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Review

Ion chromatographic characterization of toxic solutions: analysis and ion chemistry of biological liquids

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Abstract

The literature on the analysis of biological fluids by ion chromatography is reviewed herein. It has been demonstrated that ion chromatography is the method of choice for the determination of anions such as chloride, nitrite, bromide, phosphate, nitrate, sulfate, oxalate, thiocyanate, thiosulfate, citrate, isocitrate, carbonate, and similar species. Cations such as sodium, ammonium, potassium, magnesium, and calcium in various biological solutions have also been successfully identified and quantified. The technique fulfils several requirements of a reliable microanalytical method by providing sufficient speed, automation, ease of use, and accuracy. For many types of analyses, very little or even no sample preparation is required. Because of this, as presented in this review, ion chromatography is widely used not only to obtain reliable clinical data, but also to study ion chemistry. It has been an invaluable tool in nephrolithiasis and dental research. This review should provide a useful reference for analysts and researchers involved in clinical studies. The review is presented in four sections: (1) introduction, (2) methods of analysis, (3) ion chemistry and (4) critical comments and concluding remarks. Section 1, as usual, deals with the general introduction of the subject and objectives. Section 2 includes the review of the literature on ion chromatography (IC) methods developed for routine analysis of various analytes present in biological fluids. Section 3 deals with the applications of IC used in the understanding of ion chemistry of biological fluids. Specifically, it deals with the physical chemistry aspects related to nephrolithiasis and dental research, such as speciation, driving force for crystals formation and crystallization, and pathophysiology. Section 4 contains critical comments and concluding remarks.

Keywords: Reviews; Inorganic anions; Inorganic cations; Organic acids

Contents

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1. Introduction

The determination of anions such as chloride, bromide, nitrate, sulfate, phosphate, etc. in liquid matrices is the single largest area in which ion chromatography has found application [1–7]. Since the development of the technique [1] in 1975, many such application papers have been published. While this area of analytical chemistry has been periodically reviewed with respect to various topics [8–18], the literature on ion chromatographic analysis of biological fluids has not yet been so treated.

In this paper, we present a review of this literature, dealing with the ion chromatographic analysis of blood, plasma, serum, urine, and various other biological fluids and solutions. While analytes routinely determined in biological matrices are not generally considered toxic themselves, the presence of dangerous bacteria in many biological solutions effectively makes them hazardous materials. Because of this, it is reasonable to include the analysis of biological matrices in this special issue containing papers on the chromatographic analysis of toxic wastes.

The ability to analyze qualitatively and quantitatively various solutes in biological fluids, such as blood, urine, saliva, sweat, etc., may be of great benefit to the diagnosis of certain diseases. Concentration profiles of some solutes may, in certain cases, be linked to critical physiological disorders. The composition of various biological fluids may also provide important information into the cause(s) of systemic electrolyte transport disorders. For example, it has been reported that a high chloride concentration in human sweat is a hallmark of cystic fibrosis, arising from a defect in anion transport [19]. Similarly, the determination of oxalate concentration in blood is an important assessment for a number of clinical conditions in which it can be used either to diagnose disease or to monitor the success of a treatment or therapy [20]. The total concentrations (sum of the free and bound fractions) of various solutes, such as chloride, phosphate, sulfate, fluoride, citrate, sodium, potassium, magnesium, and calcium, in saliva may be used to calculate the driving force for the formation of tartar and enamel in control patients, as well as those prone to caries. In similar fashion, the total concentrations of chloride, phosphate, sulfate, oxalate, citrate, sodium, potassium, ammonium, magnesium, and calcium in urine can provide the supersaturation index for the formation of principal kidney stone minerals, such as the

calcium oxalates and calcium phosphates, as well as indications for the clinical conditions of hypercalciurea and hyperoxalurea [21]. The determination of electrolytes such as sodium, potassium, magnesium, calcium, chloride, and bicarbonate in human tears has also been reported to be of considerable clinical importance [22].

