (0.5–2.5 m*M*) was found suitable to resolve the monovalents, the 80:20 (2 m*M*) and 90:10 (1 m*M*) combinations of E_1–E_2 being more often used. The chromatogram from formate peak to the last peak is negligibly affected by this weak eluent concentration range.

For the more strongly retained analytes, a stronger eluent is needed, which is made up of E_1 (water) and E_3 (200 m*M* NaOH) (Table II). A fixed concentration of NaOH was not always effective in resolving two neighboring peaks with markedly different concentrations, for example a small PO_4^{3-} peak shouldering a large NO_3^- peak, or a small oxalate shouldering a large SO_4^{2-}. As in the weak-eluent case, an effective concentration can be easily found by simply varying E_1 (and subsequently E_3). A range of 88–96 m*M* NaOH was found effective in achieving a better resolution of such peaks (small peaks shouldering large ones), and the concentration most often used was 90 m*M* (E_1–E_3 = 55:45) or 94 m*M* (E_1–E_3 = 53:47).

TABLE II
AN ELUENT PROFILE AND MAIN STEPS OF A RUN

Time (min)	*% E_1 (Milli-Q water)*	*% E_2 (10 mM NaOH)*	*% E_3 (200 mM NaOH)*	*Load/inject (valve)*
0.0	85	15	0	Load
0.1	85	15	0	Inject
1.0	53	0	47	Inject
3.0	53	0	47	Load
4.5	53	0	47	Load
4.6	85	15	0	Load
15.0	85	15	0	Load

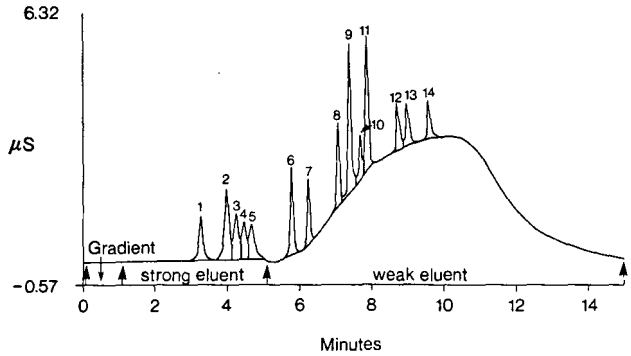

Fig. 2. Chromatogram showing each eluent spatial interval. Peaks: 1 = F^-, 0.1 ppm; 2 = acetate, 1.0 ppm; 3 = propionate, 1.0 ppm; 4 = glycolate, 0.5 ppm; 5 = butyrate, 1.0 ppm; 6 = formate, 0.5 ppm; 7 = methanesulfonate, 0.5 ppm; 8 = Cl^-, 0.2 ppm; 9 = NO_2^-, 0.2 ppm; 10 = succinate, 0.5 ppm; 11 = SO_4^{2-}, 0.5 ppm; 12 = phosphate, 0.2 ppm; 13 = NO_3^-, 0.08 ppm; 14 = citrate, 0.5 ppm.

Basically then, it is a two-eluent system, a weak and a strong one as shown in Fig. 2. The small gradient step (0.1–1 min) helps to smooth out the transition. (A similar two-eluent system was previously used for cations[10]). To maintain optimum operating conditions, a daily system cleaning with 200 m*M* NaOH for 10 min was used.

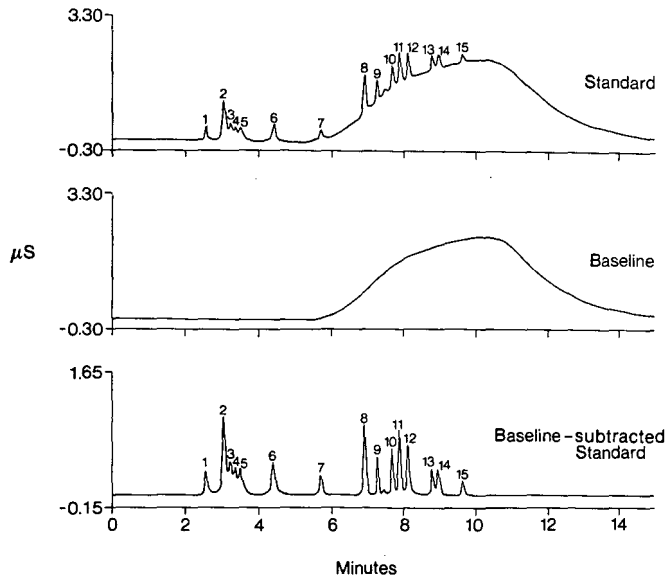

Fig. 3. Baseline-subtracted chromatogram of a standard. Peaks: 1 = F^-, 0.025 ppm;; 2 = acetate, 0.25 ppm; 3 = propionate, 0.25 ppm; 4 = glycolate, 0.125 ppm; 5 = butyrate, 0.25 ppm; 6 = formate, 0.125 ppm; 7 = methanesulfonate, 0.125 ppm; 8 = Cl^-, 0.05 ppm; 9 = nitrite, 0.025 ppm; 10 = succinate, 0.25 ppm; 11 = sulfate, 0.125 ppm; 12 = oxalate, 0.125 ppm; 13 = phosphate, 0.05 ppm; 14 = NO_3^-, 0.018 ppm; 15 = citrate, 0.125 ppm.

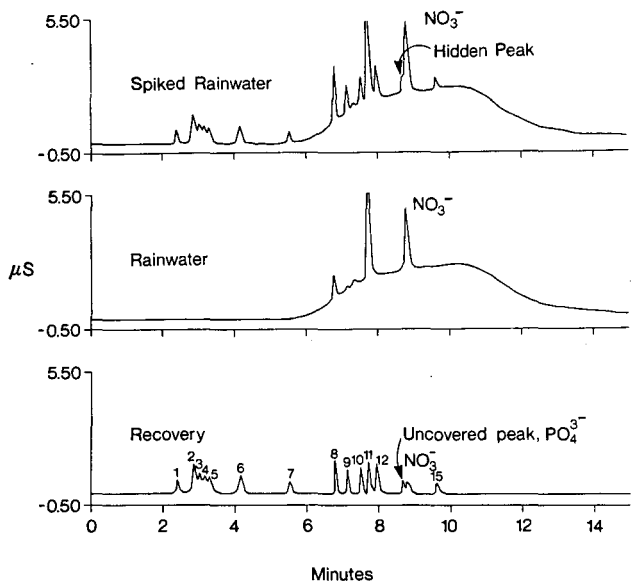

Fig. 4. Uncovering of hidden peak by background-subtraction technique. Peaks as in Fig. 3.

Baseline

Although the actual baseline is shaped as in Fig. 2, the quantitation can be carried out in the usual manner. However, the baseline-subtraction technique can be useful in checking the proper behavior of baseline (Fig. 3) or in recovery studies to uncover or sharpen hidden peaks (Fig. 4), hence facilitating identification and quantitation.

The use of mannitol–boric acid combination[13] to flatten out the baseline was tested and found to affect three things negatively: the baseline, the separator retaining capacity, and possibly the suppressor suppressing capacity. The baseline rise due to the strong eluent kept increasing to ≈ 10 μS instead of ≈ 2.5 μS as seen in Figs. 2–4. To lower the baseline back down, the two ATC columns had to be cleaned with 1 *M* NaOH for 2 h along with the whole-system cleaning. The separator retaining capacity seemed to have slightly decreased as evidenced by the early F^- elution (Figs. 3 and 4) observed after, compared to the late F^- elution (Fig. 2) observed before the mannitol–boric acid use. In these three figures the background conductivity were practically the same, ≈ 2.6 μS. Soon after, the AMMS was plugged and had to be cleaned thoroughly with NaOH, H_2SO_4, and 5% acetonitrile. The suppressor suppressing capacity slightly decreased as μ_0 increased from 2.6 to 3.6 μS, which subsequently required a more dilute weak eluent for satisfactory resolution of monovalents. The mannitol–boric acid use was abandoned.

Interferences

Of the seventeen potentially present acids, lactate coelutes with acetate, and hydroxymethylsulfonate with succinate. Fortunately, succinate is not a commonly present acid as indicated earlier, whereas lactate is found infrequently and at very low

concentration compared to acetate[20]. In general the concentration interference can occur when two analytes with close retention times have markedly different concentration levels. Nitrate and phosphate are an example as NO_3^- is often much more concentrated than PO_4^{3-}. But as explained above resolution can be achieved by using an effective E_1–E_3 ratio-or background-subtracting technique.

Sensitivity

Sensitivity has been defined and redefined by many authors as, for example: the concentration giving a signal-to-noise ratio of 2 (ref. 21); the minimum detectable concentration[22]; the response (or signal change) per unit concentration[23,24]; the ability to discern the difference between very small amounts of a substance[25]; or a measure of the effectiveness of a detector to respond to compounds entering it[26]. Based on many of these definitions, the method sensitivity is depicted here as the response in function of concentration (Fig. 5). For each acid at three to four low concentration levels of interest a line was manually drawn and the regression parameters were calculated and shown in the legend. If sensitivity is taken as the response per unit concentration, it may be equated to the slope of each line, and the sensitivities for the various acids can be easily compared as shown in Table III and Fig. 5. It is seen that the inorganic responses, particularly of the monovalent ions, are greater (more sensitive) than the organic ones, except oxalate. The response of the latter ions is close to that of sulfate and phosphate.

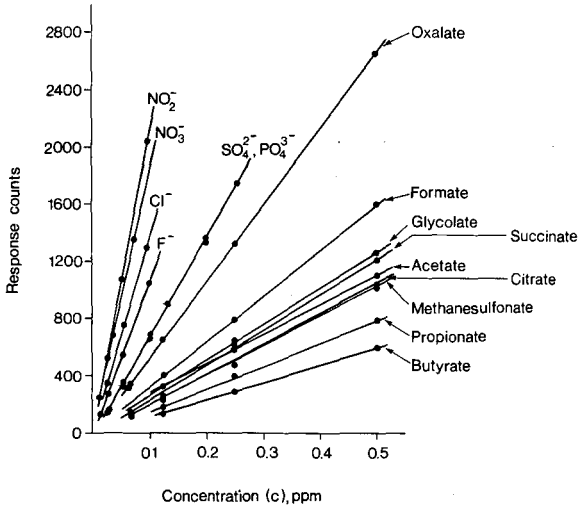

Fig. 5. Sensitivity of the fifteen acids studied. Regression parameters of equations $R = a + bc$ (r = correlation coefficient) for the acids: NO_2^- = 11.1 + 20 516c (r = 1.000); NO_3^- = −9.1 + 18 937c (r = 1.000); Cl^- = 108.7 + 11 790c (r = 0.998); F^- = 8.0 + 10 368c (r = 1.000); SO_4^{2-} = −52.4 + 7 120c (r = 0.999); PO_4^{3-} = −1.0 + 6728c (r = 1.000); oxalate = −13.2 + 5344c (r = 1.000); formate = 0.2 + 3200c (r = 1.000); glycolate = −10.3 + 2550c (r = 1.000); succinate = −23.5 + 2470c (r = 1.000); acetate = 94.0 + 2007c (r = 0.999); citrate = −19.7 + 2117c (r = 0.999); methanesulfonate = −12.3 + 2058c (r = 1.000); propionate = −11.0 + 1590c (r = 0.999); butyrate = −10.0 + 1200c (r = 1.000).

TABLE III
METHOD SENSITIVITIES FOR THE VARIOUS ACIDS (RESPONSE COUNTS/ppm)

Acid	*Sensitivity*	*Acid*	*Sensitivity*
Nitrite	20 516	Glycolate	2550
Nitrate	18 937	Succinate	2470
Chloride	11 790	Acetate	2007
Fluoride	10 368	Citrate	2117
Sulfate	7120	Methanesulfonate	2058
Phosphate	6728	Propionate	1590
Oxalate	5344	Butyrate	1200
Formate	3200		

Performance characteristics

Three types of waters were studied: a rain sample from Sibley collected by the surveillance and monitoring group, a Eulerian quality control sample (EU-ANI-1, a composite rain sample), and a rain–snow mixture collected from Burlington, which was immediately preserved with 0.2% $CHCl_3$. All samples were filtered through a 0.45-μm membrane filter. The first two samples were originally unpreserved as dictated by their protocol, but were preserved when used in recovery studies. The recovery data are presented in Tables IV–VI, showing good precision (small standard deviation of five replicate analyses) and a range of 100 $\pm$ 10% recoveries.

Table VII shows the reproducibility of retention times at two concentration levels of interest. The retenton times were obtained from spiked Milli-Q water and

TABLE IV
RECOVERY DATA FOR SIBLEY RAIN WATER, IN ppm (mg/l) AND %

Acid	*Sibley water (mg/l $\pm$ S.D., n=5)*	*Spike level 1*		*Spike level 2*	
		Recovery		*Recovery*	
		mg/l $\pm$ S.D. (n=5)	*%*	*mg/l $\pm$ S.D. (n=5)*	*%*
Fluoride	–	0.025 $\pm$ 0.001	100	0.106 $\pm$ 0.003	106
Acetate	–	0.246 $\pm$ 0.013	98	1.033 $\pm$ 0.020	103
Propionate	–	0.241 $\pm$ 0.004	96	1.056 $\pm$ 0.031	106
Glycolate	–	0.122 $\pm$ 0.003	98	0.490 $\pm$ 0.013	98
Butyrate	–	0.240 $\pm$ 0.005	96	1.027 $\pm$ 0.039	103
Formate	–	0.124 $\pm$ 0.009	99	0.470 $\pm$ 0.060	94
Methanesulfonate	–	0.130 $\pm$ 0.005	104	0.538 $\pm$ 0.021	108
Chloride	0.090 $\pm$ 0.003	0.147 $\pm$ 0.009	106	0.294 $\pm$ 0.008	105
Nitrite	0.005[a]	0.031 $\pm$ 0.004	104	0.111 $\pm$ 0.004	106
Succinate	–	0.259 $\pm$ 0.011	104	1.045 $\pm$ 0.030	104
Sulfate	1.140 $\pm$ 0.039	1.246 $\pm$ 0.032	101	1.504 $\pm$ 0.025	99
Oxalate	–	0.126 $\pm$ 0.001	101	0.541 $\pm$ 0.013	108
Phosphate, PO_4–P	–	0.053 $\pm$ 0.002	106	0.209 $\pm$ 0.005	104
Nitrate, NO_3–N	0.155 $\pm$ 0.011	0.174 $\pm$ 0.001	103	0.211 $\pm$ 0.004	100
Citrate	–	0.125 $\pm$ 0.004	100	0.531 $\pm$ 0.015	106

[a] Estimated from small peaks.

TABLE V
RECOVERY DATA FOR A EULERIAN QUALITY CONTROL SAMPLE, EU-ANI-1, IN ppm (mg/l) AND %

Acid	*EU-ANI-1*		*Spiked EU-ANI-1*	
	Found (mg/l ± S.D., n=5)	*Design value (mg/l)*	*Recovery*	
			(mg/l ± S.D., n=5)	*%*
Fluoride	–	–	0.055 ± 0.004	110
Acetate	–	–	0.481 ± 0.052	96
Propionate	–	–	0.529 ± 0.053	106
Glycolate	–	–	0.258 ± 0.025	103
Butyrate	–	–	0.511 ± 0.035	102
Formate	–	–	0.234 ± 0.019	94
Methanesulfonate	–	–	0.252 ± 0.009	101
Chloride	0.025 ± 0.001	0.02	0.131 ± 0.008	105
Nitrite	–	–	0.052 ± 0.001	104
Succinate	–	–	0.992 ± 0.021	99
Sulfate	0.063 ± 0.012	0.056	0.311 ± 0.006	99
Oxalate	–	–	0.250 ± 0.006	100
Phosphate, PO_4–P	–	–	0.099 ± 0.003	99
Nitrate, NO_3–N	0.029 ± 0.001	0.03	0.060 ± 0.001	95
Citrate	–	–	0.250 ± 0.005	100

TABLE VI
RECOVERY DATA FOR A BURLINGTON RAIN-SNOW SAMPLE, IN ppm (mg/l) AND %

Acid	*Burlington rain–snow, found (mg/l ± S.D., n=5)*	*Spiked Burlington rain–snow*		
		Expected (mg/l)	*Recovered*	
			mg/l ± S.D., n=5	*%*
Fluoride	0.064 ± 0.019	0.158	0.157 ± 0.040	99
Acetate	0.856 ± 0.006	1.770	1.691 ± 0.011	96
Propionate	–	1.000	1.055 ± 0.076	106
Glycolate	0.529 ± 0.006	0.976	0.944 ± 0.048	97
Butyrate	–	1.000	1.037 ± 0.077	104
Formate	4.716 ± 0.336	4.705	4.871 ± 0.133	104
Methanesulfonate	–	0.500	0.512 ± 0.106	102
Chloride	0.910 ± 0.023	1.019	1.043 ± 0.045	102
Nitrite	0.013 ± 0.003	0.112	0.104 ± 0.008	93
Succinate	0.227 ± 0.024	1.204	1.263 ± 0.035	105
Sulfate	8.302 ± 0.066	7.971	8.267 ± 0.048	104
Oxalate	0.280 ± 0.010	0.752	0.724 ± 0.025	96
Phosphate, PO_4–P	0.333 ± 0.016	0.500	0.533 ± 0.040	107
Nitrate, NO_3–N	1.249 ± 0.031	1.196	1.229 ± 0.032	103
Citrate	0.060 ± 0.003	0.554	0.511 ± 0.020	92

TABLE VII
REPRODUCIBILITY OF RETENTION TIMES

Acid	*Concentration level 1*		*Concentration level 2*	
	Spike 1 (ppm)	*Average retention time ± S.D. (min)*[a]	*Spike 2 (ppm)*	*Average retention time ± S.D. (min)*[b]
Fluoride	0.10	2.51 ± 0.02	0.025	2.51 ± 0.03
Acetate	1.00	2.99 ± 0.03	0.25	2.99 ± 0.03
Propionate	1.00	3.16 ± 0.03	0.25	3.16 ± 0.03
Glycolate	0.50	3.29 ± 0.03	0.125	3.29 ± 0.04
Butyrate	1.00	3.43 ± 0.03	0.25	3.43 ± 0.03
Formate	0.50	4.32 ± 0.04	0.125	4.32 ± 0.04
Methanesulfonate	0.50	5.67 ± 0.05	0.125	5.69 ± 0.05
Chloride	0.20	6.90 ± 0.04	0.05	6.92 ± 0.03
Nitrite	0.10	7.25 ± 0.03	0.025	7.27 ± 0.03
Succinate	1.00	7.63 ± 0.03	0.25	7.64 ± 0.03
Sulfate	0.50	7.81 ± 0.03	0.125	7.82 ± 0.03
Oxalate	0.50	8.04 ± 0.03	0.125	8.06 ± 0.03
Phosphate	0.20	8.64 ± 0.05	0.05	8.65 ± 0.05
Nitrate	0.072	8.85 ± 0.05	0.018	8.87 ± 0.06
Citrate	0.50	9.37 ± 0.04	0.125	9.41 ± 0.08

[a] Average of 15 retention times (9 of spiked Milli-Q water and 6 of spiked natural water), over a 15-day perod.

[b] Average of 12 retention times (6 of spiked Milli-Q water and 6 of spiked natural water), over a 15-day period.

spiked natural water samples over a 15-day period. As can be seen, the precision for all analytes is quite good (very small standard deviation) and the calculations indicate that the largest coefficient of variation is only 1.1%.

As with sensitivity, the detection limit has been abundantly written about in the literature. For example, it is often defined in terms of signal-to-noise ratio[22], standard deviation of short-term noise[24], or standard deviation of several replicate analyses of a sample containing a low level of analyte, usually 1–10 times the concentration of the estimated detection limit[27,28]. The detection limits listed in Table VIII were obtained following the last type of definition; they were equated to twice the standard deviation

TABLE VIII
DETECTION LIMIT OF THE ACIDS, IN ppm (mg/l)

Acid	*Detection limit (ppm)*	*Acid*	*Detection limit (ppm)*
Fluoride	0.010	Nitrite	0.011
Acetate	0.059	Succinate	0.080
Propionate	0.057	Sulfate	0.040
Glycolate	0.040	Oxalate	0.067
Butyrate	0.074	Phosphate	0.028
Formate	0.040	Nitrate	0.012
Methanesulfonate	0.053	Citrate	0.071
Chloride	0.022		

of eight replicate analyses of a sample containing analytes with concentration equal to eight times the estimated detection limit. The latter was estimated as the concentration giving a signal equal to 2–3 times that of the noise. It is expected that these detection limits (Table VIII) would be lower if a concentrator or a better detector featuring the temperature-controlled multielectrode flow cell are used. It was unnecessary to study the upper limits as the precipitation samples do not contain very high concentrations of these acids to surpass the column capacity.

Preservation

Tables IV–VI indicate two things: (1) the two unpreserved samples do not contain any organic acids as opposed to the preserved one (Table VI), which seems to confirm the need to preserve samples[1,3] if organics are to be accountable; and (2) F^- analyses by AS4- or AS4A-CO_3^{2-}/HCO_3^- on the two unpreserved samples (or possibly on any unpreserved sample after 62 days storage in a 4°C room[1]) would be free from acetate–formate interference.

When $CHCl_3$ is used, the analysis for Cl^- may be affected[3]. Furthermore, although $CHCl_3$ prolongs the holding time or may prevent some preferential conversion between organic acids[29], it may produce some decomposition products; for example, an extra peak between nitrite (peak 9) and succinate (peak 10) appeared in the preserved standard (Fig. 3), but not in the preserved Milli-Q water.

While it is best to analyse immediately after sample collection, such practice is hardly a practical reality. Some form of preservation is required. A detailed study using $CHCl_3$ or some other biocides seems necessary.

CONCLUSION

A method has been developed for routine analysis of major inorganic and organic acids as well as several other acids commonly cited in the literature on precipitation samples.

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Note

Effect of eluent impurities and sample matrix on quantitative analysis by ion chromatography

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Conventional liquid chromatography is an impulse method[1] in terms of the mode of sample injection, *i.e.*, eluent is allowed to flow continuously, sample is injected into the flow as a pulse and a chromatogram is obtained. Conversely, when sample is allowed to flow continuously as in gas chromatography[1], and gas without a sample constituent is injected into the flow as a pulse, negative chromatography, *i.e.*, "vacancy chromatography"[2] is obtained. If the unknown sample is allowed to flow continuously and a reference sample is injected as a pulse, then if there are some differences in the constituent or its concentration between those two samples, positive and/or negative peaks can be observed. If there is no difference between the samples, no peak will appear on the chromatogram. This method is effective in recognizing clearly the difference between a standard and an unknown sample in which the latter contains constituents very close to those in standard sample. Gel permeation chromatography[2,3] for example, allows the detection of differences as small as 1% in the molecular weights of the components and it is useful for process control.