Rapid and accurate determination of the solute profiles of various biological fluids presents a technical challenge for all but the most abundant components. Reliable microtechniques are necessary to obtain clinically relevant data regarding the composition of various biological fluids. Ion chromatography, which fulfils several of the requirements of microanalytical techniques and provides sufficient speed, ease of use, and accuracy, is widely applied for this purpose. This review will therefore provide a useful reference tool for analysts and researchers involved in clinical studies or its applications.

2. Methods of analysis

2.1. Analysis of blood, plasma, and serum

2.1.1. Analysis of sodium (Na^+), ammonium (NH_4^+) and potassium (K^+)

Several papers are reported on the ion chromatographic determination of sodium and potassium [23,24] and sodium, ammonium, and potassium [1,25–27]. Small et al. [1] were the first to demonstrate the application of ion chromatography in the analysis of blood serum for sodium, ammonium, and potassium levels. Shintani and Ube [26] and Shintani [27] reported on the determination of sodium, ammonium, and potassium in serum using different ion chromatographic schemes. Using non-suppressed IC, it was demonstrated that serum cations can be determined simultaneously with serum anions and uremic toxins [26]. Separations were achieved on a Wescan cation-exchanger column with phosphoric acid as mobile phase run at 1.0 ml min^{-1} flow-rate. Serum was ultrafiltered and injected undiluted except for sodium determination which required 100 times dilution. Conversion of urea to ammonium ions was carried out with immobilized urease which was contained in a column preceding the injection port.

More recently, Thienport et al. [23] reported that ion chromatography can be considered as a valuable reference methodology for the determination of the total concentrations of sodium and potassium in human serum. Determinations by these authors were made with a conductivity detector with sample pretreatment consisting of acidic dilution and filtration. Methods for the individual and simultaneous determination of both analytes were investigated. The effect of the calibration method used, either single point or standard curve, on method imprecision was studied. The best performance was achieved by performing separate analyses each using single-point calibration with a bracketing analysis scheme. Under these conditions, the mean total coefficient of variation for sodium and potassium was 1.0%. The mean method bias was −0.2% for sodium and 2% for potassium as determined with three control materials obtained from the National Institute of Standards and Technology (NIST). The IC results obtained by Thienport et al. compared well with those obtained by reference methods based on flame atomic-emission spectrometry. In another recent study Watanabe et al. [24] reported that a small potentiometric ion-sensing probe (an ion-sensitive field effect transistor) could be used as a detector for the ion chromatographic determination of sodium and potassium in blood serum. A good comparison between the IC results and those obtained using a flame-photometric method was observed.

2.1.2. Analysis of magnesium (Mg^{2+}) and calcium (Ca^{2+})

Anderson [25] demonstrated the application of ion chromatography to the determination of divalent cations such as magnesium and calcium in blood serum. Since then, several other papers [28–30] have been reported on this topic. Smith [28] reported a single-column ion chromatographic method for the determination of the total and free ionic concentrations of calcium and magnesium in serum. In this paper, the separation of the two cations was achieved on a sulfonated polysytrene–divinylbenzene column with 0.12 mol l^{-1} $HClO_4$ as eluent. The ions were detected spectrophotometrically at 590 nm following post-column reaction with Arsenazo I. The method is reported to be rapid, reproducible, accurate and relatively free of interference. The total concentration of Mg^{2+} and Ca^{2+} in blood serum was

determined by sample acidification followed by centrifugation. The liquid layer was then injected into the chromatograph for the determination of analytes. For the determination of the free concentrations of calcium and magnesium, the ions were separated from the protein matrix using a small column filled with XAD-4 resin sulfonated to a capacity of 2.8 mequiv. g^{-1}. Elution from the small resin column was accomplished using 4 mol l^{-1} HCl at a flow-rate of 35 ml min^{-1}. The eluate was buffered to pH 10 prior to injection into the ion chromatograph.