In ion chromatography, Okada and Kuwamoto[4] added various ions to the eluent in the process of examining the formation mechanism of a "dip peak", and concluded that "absent peaks" of those ions are one of the causes. However they suggested that the vacancy chromatogram, which is a mirror image of the normal chromatogram, is difficult to obtain because of the slow "transfer rate" of divalent ions such as sulphate.

It has been found that when an eluent mixed with sample is allowed to flow in a column continuously, and solution that contains no sample constituent is injected through the sample injection port as a pulse, a "vacancy chromatogram" can be obtained[5,6]. Also, the peak height or area depends closely on the amount and type of the solution to be injected. This means, in conventional ion chromatography, that if the component to be measured is present in the eluent as an impurity, a negative error corresponding to the vacant peak would appear, and its size would depend on the quality of the sample, *i.e.*, the concentration of the matrix in the sample.

In this paper, the effect of impurities in the eluent and of the matrix concentration in the sample on the accuracy of quantitative analysis is discussed.

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EXPERIMENTAL

Apparatus

A conventional non-suppressed type of ion chromatograph consisting of a Hitachi Model L-6200 pump, a 2720IC packed column (cation-exchange resin, particle size 10 μm, capacity 0.01 mequiv./ml; 50 mm × 4 mm I.D.), an L-3700 conductivity detector, a 655A-52 column oven and a Rheodyne Model 7125 sample injector was used. The conductivity cell, with the column, was placed in the column oven. The temperature was set at 40°C.

Reagents

In order to separate alkaline earth metal ions, 0.5 m*M* tartaric acid–0.5 m*M* ethylenediamine solution (pH 4.8) with and without 1 ppm each of the alkaline earth metal ions, and to separate alkali metal ions, 1.6 m*M* nitric acid solution with 2 ppm each of the alkali metal ions, were used as eluents. The other reagents were commercially available guaranteed-reagent grade materials.

RESULTS AND DISCUSSION

Ion chromatography was performed on various standard sample solutions containing 1 ppm each of magnesium, calcium, strontium and barium. Fig. 1 shows chromatograms of the samples prepared by dissolving these alkaline earths in 0.5–20 m*M* tartaric acid–ethylenediamine (matrix) solutions for which the composition ratio was the same as that of the solute in the eluent. In Fig. 1, 0.5 m*M* means that the chromatogram of a sample of 1 ppm alkaline earth metal ions–0.5 m*M* tartaric acid–0.5 m*M* ethylenediamine solution is shown. The peak height of calcium ion decreases considerably with increasing matrix concentration, and at 5 m*M*, it is a negative peak. When the concentration reaches 20 m*M*, the peak becomes very broad and the peaks of magnesium and strontium cannot be distinguished.

The peak areas in these chromatograms are plotted in Fig. 2. It seems that the quantitative value at zero matrix concentration is the correct one and the other values

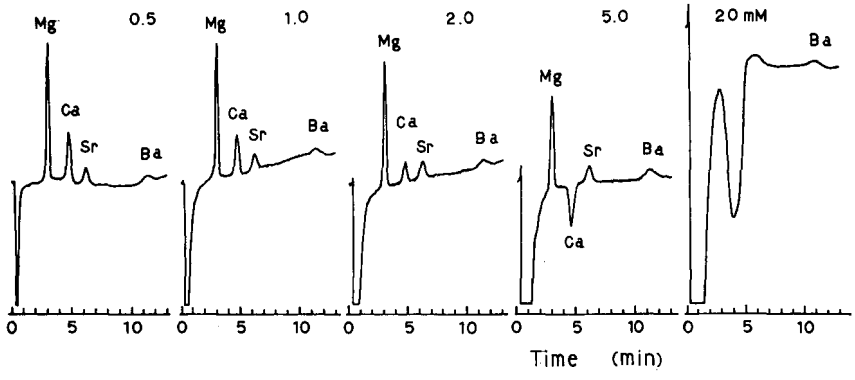

Fig. 1. Chromatograms of standard solutions. Samples, 1 ppm Mg, Ca, Sr, Ba–*x* m*M* tartaric acid–*x* m*M* ethylenediamine, 20 μl; column, Hitachi 2720IC packed column, 50 mm × 4 mm I.D., 40°C; eluent, 0.5 m*M* tartaric acid–0.5 m*M* ethylenediamine; flow-rate, 1.0 ml/min; detector, conductivity.

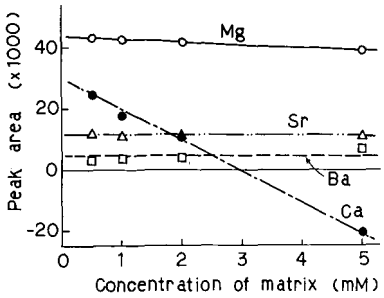

Fig. 2. Effect of sample matrix concentration on peak area for conditions as in Fig. 1.

contain negative errors. It is considered that a negative error that becomes larger with increasing matrix concentration is caused by a vacant peak, based on impurities such as magnesium and calcium in the eluent. The concentration of the impurities was measured using atomic absorption spectrometry. The eluent that remained in the vessel used before the eluent was transferred into the eluent container contained 16 ppb of calcium.

The chromatograms of the standard sample solutions using an eluent that contained 1 ppm each of alkaline earth metal ions were also obtained. The standard sample solutions which were 1 ppm (2 ppm in pure water) solutions of alkaline earth metal ions were injected in 20-μl volumes. Some of the chromatograms obtained are shown in Fig. 3. Only the standard sample dissolved in pure water gives a normal chromatogram, and when the matrix concentration is 0.5 mM, which is the same as the eluent, peaks of the sample constituents do not appear at all, and at higher concentrations negative, vacant peaks appear. This behaviour is shown in Fig. 4. The

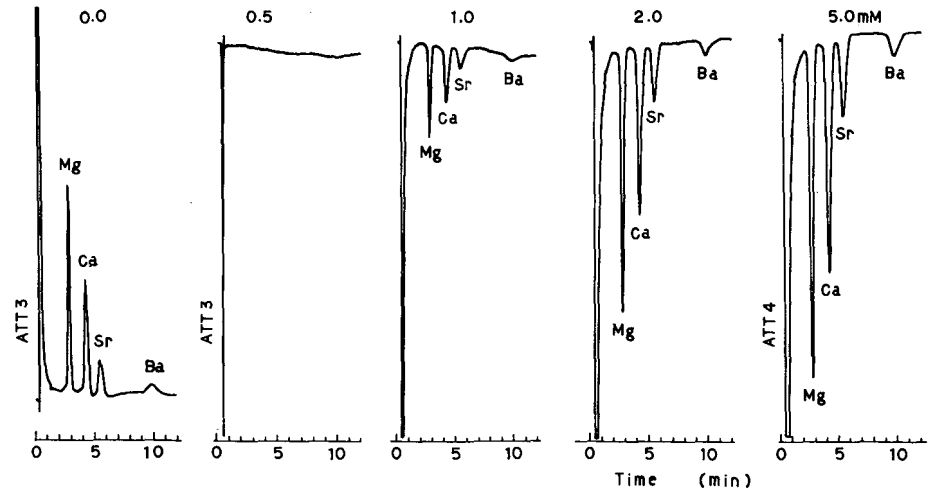

Fig. 3. Sample matrix concentration and chromatographic response profiles. Samples, 2 ppm Mg, Ca, Sr, Ba–water and 1 ppm Mg, Ca, Sr, Ba–x mM tartaric acid–x mM ethylenediamine, 20 μl; eluent, 1 ppm Mg, Ca, Sr, Ba–0.5 mM tartaric acid–0.5 mM ethylenediamine. Other conditions as in Fig. 1.

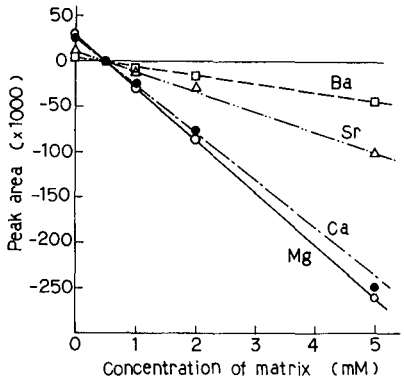

Fig. 4. Effect of sample matrix concentration on peak area for conditions as in Fig. 3.

relationship between matrix concentration and peak area is almost linear, but contamination by calcium is easy and there are large fluctuations of the data.

Samples that contained only matrix and no alkaline earths were injected. The results are shown in Fig. 5, with the vacant peak area plotted against matrix concentration, the same as above. Good linearity is found up to a matrix concentration of 20 m*M*. Typical chromatograms are shown in Fig. 6. When only water was injected, positive peaks corresponding to 0.02 ppm of magnesium and 0.1 ppm of calcium are detected. The cause is thought to be additional adsorption of alkaline earth metal ions in the eluent on the column owing to the change in equilibrium on dilution of the eluent.

Because the slopes of the lines in Figs. 4 and 5 are almost the same, and the relationship between concentration of impurity in the eluent and vacant peak area of the impurity is linear[5], the slope should indicate the amount of the impurity present in the eluent. Incidentally, the concentrations of magnesium and calcium in the eluent, as obtained from the slopes of the lines in Fig. 2, are 0.016 and 0.19 ppm, respectively. The

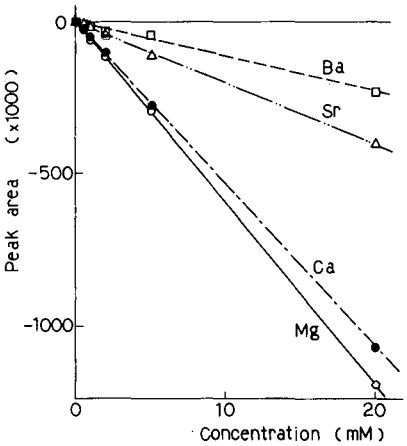

Fig. 5. Effect of sample matrix concentration on peak area for water and x m*M* tartaric acid–x m*M* ethylenediamine (no alkaline earth metal ions) as samples and other conditions as in Fig. 3.

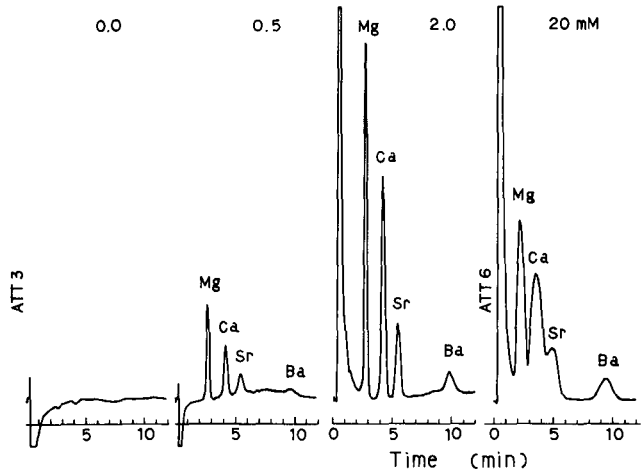

Fig. 6. Example of vacancy chromatograms. Conditions as in Fig. 5.

calcium value is about ten times higher than the initial one. This suggests that contamination arises from the chromatographic process.

The same phenomena were observed for the separation of alkali metal ions. In this instance 1.6 m*M* nitric acid with 2 ppm each of alkali metal ions was used as the eluent and 2 ppm each of alkali metal ions in hydrochloric acid of different concentrations as the samples. The results are shown in Fig. 7 as peak area *vs.* concentration of hydrochloric acid (sample matrix). Positive peaks were observed when the concentration of hydrochloric acid in the sample was less than 1.6 m*M* and negative (vacant) peaks at concentrations above 1.6 m*M*.

The impulse chromatographic response, R_t, is given by the equation[1]

$$R_t = R_0 + \sum_j \frac{C_j}{\sqrt{2\pi}\sigma_j} \cdot \exp\left[-\frac{(t - t_{R_j})^2}{\sigma_j^2}\right] \quad (1)$$

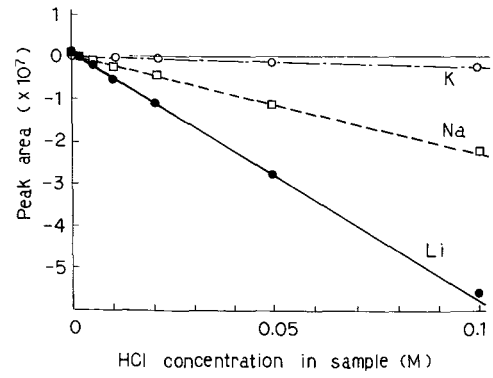

Fig. 7. Effect of hydrochloric acid concentration on vacant peak area. Sample, 2 ppm alkali metal ions–*x* m*M* hydrochloric acid, 20 μl; column, cation-exchange resin of *ca.* 20 μequiv./ml, 50 mm × 4 mm I.D., 40°C; eluent, 1.6 m*M* nitric acid–2 ppm alkali metal ions; flow-rate, 1.5 ml/min; detector, conductivity.

where R_0 is the baseline response (steady-state value), σ is the peak standard deviation, t is time and t_{R_j} is the retention time of component j. The concentration can be expressed as

$$C_j = K(C_{js} - \alpha C_{je}) \tag{2}$$

where K is a constant, C_{js} is the concentration of component j in the sample solution and C_{je} that in the eluent. When the matrix of the sample is the same as that of the eluent, $\alpha = 1$. Fig. 4 shows that if the sample matrix composition ratio is the same as the eluent but only its concentration is different, α will be equal to C_{e_0}/C_{s_0}, where C_{e_0} is the eluent concentration and C_{s_0} is the matrix concentration in the sample. In the other case, where the sample matrix component is different from the eluent, as shown in Fig. 7, α depends on the affinity of the sample matrix component to the column packing material. When C_j is positive a positive peak, when C_j is zero no peak and when C_j is negative a vacant peak are observed on the chromatogram.

It appears that the negative error might be attributed the fact that the impurities in the eluent enriched on the resin in a separation column are partially eluted at the front owing to the equilibrium between the concentration of the impurities in the resin in the column and that of the constituents in the matrix of the sample. The constituents of the sample were partially readsorbed on the resin in the equilibria between the resin and the eluent. Therefore, the concentration of constituents in the sample decreases and causes large negative errors in quantitative analysis. This negative error can be eliminated by complete removal of the impurities from the eluent and avoiding contamination.

Removal of the sample matrix component is also effective, as reported for the determination of alkali metal ions in hydrochloric acid, where the chloride ion as matrix was removed by using silver carbonate[7].

CONCLUSION

In ion chromatography, when components to be analysed are present in the eluent as impurities, the quantitative analytical results include a small positive error or a large negative error depending on the concentration of the sample matrix. Because the positive error does not exceed the concentration of the impurities in the eluent, the error may sometimes be negligible. The negative error, however, in an analytical system using an ion-exchange resin of very low ion-exchange capacity and a dilute eluent could reach more than 100 times the concentration of the impurity in the eluent. This problem is especially serious when using a resin of extremely low capacity, a low concentration of eluent and a high concentration of sample matrix. The solution is to improve the purification of the eluent. In some instances, it is effective to remove matrix components from the sample. Some instances in which a negative peak appears unexpectedly might be treated by assuming the appearance of this vacant peak.

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CHROM. 21 832

ALUMINA AS STATIONARY PHASE FOR ION CHROMATOGRAPHY AND COLUMN-COUPLING TECHNIQUES

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SUMMARY

Columns packed with alumina were combined with common anion-exchange columns and applied to the ion chromatographic determination of sulphate in brines and biological fluids and for the trace determination of iodide in mineral waters and fruit juice samples. Alumina was found to be a highly selective stationary phase for the preconcentration of sulphate from complex matrices. Further, owing to its selectivity, which is different from that of R_4N^+-type anion-exchange materials, it is well suited for on-line column-coupling techniques. In this way sample clean-up can be minimized, and the sensitivity of the chromatographic system allows determinations of iodide down to the low ppb range.

INTRODUCTION

The chromatographic separation of inorganic anions is carried out mainly with strongly basic anion exchangers of the tetraalkylammonium type (R_4N^+) or by ion interaction chromatography using ordinary C_{18} reversed-phase columns and hydrophobic ion-pairing reagents in the mobile phase. The selectivities of these systems are similar, but for some applications it might be advantageous to have a chromatographic system with different selectivity as an alternative.

Major changes in the elution order relative to R_4N^+-type ion exchangers have been reported for amine-modified silica[1,2] and for alumina[3,4]. Especially alumina might be the stationary phase of choice for sample preconcentration and clean-up and for column-coupling techniques in combination with conventional ion-exchange columns.

In this paper, the use of alumina-filled cartridge columns combined with a polymer-based R_4N^+-type ion-exchange column is described for the determination of trace amounts of sulphate in brine (containing about 20 g/l of sodium chloride) and of sulphate in complex biological matrices such as serum.

The advantages of coupling an alumina column with silica-based R_4N^+-type ion-exchange columns are demonstrated for the determination of iodide in mineral

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water and juice samples. Multi-column analysis is a well established method in high-performance liquid chromatography (HPLC). In ion chromatography, there have been few reports of coupling columns with different stationary phases, such as the combination of ion-exchange chromatography with ion-exclusion chromatography[5,6]. The whole range of benefits of column-switching techniques in ion chromatography seem not yet to have been fully exploited.

EXPERIMENTAL

Instrumentation and reagents

The chromatographic instrumentation consisted of Waters M510 HPLC pumps, Rheodyne 7010 injection valves with a 20-, 50- or 200-μl loop or a precolumn (40 × 4 mm I.D.) filled with a Vydac anion exchanger (30-μm particle size), a Waters M430 conductivity detector and a Waters M481 UV detector. The following separation columns and mobile phases were used: a Beckman XL column (70 × 4.6 mm I.D.) packed with 3-μm Ultraspher-ODS and 0.01 *M* octylamine (adjusted to pH 6.5 with phosphoric acid)–methanol (37:4, v/v) as the mobile phase; a Hamilton column (250 × 4.1 mm I.D.) packed with 10-μm PRP-X100 and 2m*M* potassium hydrogenphthalate as the mobile phase; a Vydac 302-IC column (250 × 4.6 mm I.D.) and 10 g/l of methanesulphonic acid (adjusted to pH 4 with sodium hydroxide) as the mobile phase; and a Stagroma column (125 × 4.6 mm I.D.) packed with 5-μm Spherisorb alumina and 1 g/l of methanesulphonic acid (adjusted to pH 4 with sodium hydroxide) as the mobile phase.

Samples for sulphate determination were preconcentrated or cleaned up with Waters Sep-Pak cartridges filled with neutral alumina. Before use these cartridges were washed with 1 ml of 1 *M* ammonia, 2 ml of water and 1 ml of 0.7% hydrochloric acid.

Determination of sulphate in brine samples

About 600 ml of the sample were passed through a glass column (150 × 15 mm I.D.) filled with a Dowex 50W-X-8 (H^+) cation exchanger (50–100 mesh). A 500-ml volume of the eluate was passed through a Sep-Pak alumina cartridge, followed by 1 ml of 0.7% hydrochloric acid and 1 ml of water, then sulphate was eluted with 4 ml of 1 *M* ammonia solution and 1 ml water. The eluate was passed through a cartridge (15 × 10 mm I.D.) filled with Dowex 50W-X-8 (H^+) and 20 μl were injected on to the Hamilton PRP-X100 column. Detection was effected by conductivity measurement.

Determination of sulphate in serum

Serum (5 ml) was diluted to about 50 ml with 0.7% hydrochloric acid and passed through a Sep-Pak alumina cartridge. The subsequent treatment was the same as for brine samples.

Determination of iodide in mineral water and fruit-juice samples

For screening purposes, 50 μl of mineral water were directly injected on to the RP-18 column in combination with UV detection at 227 nm. Samples that possibly contained more than 20 ppb of iodide were analysed a second time by injecting 200 μl on to the alumina column with UV detection at 227 nm. With complex matrices the

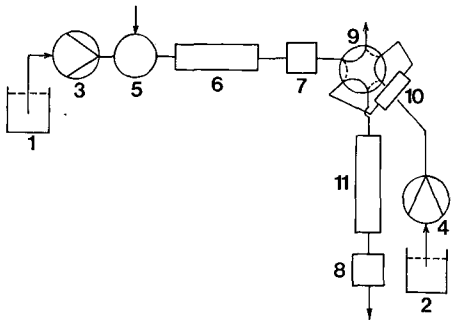

Fig. 1. Experimental set-up for column switching. 1, 2 = Reservoirs for mobile phases; 3, 4 = HPLC pumps; 5 = injection valve with 200-μl loop; 6 = separation column packed with alumina; 7, 8 = UV detectors; 9 = column-switching valve; 10 = precolumn packed with 30-μm Vydac anion exchanger; 11 = Vydac 302-IC separation column.

alumina column was coupled with the Vydac column (Fig. 1); the iodide-containing fraction was eluted on to the Vydac precolumn, which was then switched into the flow of the Vydac separation column. UV detection at 227 nm was used.

RESULTS AND DISCUSSION

Brines may be used for balneotherapeutic purposes[7], which makes the determination of all components of such samples necessary. In general, the ion chromatographic determination of trace amounts of sulphate at levels down to less than 1 mg/l in the presence of about 20 g/l of sodium chloride is not possible by direct injection. Dilution of the sample might be the method of choice if the sulphate-to-chloride ratio is not too low, but in our case dilution would prevent the detection of trace concentrations of sulphate. This problem can be circumvented either by elimination of chloride in the injected sample by on-line precipitation on a silver-loaded cation-exchange column coupled with the separation column[8] or by selective preconcentration of sulphate. The latter method was chosen for our investigations.

Fritz *et al.*[9] have already reported the use of alumina-filled columns for the separation of sulphate from other anions before titrimetric determination of sulphate. Under acidic conditions, sulphate is strongly retained, whereas chloride is not. The anion-exchange capacity of alumina is pH dependent and alumina will change from an anion exchanger to a cation exchanger on altering the pH from acidic to basic conditions[3]. Therefore, after the enrichment step sulphate can be eluted with a small volume of ammonia solution. Such an approach is practicable, because nowadays disposable cartridge columns filled with alumina are commercially available. The pH of the brine sample was lowered to an appropriate value by running it through a strongly acidic cation-exchange column. We believed this method to be better than the addition of an suitable acid, because in this way an increase in the ratio of the total concentration of anions to the concentration of sulphate could be avoided; otherwise, the recovery of sulphate might decrease. After preconcentration on alumina and subsequent elution, the eluate was neutralized by passing it through a cation-exchange cartridge.