Thienport et al. [29] reported on the development of an ion chromatographic method with conductivity detection as a reference method for the determination of the total concentrations of magnesium and calcium in blood serum. Serum samples were acidified and filtered prior to injection into the chromatograph. The mean total coefficients of variation for Mg^{2+} and Ca^{2+} were reported at 0.91 and 1.2%, respectively. The mean bias was found to be +0.06% for calcium and −0.35% for magnesium, as determined with five control materials from the Community Bureau of References and NIST.

2.1.3. Analysis of oxalate ($C_2O_4^{2-}$)

Skogerboe et al. [20] described a suppressed ion chromatographic method for the determination of oxalate in blood plasma. Samples were analyzed on a Dionex Model 4500i LCM2 which included a dual gradient pump and a CDM2 conductivity detector. Separation of oxalate was achieved on a Dionex AS4A anion separator column preceded by an AG4A anion guard column. A mixture of 0.85 mmol l^{-1} HCO_3^- and 1.0 mmol l^{-1} CO_3^{2-} was used as an eluent at a flow-rate of 2.0 ml min^{-1}. Eluent conductivity was suppressed by an anion micromembrane suppressor (AMMS). Sample treatment included deproteination of acidified blood plasma samples which were then filtered through a cation-exchange resin in the Ag^+ form (Dionex ONGUARD-Ag) to remove chloride. The detection limit of the IC method was 0.5 μmol l^{-1} or 4.4 ng oxalate measured using a sensitivity of 0.3 μS full scale. The oxalate recovery in plasma samples was found to be 93±18% (n=4 trials) using the method of standard addition. The IC method compared well with enzymatic determination. Several other papers have also reported on the determination of blood plasma oxalate [31–33].

2.1.4. Analysis of chloride (Cl^-), phosphate (HPO_4^{2-}) and sulfate (SO_4^{2-})

Sulfate and phosphate, together with chloride, constitute the major anions of blood. A knowledge of the turnover of some of these ions may be helpful in understanding their roles in certain clinical conditions. For example, the determination of the concentration of serum sulfate and urinary sulfate excretion may be useful in the understanding of sulfate conjugation or metabolism, especially after administration of sulfa drugs [34]. Ion chromatography has been established as a workhorse instrument for the determination of common anions, such as chloride, bromide, nitrite, phosphate, sulfate, oxalate, etc., in a large number of matrices including biological fluids. Several papers, as summarized below, have reported on the determination of chloride, sulfate, phosphate, and other common anions in human blood serum.

Salas-Auvert et al. [35] have reported on the simultaneous determination of chloride, phosphate, nitrate, and sulfate in blood serum by suppressed ion chromatography. Samples were analyzed on a Dionex Model 2000i/SP ion chromatograph equipped with a conductivity detector. The IC conditions used included a Dionex IonPac AG4A guard column, an AS4A anion separator column, and an anion micromembrane suppressor, AMMS-II. A mixture of 1.7 mmol l^{-1} $NaHCO_3$ and 1.8 mmol l^{-1} Na_2CO_3 was used as eluent at a flow-rate of 2.0 ml min^{-1}. The relative standard deviation value for each anion was less than 1.5%. Analyses were made of human, black vulture, and chicken blood sera without any pretreatment which necessitated some column maintenance.

Reiter et al. [36] reported a similar method for the determination of inorganic sulfate in human serum. These authors reported that treatment of serum samples with perchloric acid was an effective method of protein precipitation. Residual perchloric acid, after protein removal, was removed by potassium carbonate. This sample treatment procedure generated serum samples which no longer affected the IC column. De-proteinated serum samples were diluted 200 times prior to injection into a Dionex 2000i system which was calibrated daily for sulfate stan-

dards containing 1.8–14 μmol l^{-1} sulfate. The recovery of added sulfate ranged from 86 to 113%. Their method of serum sulfate determination compared well with determinations made using turbidimetry and atomic absorption spectrometry techniques.