A sodium chloride content of the brine sample of up to at least 20 g/l does not affect the recovery of the preconcentration procedure. The recovery is also independent of the volume of the brine sample up to at least 500 ml, which was checked by loading a constant amount of 0.55 mg of sulphate in different volumes of sodium chloride solution on to the cartridge. The recovery as a function of the sulphate concentration was 86.0% for 0.72 ppm, 89.9% for 1.1 ppm, 95.3% for 2.4 ppm and 97.7% for 4.8 ppm of sulphate (brine sample volume 500 ml). The lower recovery of sulphate at lower concentrations must be attributed to incomplete elution from the alumina cartridge by ammonia; to a small extent sulphate might be adsorbed on alumina by mechanisms different from ion exchange.

A typical chromatogram of a brine sample containing 0.49 mg/l of sulphate and 20.99 g/l of sodium chloride is shown in Fig. 2. The sensitivity of the chromatographic system would permit the determination of concentrations considerably lower than 500 ppb, but then the recovery would decrease to unacceptable levels. The relative standard deviation was 2.1% (n = 4) for a sample containing 1 ppm of sulphate.

Alumina is not only well suited for preconcentration but also for sample clean-up of complex biological matrices. This is shown for a serum sample in Fig. 2. The sample was diluted with 0.7% hydrochloric acid and passed through the alumina cartridge column. In this way a chromatogram free from any interferences can be obtained.

All the applications may be automated by commercially available sample preparation devices, so that, at least partially, the alumina-filled cartridges can be used on-line coupled with conventional anion-exchange separation columns.

The applications described above are based on the use of alumina-filled columns as a means of retaining or not retaining some ions. Nevertheless, the advantages of alumina in ion chromatography can only be fully exploited if alumina is used as stationary phase in a true separation column. Then the coupling with R_4N^+-type columns will result in a significantly increased separation power owing to the different selectivities of the stationary phases. Such a column-coupling technique was developed for the determination of trace amounts of iodide in mineral water and fruit-juice samples. Iodide is an essential micronutrient, but in some regions of Europe the alimentary iodide supply is insufficient[10]. Recently in Austria it has been reported

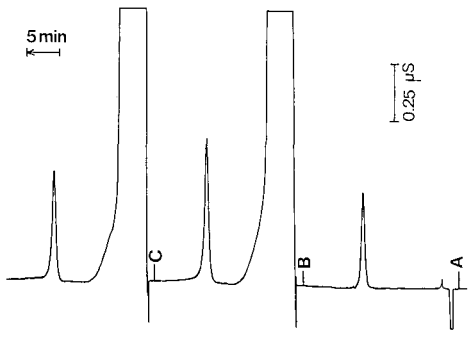

Fig. 2. Typical chromatograms for the determination of sulphate in brine and serum. Flow-rate, 2 ml/min. (A) Sulphate standard; (B) brine sample containing 0.49 ppm of sulphate, 100-fold preconcentrated; (C) human serum containing 31.4 mg/l of sulphate.

that mineral waters and several fruit juices can substantially contribute to a sufficient iodide supply[11]. Unfortunately, the data available on the iodide contents of mineral waters differ greatly (sometimes by more than one order of magnitude for the same sample), so that a reinvestigation of the iodide concentrations of such samples seemed necessary.

First we looked for a rapid screening method to differentiate between samples containing less than 20 ppb of iodide and those containing higher concentrations, because only the latter could contribute substantially to a sufficient iodide supply. For that purpose ion-interaction chromatography with RP-18 as the stationary phase, octylammoniumphosphate as the ion-pairing reagent and UV detection at 227 nm was used. Typical chromatograms for two mineral water samples are given in Fig. 3; while sample 1 unequivocally has a content of less than 20 ppb, the content of sample 2 might be higher, but an exact determination is difficult owing to some interferences. The sample was therefore injected a second time on to an alumina-filled separation column. This gave a clear chromatogram without interferences, as is shown in Fig. 3. An alumina column also proved advantageous as it can be loaded with an injection volume of 200 μl, whereas only up to 50 μl could be injected on to the RP-18 column without peak distortion. On the other hand, the disadvantage of the alumina column is that sulphate, which commonly occurs in mineral waters in high concentrations, is accumulated on the column, so that frequent flushing of the column with buffers of appropriate strength and pH might be necessary. To avoid this, we injected on to the alumina column only those samples in which elevated iodide levels could be expected from the initial screening procedure. The number of samples that can be processed without reconditioning the alumina column depends on the type of mineral water (*i.e.*, the concentration of sulphate). In general, the column was used for 1 day (about 20 injections of samples) and flushed overnight with an eluent containing 5 g/l of methanesulphonic acid adjusted to pH 6.

Serious interferences in the chromatograms were observed with fruit-juice samples. This could be circumvented by coupling the alumina column with a silica-based R_4N^+-type anion-exchange column, as shown in Fig. 1. Methanesulphonic acid,

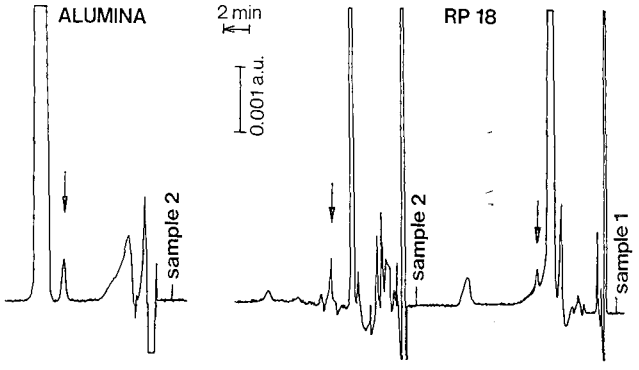

Fig. 3. Typical chromatograms for iodide in mineral waters using ion-pair chromatography on RP-18 or ion chromatography on alumina. Flow-rate, 0.8 ml/min. Sample 1 contains less than 20 ppb of iodide, sample 2 contains 25 ppb of iodide.

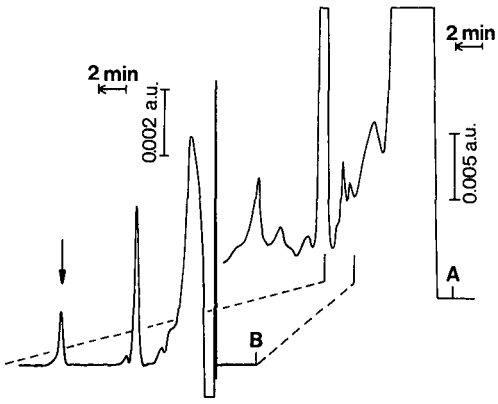

Fig. 4. Determination of iodide in a fruit-juice sample containing 72 μg/l of iodide. (A) Separation column packed with alumina (flow-rate, 0.8 ml/min); (B) column coupling with Vydac 302-IC separation column (flow-rate, 1 ml/min).

adjusted to pH 4, as the mobile phase is compatible with both columns. Its concentration for the alumina column needs to be only about one tenth of that necessary for the R_4N^+-type ion-exchange column. In this way a favourable peak compression could be obtained in the column-switching procedure. A typical chromatogram without and with column coupling is shown in Fig. 4.

The examples presented in this paper demonstrate some favourable aspects of alumina as a stationary phase in ion chromatography. It is unlikely that alumina will replace existing ion-exchange stationary phases, but it turned out as a valuable complement, which in some instances can facilitate the determination of ions in difficult matrices.

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Note

Determination of cations in tear fluid samples by non-suppressed ion chromatography with indirect UV detection

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The determination of electrolytes (such as sodium, potassium, magnesium, calcium, chloride and hydrogencarbonate) in tear fluid is of considerable clinical importance. Increased tear osmolarity is responsible for "dry eye" symptoms (ceratoconjunctivitis sicca)[1,2], which may result from a disturbed equilibrium between tear secretion and evaporation. Increased tear osmolarity has also been demonstrated in wearers of contact lenses[3] and might explain several complaints associated with contact lens wear. Further, ions of the tear film play an important role in the protection of the epithelium of the cornea[4] and corneal transparency is strongly dependent on transport processes of electrolytes between the tear film and the cornea[5].

The determination of normal values of electrolytes in the tear fluid is difficult, because during sampling the normal physiological secretion of tear fluid should be maintained and any stimulation should be avoided. These requirements can only be fulfilled if the amount to be sampled is reduced to 1–2 μl. Therefore, reliable microanalytical techniques are necessary to obtain clinically relevant data about the composition of tear fluid.

In this paper, the application of ion chromatography to the determination of cations in tear fluid is described. This technique shows some advantages over spectroscopic methods with limited amounts of sample and could therefore be the method of choice for routine work and diagnostic purposes. Further, as it is a non-destructive method, it allows the determination of anions from the same sample after cation chromatography.

EXPERIMENTAL

Instrumentation and reagents

The chromatographic instrumentation consisted of a Waters Assoc. M510 high-performance liquid chromatographic pump, a Rheodyne 7125 injection valve with a 20-μl loop and a Waters Assoc. Model 481 UV detector. An Apple IIe computer with Chromatochart software (Interactive Microware) was used for recording the chromatograms and determining peak areas.

Cations were separated on a 100 × 3.2 mm I.D. Polyspher IC CA column (Merck) with a mobile phase of 0.05 m*M* cerium(III) sulphate solution, prepared from cerium(III) sulphate octahydrate (Aldrich). The flow-rate was 1 ml/min. Indirect UV detection at 254 nm was used.

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Chloride determination by anion chromatography was carried out on a 50 × 6 mm I.D. Polyspher IC AN column. The mobile phase was 0.5 m*M* potassium hydrogenphthalate (containing 53 m*M* ethylene glycol)–2-propanol (1000:27). The flow-rate was 1 ml/min. Indirect UV detection at 260 nm was used.

Atomic absorption spectrometric (AAS) experiments were carried out with a Perkin-Elmer Model 2380 instrument. AAS signals were recorded by a BBC SE120 chart recorder and evaluated from their peak heights.

Determination of cations and chloride in tear fluid

Tear samples were collected by placing a disposable 2-μl glass capillary on the lower conjunctival sac, where it was kept until filled with tear fluid. After collection, the capillary was blown out into a polypropylene micro-tube containing 30 μl of 0.05 m*M* cerium(III) sulphate solution. The tube was centrifuged at 800 *g* 2 μl of this solution were further diluted with 50 μl of 0.05 m*M* cerium(III) sulphate solution and used for determination of sodium and potassium by cation chromatography. The remainder was used for the determination of calcium and magnesium by cation chromatography; 10 s after injection of this solution, the eluate was collected for 30 s (corresponding to a volume of 0.5 ml) and 20 μl of this fraction were injected on to the anion-exchange column for determination of chloride.

AAS analysis was carried out with 2 μl of tear fluid diluted to 250 μl (measurement of calcium and magnesium) and 20 μl further diluted to 1000 μl (for measurement of sodium) or 100 μl (for measurement of potassium). All dilutions were made with a solution containing 11.4 m*M* strontium chloride and 7.5 m*M* caesium chloride. In each instance 90 μl of the dilution were injected into the flame and wavelengths of 422.7 nm (calcium), 285.2 nm (magnesium), 589.0 nm (sodium) or 766.5 nm (potassium) were used.

RESULTS AND DISCUSSION

From the practical point of view, it was desirable to carry out the separation of alkali and alkaline earth metal ions in one run under isocratic conditions. This can be achieved with different types of cation exchangers: a silica-based polymer-coated weakly acidic cation-exchange column[6], with tartaric acid or other organic acids as the mobile phase, or a polymer-based strongly acidic cation-exchange column with cerium(III) sulphate solution as the mobile phase[7]. The latter column, with its inherently greater sensitivity than non-suppressed conductimetric detection, also allows indirect photometric detection[8]. Therefore, we chose this column for cation analysis of tear samples.

Sodium ions are present in a large excess over calcium and magnesium ions in tear fluid. Therefore, a simultaneous analysis of all ions from one sample dilution suitable for calcium and magnesium determination was not possible, because owing to overloading effects the resolution between sodium and potassium was poor. Therefore, these two ions were analysed in a second run after further dilution of the sample (with all chromatographic parameters unchanged).

Typical chromatograms of a standard and a tear fluid sample are shown in Figs. 1 and 2. As can be seen, ammonium could also be detected in the tear fluid, but this ion was not determined because it was of only minor interest for these clinical investigations.

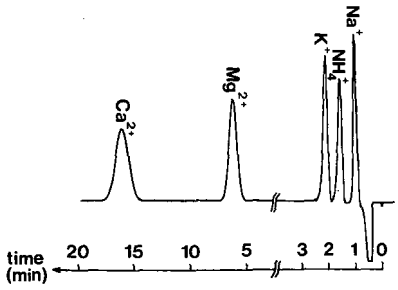

Fig. 1. Separation of a standard mixture of cations. Column, Polyspher IC CA (100 × 3.2 mm I.D.); eluent, 0.05 m*M* cerium(III) sulphate solution; flow-rate, 1 ml/min; indirect UV detection at 254 nm. Attenuation: 0.02 a.u.f.s.

The limits of detection (signal-to-noise ratio = 3) were about 0.07 mg/l for sodium, 0.12 mg/l for potassium, 0.25 mg/l for magnesium and 0.60 mg/l for calcium (injection volume 20 μl). For all ions linearity was found over two orders of magnitude of concentration, beginning at the detection limits (r better than 0.9995, n = 7); at higher concentrations peak asymmetry due to overloading of the column was observed.

Evaluation of the reproducibility, carried out with a pooled tear fluid sample, gave a relative standard deviation (n = 5) of 3–4% for sodium (2.62 g/l), potassium (206 mg/l) and magnesium (12.5 mg/l) and 7% for calcium (25 mg/l).

As can be seen from Fig. 2, the calcium levels in tear fluid are low, so that the analysis had to be carried out near the detection limit of the method. This might be overcome by using a narrow-bore column (1–2 mm I.D.); sensitivity should be inversely proportional to the square of the diameter, provided that the same amount of sample can be injected (which can be expected to be practicable in this instance).

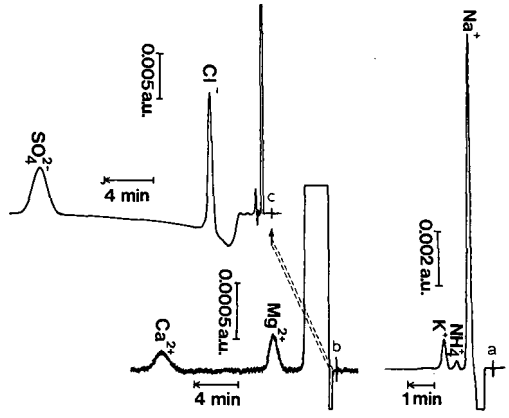

Fig. 2. Chromatograms of a tear fluid sample containing 2.62 g/l of sodium, 206 mg/l of potassium, 12.5 mg/l of magnesium, 25 mg/l of calcium and 3.94 g/l of chloride. (a) Sample diluted 1:416; (b) sample diluted 1:16; (c) anion chromatogram of the fraction collected from cation chromatography. Conditions for cation chromatography as in Fig. 1. Conditions for anion chromatography: column, Polyspher IC AN (50 × 6 mm I.D.); eluent, 0.5 m*M* potassium hydrogenphthalate (containing 53 m*M* ethylene glycol)–2-propanol (1000:27); flow-rate, 1 ml/min; indirect UV detection at 260 nm.

Prepacked narrow-bore columns were not commercially available, so work is in progress to prepare and test such narrow-bore columns.

On the other hand, the detection limits reported in the literature[7] are about one order in magnitude lower than those found in our investigations. By careful selection of a highly stable type of UV detector, the necessary improvements in detection might therefore easily be achieved [to avoid confusion, it should be mentioned that in the paper cited[7] there are erroneous data on detection limits (amounts injected) of magnesium and calcium, which should be 1 and 2 ng rather than 0.1 and 0.2 ng].

Indirect fluorescence detection is also applicable when a cerium(III) solution is used as the mobile phase. Unfortunately, the detection limits reported in the literature[9] are similar to those for indirect UV detection, so that fluorescence was not further investigated in this work.

In addition to ion chromatography, flame AAS was tested for its applicability to the analysis of electrolytes in tear fluid. In continuation of previous work[10], a flame injection technique was used after appropriate dilution of the sample. The reproducibility of this method was approximately the same as that of ion chromatography. Likewise, the sensitivity for calcium was unsatisfactory, so that in general AAS did not exhibit major advantages over the chromatographic method.

On the other hand, a disadvantage of AAS is the risk of contamination by the addition of caesium and strontium chloride, which is necessary to avoid ionization or chemical interferences in the flame. Further, AAS measures the total amount of an element, but ion chromatography allows the determination of the free ions; similarly to blood, in tear fluid calcium will be present only partly in the ionized form. In principle, speciation analysis can be done by ion chromatography, although investigations are still necessary to check how far the equilibrium between the free and protein- or complex-bound ion is maintained during chromatography. In addition, AAS is a destructive method, so that sample is lost after analysis, whereas in ion chromatography fractions of the eluate can be collected and used for anion chromatography. This is shown by the chromatogram in Fig. 2 for the determination of chloride, which elutes in the void volume of the cation-exchange column (the sulphate peak is caused by the mobile phase in cation chromatography).

The relative standard deviation of the determination of chloride in a pooled tear fluid sample containing 3.94 g/l of chloride was 1.4% ($n = 5$). The amount of chloride in the sample is high, so that chloride might be determined just as well by injecting 20 μl of the dilution used for sodium and potassium. Nevertheless, the determination of chloride in fractions collected from cation chromatography should demonstrate the compatibility of both the cation and the anion chromatographic systems. This is important for on-line column-coupling techniques for the determination of other anions present in low concentrations. Such anions must be determined in the same injection as calcium and magnesium, as the sample size is limited. In this instance on-line transfer of the whole fraction containing the anion of interest to the anion-exchange column is advisable.

In conclusion, these investigations have shown that ion chromatography is a useful microanalytical technique for the diagnosis of ceratoconjunctivitis sicca. Other routine diagnostic methods for detecting changes in tear-film osmolarity, such as the "tear mucus ferning test"[11], can be supplemented and confirmed by this chromatographic method.

ACKNOWLEDGEMENT

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MODIFICATION OF A DIONEX SYSTEM 12 ION CHROMATOGRAPH FOR SEQUENTIAL DETERMINATION OF THE MAIN COMPONENTS IN ATMOSPHERIC PRECIPITATION

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SUMMARY

A modification of a Dionex System 12 ion chromatograph is described which enables organic anions (acetate and formate), inorganic cations (ammonium, sodium and potassium) and inorganic anions (chloride, nitrate and sulphate) to be determined sequentially in one measuring procedure. The modified instrument consists of a programmable controller unit, a conductimetric meter, two conductimetric detectors of the Dionex System 12 ion chromatograph, the HPIC-AS4A and HPIC-CS3 modern separation units, AMMS-1 and CMMS-1 micro-membrane suppressor columns, a unique system of valves from Dionex and two dual pumps from Biotronik. The limits of detection are between about 1 and 3 μg/l for chloride, nitrate and sulphate and between about 2 and 10 μg/l for acetate, formate, ammonium, sodium and potassium. The reliability of the method was demonstrated by analysing two NBS simulated rain water Standard Reference Materials. Some examples are given of the application of the method to the sequential determination of the main precipitation components in typical samples from urban and rural regions of the F.R.G. The ion concentrations varied between about 0.02 and 300 mg/l.

INTRODUCTION

Industrialization has caused an increase in the release of anthropogenic pollutants into the atmosphere. Sulphuric and nitric acid are atmospheric contaminants resulting to a large extent from the oxidation of sulphur and nitrogen oxides (SO_2 and NO_x)[1,2]. The wet pollutants from the atmosphere and the deposition of toxic substances on the surface of plants, water and soil are therefore topics of interest. For investigating the composition of atmospheric precipitation, ion chromatography is an attractive technique for the determination of ions in solution[3–5]. The determination of inorganic anions, cations and also organic anions such as formate and acetate by ion chromatography has been reported[6–11]. However, the routine determination of the main components in precipitation is associated with several problems:

(1) Negative "dips" or deviations from the baseline can interfere with fluoride and chloride determinations. The addition of the same eluent ion concentration as in

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the samples helps to avoid such dip interferences[5]. However, this procedure requires more time for sample preparation.

(2) For a rapid determination of acetate and formate in precipitation samples, the separation column must be automatically cleaned up.

(3) The appropriate columns and eluents must be exchanged between the determination of anions and cations when using one measuring instrument. Then the measuring system must be cleaned to obtain a baseline, which requires a long waiting period.

(4) In contrast to new developments of Dionex ion chromatographic systems, the original Dionex ion chromatograph, Auto IonTM, System 12, equipped with two Type NSI 33-R reciprocating liquid pumps, a P/N 30827 separation column and a P/N 30828 suppressor column, gives unsatisfactory results.

In this paper, a modified Dionex System 12 ion chromatograph is described and routine measurements are reported for the main precipitation components in samples from Schauinsland, Jülich, Berchtesgaden, Essen, Dortmund and Hamburg (F.R.G.). The application of this modified method avoids dip interferences. Moreover, the automatic clean-up of the separation column for the determination of acetate and formate reduces the analysis time.

EXPERIMENTAL AND RESULTS

Apparatus

The modified ion chromatograph was constructed with the following components: a programmable controller unit and conductivity meter of the AutoTM System 12 Analyzer, HPIC-AG4A and HPIC-CG3 guard columns, HPIC-AS4A and HPIC-CS3 separation columns, AMMS-1 and CMMS-1 micro-membrane suppressor columns, Dionex valves, two BT 0512 high-performance liquid chromatographic (HPLC) eluent pump units, an BT 7040 sample injector (Biotronik), an Shimadzu C-R1B integrator, an MGW Lauda RC6 thermostat and two Gilson minipuls 2 tube pumps for the transport of regenerants. Sampling of rain water and snow, free from contamination, is achieved by using an automatic sampler of our own construction, which is now commercially available[12]. The rain water and snow samples were filtered during the sampling in a Satorius Type SM 16511 filtering device through a membrane filter (pore size 0.45 μm).