Hoffman et al. [34] reported a single column ion chromatographic method for the determination of inorganic sulfate in human plasma. Plasma samples were ultrafiltered to remove proteins and diluted prior to sample injection into the chromatograph. The method, described as simple, fast, sensitive, and reproducible, was applied to study the effect of subchronic administration of acetominophen on the plasma concentration and urinary excretion of inorganic sulfate in healthy volunteers. A similar method was reported by Politi et al. [32] for the rapid determination of phosphate and sulfate together with oxalate in blood serum. Samples were deproteinated by passing through a Centrifree filter by centrifugation before injection into an ion chromatograph equipped with anion-exchange column.

Michigami et al. [37] used an ODS (octadecyl silica) column dynamically coated with cetylpyridinium chloride for the ion chromatographic determination of sulfate simultaneously with nitrite, bromide, and nitrate in human blood serum. Interfering proteins were removed from serum by filtration through an ultrafilter-paper. Inorganic and organic anions commonly found in serum had no effect on the determination of sulfate and other ions of interest. The anions were separated using a 1.0 mmol l^{-1} citrate solution, containing 2.5% methanol, buffered to pH 6.5. The method employed UV detection and had a reported sulfate recovery of 94–106%.

2.1.5. Analysis of bicarbonate (HCO_3^-) and organic acids

Ion-exclusion chromatography is the method of choice for the determination of bicarbonate [38] in blood plasma. Ion-exclusion chromatography coupled with IC is also used for the separation and determination of some organic acids, such as lactate and pyruvate, in blood plasma. The determination of these species in blood is reported to be clinically important. For example, bicarbonate plays a significant role in the transport of carbon dioxide and in the pH management of blood. Plasma lactate is important in the diagnosis of lactic acidosis in diabetic patients and in the prognosis of acute myocardial infarction complicated by shock. Plasma pyruvate is reported to be useful in the diagnosis of thiamine deficiency and heavy metal poisoning.

Through the use of a Dionex HPICE-AS1 analytical column, Kreling and DeZwaan [38] were able to quantify bicarbonate in blood plasma. Analyses were made on a Dionex Model 2010i ion chromatograph with a conductivity detector. The ion chromatographic method was reported to be very selective and free of interference. Doubly-distilled water that was degassed and continuously purged with helium was used as eluent at a flow-rate of 1.5 ml min^{-1}. The method was able to determine bicarbonate levels in blood plasma to a limit of 0.1 mmol l^{-1}. No sample preparation or handling prior to analysis was required, except for sample dilution with water. Results using the IC method compared well with those obtained using conventional titrimetric analysis. Good precision, small sample requirements, reliability and ease of operation were listed as additional advantages of the IC method.

Shintani and Ube [26] described a non-suppressed IC method for the determination of carbonate in blood serum. Serum was ultrafiltered and diluted by 2 times before injection into a Wescan anion-exchange column. A 4.0 mmol l^{-1} potassium phthalate solution adjusted to pH 4.5 was used as eluent.

Rich et al. [39] reported the use of a hyphenated technique for the determination of pyruvate and lactate in blood plasma. The authors used ion-exclusion chromatography coupled to ion chromatography and concluded that the ion-exclusion chromatographic–IC method may be used as a clinical diagnostic tool for the assay. Pyruvate and lactate were extracted from de-proteinized plasma by the use of ion-exclusion resin. After elution from the resin, the plasma extract is chromatographed on an anion-exchange column with 0.66 mmol l^{-1} sodium carbonate as mobile phase. Peaks were detected with a conductivity detector after suppression of eluent's conductivity. As little as 100 ng of pyruvate could be detected in a single 0.2-ml sample.