Chemicals

An eluent consisting of 3.6 m*M* $NaHCO_3$–4 m*M* Na_2CO_3 and 25 m*M* H_2SO_4 as regenerant were prepared for the determination of chloride, nitrate and sulphate. For the determination of acetate and formate and for clean-up of the separator, an eluent $NaHCO_3$ 1 m*M* and a stronger 10 m*M* $NaHCO_3$ eluent, respectively, were used. The eluent and regenerant solutions for determining cations were 70 m*M* HCl and 40 m*M* tetramethylammonium hydroxide solution (TMAH), respectively. Before flowing through the columns, the initial concentration of eluent was diluted by a factor of 2 with deionized water or sample. Deionized water (Millipore) and Merck analytical-reagent grade chemicals were used to prepare all solutions.

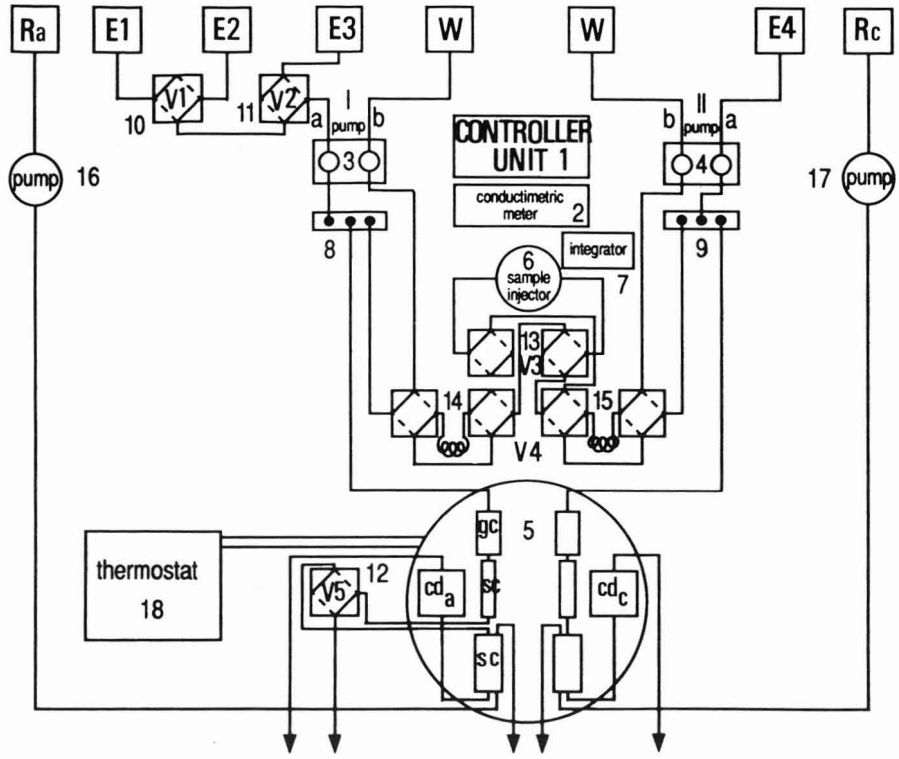

Fig. 1. Flow scheme of modified ion chromatograph.

Modification of ion chromatograph

The flow scheme of the modified ion chromatograph is shown in Fig. 1. It consists of one programmable controller unit (1), one conductivity meter (2) from the Dionex System 12, two eluent pumps Biotronik (3, 4), a thermometric box (5) containing the column system and two Dionex conductimetric detectors (cd_a and cd_c), a Biotronik sample injector (6) and a Shimadzu integrator (7). The Biotronik 0512 HPLC pump unit (3, 4) with a dual liquid pump can transport in one pump line (a) a double eluent concentration and in the second pump line (b) the deionized water or sample into a three-way mixer (8, 9) before flowing through the columns. In this instance, the dip interferences can be automatically avoided during the measuring procedure.

The flow path on the left of Fig. 1 with pump I (3) was used to determine acetate, formate, chloride, nitrate and sulphate anions. With the aid of valves V1 (10) and V2 (11) and the programmable controller unit (1), the appropriate eluents 1 m*M* $NaHCO_3$ (E1), 10 m*M* $NaHCO_3$ (E2), 3.6 m*M* $NaHCO_3$–4 m*M* Na_2CO_3 (E3) were automatically provided for the determination of organic or inorganic anions. By using a stronger eluent, 10 m*M* $NaHCO_3$ (E2), through valves V1 (10) and V5 (12), the separation column can be cleaned up automatically after the analysis cycle of acetate and formate in *ca.* 8 min. Under these conditions, the analysis time was less than 20 min.

At the right hand side of Fig. 1, the analysis scheme for cations is shown. Pump II (4) and suitable eluents and columns were used to perform the determination of ammonium, potassium and sodium cations. By monitoring the programmable controller unit (1), the valve V3 (13) could be switched to bring precipitation samples from the sample injector (6) into the sample loop of 127 or 121 μl (14 or 15). All these processes are automatically achieved by action of the controller unit and valves. Moreover, two Gilson pumps (16 and 17), which deliver a constant volume with time, are used to transport 25 m*M* H_2SO_4 (Ra) and 40 m*M* TMAH (Rc) regenerant through the micro-membrane suppressors AMMS-1 and CMMS-1. A thermostat (18) regulates the temperature of the contents of the box during the measuring time.

A photograph of the modified ion chromatograph is shown in Fig. 2.

Analytical procedure

A flow chart of the whole analytical procedure is shown in Fig. 3. Standard solutions and filtered precipitation samples are first deposited in the sample injector. Before starting the analytical procedure, three measuring programs for the determination of anions and cations are entered in the program controller of the modified ion chromatograph. The selected measuring parameters were written separately. The program for the determination of acetate and formate is started first. After calibration with a standard solution, the rain water samples are measured and the results calculated automatically by using the Shimadzu integrator. Pump unit II can be switched on manually or automatically before the end of the acetate and formate determination cycle to obtain a constant measuring baseline as required for the determination of Na^+, NH_4^+ and K^+. Subsequently, the measurement can be followed by the

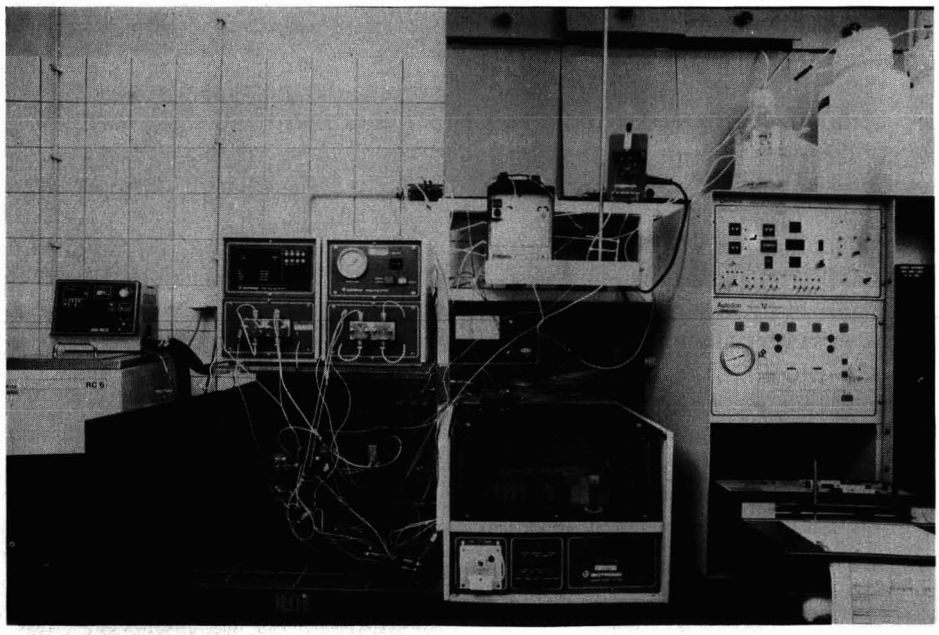

Fig. 2. Modified ion chromatograph system.

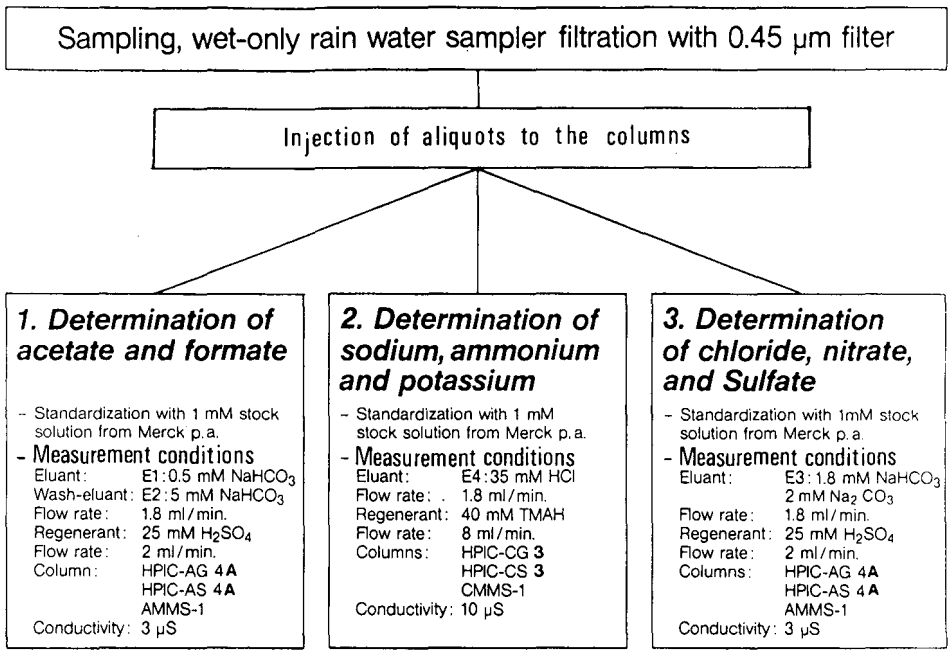

Fig. 3. Flow scheme of the analytical procedure.

controller unit of the ion chromatograph. Finally, in a similar way, the determination of chloride, nitrate and sulphate is automatically performed. In this instance, the sequential determination of main components Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, NH_4^+ and the organic anions CH_3COO^- and $HCOO^-$ is possible in one measuring procedure. The limits of detection are 1 µg/l for chloride, 2 µg/l for nitrate and sodium, 3 µg/l for sulphate, 5 µg/l for formate, ammonium and potassium and 10 µg/l for acetate.

Application of the method to the determination of the main precipitation components

Measurement of NBS Standard Reference Material. The NBS simulated rain water Standard Reference Materials (SRMs) 2694-I and 2694-II were used to test the method. Table I compares results obtained by using the modified ion chromatograph with the NBS certificate values[13]. It can be seen that the agreement is good.

Measurement of the main components of precipitation samples. The following examples demonstrate the suitability of the modified ion chromatograph for the sequential analysis of main components in the case of typical precipitation samples collected in the winter of 1988–89 at Schauinsland, Essen, Jülich, Berchtesgaden, Dortmund and Hamburg (F.R.G.).

Fig. 4A shows a typical chromatogram of fluoride, chloride, nitrate and sulphate. It can be seen that the dip interferences have disappeared. Therefore, the peak height of chloride in the chromatogram can be more accurately determined.

The ion concentrations of the precipitation samples varied between about 0.020 and 300 mg/l. The results for the rain water samples from urban and rural regions in

TABLE I

MEASUREMENT OF THE COMPONENTS OF NBS SRM 2694-I AND SRM 2694-II

$n = 5$.

SRM	*Value (mg/l)*	*Sodium*	*Potassium*	*Fluoride*	*Chloride*	*Nitrate*	*Sulphate*	*Ammonium*
SRM 2694-I	Certified	0.205 ± 0.009	0.052 ± 0.007	0.054 ± 0.002	(0.24)[13]	–	2.750 ± 0.050	–
	Measured	0.196 ± 0.005	0.050 ± 0.003	0.064 ± 0.007	0.25 ± 0.050	–	2.680 ± 0.090	–
SRM 2694-II	Certified	0.419 ± 0.015	0.106 ± 0.008	0.098 ± 0.007	(1.0)[13]	7.060 ± 0.150	10.90 ± 0.200	(1.00)[13]
	Measured	0.418 ± 0.030	0.118 ± 0.002	0.099 ± 0.001	0.980 ± 0.060	7.150 ± 0.010	10.70 ± 0.480	1.100 ± 0.080

TABLE II

AVERAGE CONCENTRATIONS (mg/l) OF THE MAIN COMPONENTS OF RAIN AND SNOW SAMPLES FROM SIX REGIONS IN THE F.R.G.

Region	*Acetate*	*Formate*	*Chloride*	*Nitrate*	*Sulphate*	*Ammonium*	*Sodium*	*Potassium*
Schauinsland, Dec. 1988 ($n = 3$)	–	–	0.87	1.79	1.54	0.14	0.48	0.33
Berchtesgaden, Jan. 1989 ($n = 3$)	0.10	0.02	1.32	3.79	3.40	1.25	0.95	0.37
Jülich, Jan. 1989 ($n = 3$)	0.10	0.01	2.13	4.40	6.57	2.58	0.98	0.16
Essen, Jan. 1989 ($n = 4$)	0.21	0.27	1.89	4.06	5.21	1.19	0.72	0.20
Dortmund, Dec. 1988 ($n = 2$)	0.07	0.10	3.11	2.08	3.29	0.69	1.33	0.12
Hamburg, Jan. 1989 ($n = 4$)	0.22	0.17	6.83	4.27	7.09	0.98	2.91	2.09

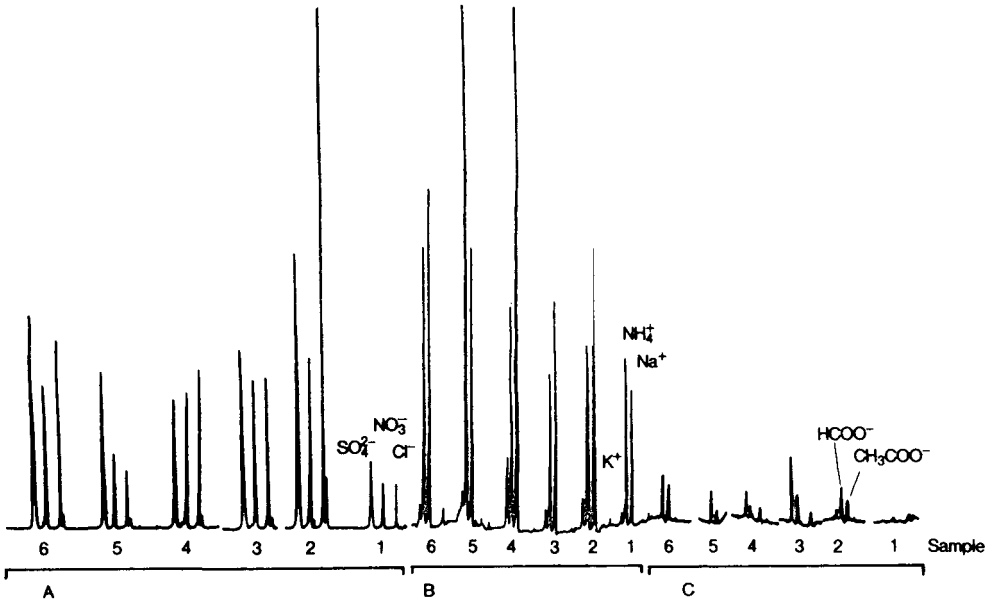

Fig. 4. Typical chromatograms of the main components of precipitation samples from (1) Schauinsland (December 1988), (2) Berchtesgaden (January 1989), (3) Essen (January 1989), (4) Dortmund (December 1988), (5) Jülich (January 1989) and (6) Hamburg (January 1989) (F.R.G.). (A) Determination of chloride, nitrate and sulphate; (B) determination of sodium, ammonium and potassium; (C) determination of acetate and formate.

Table II reveals a difference in the concentrations of the components. High average concentrations were observed in the urban regions (Essen, Dortmund and Hamburg). The lower values from hilly regions (Schauinsland and Berchtesgaden) indicate that the removal of the main precipitation components from the atmosphere by wash-out and rain-out processes depends on the altitude. The amount of precipitation at these sites is in general higher by a factor of 2–2.5. Therefore, the average concentration decreases with the amount of precipitation. It can be seen from Table II that the highest values for chloride, sulphate, sodium and potassium were found in the rain water samples from Hamburg, a typical urban conglomeration with a number of industrial sources. The highest concentration of ammonium, at the Jülich site, is caused by the surrounding agricultural activity of the sampling station. Moreover, a large lignite coal-fired power station of about 2300 MW is located 10 km south-west of the sampling site and also a heavily industrialized area to the west. These two important areas are sources of acid precursors, which explains the high concentrations of nitrate and sulphate in the rain water from the Jülich site. The low concentration of acetate and formate in the samples can be interpreted as resulting from bacterial activity, which detroys most of these organic components during the sampling and storage time under the original conditions. However, this loss of acetate and formate can be avoided if a small amount of chloroform (40 μl per 10 ml of sample) is added to the rain water samples, thus stopping bacterial growth[14].

Table III presents a comparison between the stability of organic anions in treated and untreated samples of rain water sample collected at Jülich. The accuracy

TABLE III

COMPARISON BETWEEN THE STABILITIES OF ORGANIC ANIONS IN TREATED AND UNTREATED ALIQUOTS OF RAIN WATER SAMPLES COLLECTED IN JÜLICH, JANUARY 1989

Storage time (days)	*A*[a]		*B*[b]	
	Acetate (mg/l)	*Formate (mg/l)*	*Acetate (mg/l)*	*Formate (mg/l)*
0	0.52	0.40	0.53	0.39
1	0.53	0.39	0.50	0.37
4	0.51	0.37	–	–
5	0.50	0.35	–	–
7	0.50	0.36	–	–
14	0.49	0.35	–	–

[a] A: aliquots treated by adding 40 μl of chloroform per 10 ml of rain water sample, stored at + 7°C in darkness.

[b] B: untreated aliquots stored at room temperature.

and precision were calculated after replicate analyses of several samples. Table IV shows the results of five measurements of the main components in two rain water samples. As can be seen, the performance of the modified method (with a relative standard deviation less than 8%) is very satisfactory.

With an investment of about US $2000 for the dual pumps, the modern columns, valves and accessories, the Dionex System 12 ion chromatograph can be modified as desinbed to determine sequentially the main components of precipitation samples in one measuring procedure. By using this automatic ion chromatograph, precipitation samples can be measured continuously.

TABLE IV

USE OF THE MODIFIED ION CHROMATOGRAPHIC SYSTEM FOR THE DETERMINATION OF COMPONENTS IN RAIN WATER

Each sample was analysed five times.

Component	*A*[a]		*B*[b]	
	Average concentration (mg/l)	*Standard deviation (mg/l)*	*Average concentration (mg/l)*	*Standard deviation (mg/l)*
Chloride	1.35	0.06	2.78	0.10
Nitrate	1.88	0.07	1.11	0.05
Sulfate	1.25	0.03	1.30	0.06
Ammonium	1.54	0.08	0.99	0.04
Sodium	0.46	0.03	1.21	0.02
Potassium	0.12	0.01	0.13	0.01
Acetate	0.67	0.02	0.42	0.01
Formate	0.72	0.02	0.28	0.02

[a] A: rain water sample from Jülich, January 1989.

[b] B: rain water sample from Dortmund, December 1988.

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Analytical Absorption Spectrophotometry in the Visible and Ultraviolet: The Principles

by **L. Sommer,** *J.E. Purkyne University, Brno, Czechoslovakia*

(Studies in Analytical Chemistry, 8)

Despite the many competitive analytical techniques, molecular absorption spectrophotometry is still very popular in practice, particularly in biochemical, clinical, organic, agricultural, food and environmental analyses. This is due mainly to the inherent ease and relative simplicity of spectrophotometric procedures and the availability of reliable, highly-automated instruments. Moreover, both the method and its instrumentation have recently undergone considerable development resulting in some new special approaches of spectrophotometry in the ultraviolet (UV) and visible (VIS) regions. Although there are several comprehensive textbooks on UV/VIS spectrophotometry, they tend to describe historical aspects or contain collections of detailed procedures for the determination of analytes and do not reflect sufficiently the present state of the method and stage of development reached.

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The text is supported by tables and figures, and there are numerous references for each topic treated. The book is written for all those who use UV/VIS spectrophotometry in the laboratory and will also be useful to students as supplementary reading.

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Carteolol	1.86	OD
Chlophedianol	2.82	OJ
Chlormezanone	1.47	OJ
Cyclopentolate	2.47	OJ
Diltiazem	1.46	OD
	2.36	OF
	1.75	OG
Disopyramide	2.46	OF
Ethiazide	1.54	OF
Ethotoin	1.40	OJ
Fenoprofen	1.35	OJ
Glutethimide	2.48	OJ

SUBSTANCE	α	column
Gauifenesin	2.40	OD
Hexobarbital	1.7	CA-1
Homatropine	3.13	OD
Hydroxyzine	1.17	OD
Indapamide	1.58	OJ
Ketamine	complete resolution	CA-1
Ketoprofen	1.46	OJ
Mephobarbital	5.9	OJ
	2.3	CA-1
Methaqualone	2.8	CA-1
	7.3	OJ
Methsuximide	2.68	OJ
Metoprolol	complete resolution	OD
Mianserin	1.75	OJ
Nilvadipine	complete resolution	OT

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Oxapadol	complete resolution
Oxazepan	4.36
Oxazolam	1.67
Oxprenolol	6.03
Perisoxal	1.33
	1.27
Pindolol	5.07
Piprozolin	1.7
Praziquantal	complete resolution
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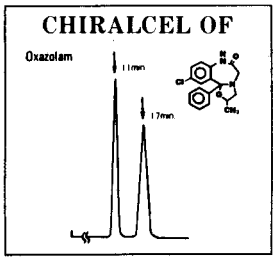

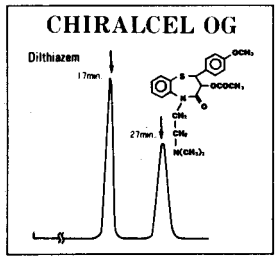

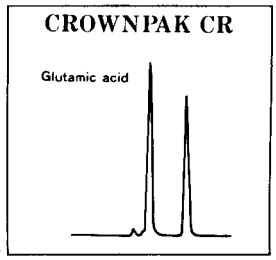

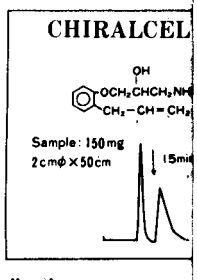

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