2.1.6. Analysis of other ionic species

2.1.6.1. Alkanesulfonates

Moro et al. [40] reported a rapid, specific and reproducible method for the determination of 1,4-

butanedisulfonate in blood plasma. The method initially requires the removal of proteins by precipitation with perchloric acid, followed by the removal of perchlorate ions by addition of potassium carbonate. IC, carried out using conductivity detection, gave linear calibration over the concentration range of 2.5–25 μg ml^{-1}. The intra- and inter-assay percent relative standard deviations were, respectively, 3.6 and 5.8%. The analyte was found to be stable in plasma and in perchloric acid at 37°C for 24 h. The IC method was applied to monitoring plasma levels in animals receiving chronic intravenous oral administration of 1,4-butanedisulfonate.

2.1.6.2. Bromide

Wong et al. [41] used plasma bromide dilution for the determination of extracellular body water. Separation and determination of bromide was carried out by IC with UV detection at 210 nm. Sample preparation required removal of plasma proteins by ultracentrifugation. Interference by the large concentration of plasma chloride was overcome by sample dilution and the use of 5 mmol l^{-1} NaCl as eluent. Human plasma samples were spiked with known quantities of bromide in the range of 37.54–125.14 μmol l^{-1} and were determined by ion chromatography. The results were reproducible within 0.72 nmol l^{-1} (S.D.) and differed from results obtained using gravimetry by -1.88 ± 4.27 nmol l^{-1} (mean±S.D.). The difference between the IC results and those of the gravimetric analysis was not statistically significant ($p=0.19$). Results of the extracellular water volumes of 10 newborn minipigs made by the plasma bromide dilution IC method were found to be 400 ± 63 ml kg^{-1}, comparable with literature values reported for premature infants. In another paper Abuku et al. [42] reported on the determination of bromide ions in total blood and plasma by IC with amperometric detection. No sample preparation was required except dilution by 10–20 times with eluent. Calibration curves were obtained in the range 0.05–5 μg ml^{-1} of bromide ions. IC results of bromide determination in total blood and plasma compared well with those obtained by neutron activation analysis.

2.1.6.3. Nitrite and nitrate

Everett et al. [43] developed a versatile method for the simultaneous determination of nitrite and nitrate in plasma by IC using spectrophotometric detection at 214 nm. The method compared well with capillary electrophoresis, exhibiting an equivalent sensitivity for each ion. Total run times for the IC method were reported at less than 4 min, shorter than that required for analysis by capillary electrophoresis.

2.1.6.4. Trichloroacetate

Itoh [44] developed an IC method for the determination of trichloroacetate in human serum. Two short (50×5 mm I.D.) low-capacity anion-exchange columns were placed in series and used as the analytical column. Elution and separation of trichloroacetate in human serum were obtained with a mixture of 35 mmol l^{-1} NaOH, 20 mmol l^{-1} *p*-hydroxybenzonitrile, and 2% (v/v) acetonitrile (pH 12.3) as eluent. The calibration curve was rectilinear from 0.3 to 33 mg l^{-1} of trichloroacetate at 10-μS sensitivity with a detection limit (3σ) of 0.08 mg l^{-1}. The recovery of trichloroacetate added to serum samples ranged from 98.2 to 101%, with a relative standard deviation (R.S.D.) less than 3.0%.

2.1.6.5. Bisphosphonates

Using a high-performance ion chromatograph and a Dionex AS7 column with nitric acid as the mobile phase, Daley-Yates et al. [45] reported that 1-hydroxy-3-aminopropylidene-1,1-bisphosphonate and related compounds in human urine and plasma may be determined. Following precipitation with calcium chloride and centrifugation, the extracted bisphosphonates were redissolved in acetic acid prior to separation. The analytes were oxidized to orthophosphate by the post-column addition of ammonium persulfate. A second post-column reaction was performed using molybdenum ascorbate to yield the phosphomolybdate chromophore detected at 280 nm. The authors reported a detection limit of 10 ng ml^{-1}.

2.2. Analysis of urine

The determination of major and minor ionic species, such as oxalate, citrate, isocitrate, phosphate, sulfate, chloride, sodium, ammonium, potassium, magnesium, and calcium, in urine is of considerable importance. The concentrations of some urinary species, such as organic acids, may be correlated with the presence of various, specific diseases. In addition, the concentration of urinary ions may be

monitored to study the efficacy of a particular diet or drug therapy and the determination of the total concentrations of the major and minor ionic species, such as those cited above, is necessary for the calculation of the supersaturation indices, the driving forces, for the formation and nucleation of kidney stone minerals, such as calcium oxalate and calcium phosphate.

2.2.1. Determination of oxalate ($C_2O_4^{2-}$)

Analysis of the oxalate anion in urine has been recognized as one of the most important determinations in urolithiasis research. Significantly higher levels of urinary oxalate have been reported for patients with active kidney stone disease than in normal, control subjects. The determination of oxalate is also important for the calculation of the supersaturation of a urine sample with respect to kidney stone minerals, including the calcium oxalates and phosphates. The calculation of urinary supersaturation is also essential when modeling mineralization and demineralization processes of urinary samples in vitro.

There are several reports on the ion chromatographic determination of urinary oxalate. Mahle and Menon [46] performed the initial method development, using suppressed ion chromatography. These authors used two anion separator columns, along with a cation-exchange column as the suppressor, to resolve the oxalate peak in a urine sample. The total analysis time for oxalate determination by their method was approximately 35 min. Later methodology, however, showed considerable improvement in reducing total analysis time while maintaining accuracy and peak resolution due mainly to the advancements in column/separations technology and in IC instrumentation. For example, Robertson et al. [47] reported that oxalate could be resolved in 17 min by the use of an HPIC anion G4 guard column in series with an anion S4 separator and fiber suppressor columns. By their method, oxalate in diluted urine samples could be analyzed in approximately 20 min, with an intra-run R.S.D. of 3.8%. Overall intra- and inter-run R.S.D.s, inclusive of the sampling and sample (urine) dilution, were found to be 6.5 and 8.3%, respectively. Standard addition recoveries, carried out through the addition of 300 μmol l^{-1} sodium oxalate to each of 10 urine samples, showed a mean recovery of 102±6.8%, with a 6.7% R.S.D. Singh and Nancollas [48] also reported a rapid method for the analysis of urinary oxalate. Providing good accuracy and precision (mean recovery 100.6±2.7%), the suppressed IC method required only 15 min of analysis time and minimal sample preparation. Urine samples were acidified and diluted 100-fold. Diluted urine samples were filtered through 0.22-μm Millipore filters before injection into a Dionex QIC analyzer. The minimum detectable oxalate in urine was 0.11×10^{-3} mol l^{-1} with a relative standard deviation of about 10%. Good agreement was found between the IC method and the use of isotope dilution for urinary samples containing widely varying oxalate concentrations. Schwille et al. [32], Von Schnakenburg et al. [49], Singh [50] and Toyota [51] have also reported on oxalate determination in urine.

2.2.2. Analysis of phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) and oxalate ($C_2O_4^{2-}$)

Determination of the total concentrations of anions, such as chloride, phosphate, sulfate, and oxalate, in urine is required for the calculation of the free ionic concentrations for the species of interest in nephrolithiasis research. Singh and Nancollas [52] developed a method for the simultaneous determination of urinary phosphate, sulfate, and oxalate. By using a Dionex HPIC AG3 guard column, an HPIC AS3 anion separator column, and an anion micromembrane suppressor, all three ions could be analyzed within 20 min. The eluent, a mixture of 2.42 mmol l^{-1} $NaHCO_3$ and 1.81 mmol l^{-1} Na_2CO_3, was run isocratically at 132 ml h^{-1}. Urine samples were diluted 40-fold and filtered through 0.22-μm Millipore filters before injection into IC. The limit of detection for oxalate was 5.0×10^{-6} mol l^{-1} in urine (prior to dilution) with an R.S.D. of less than 10%. Methods for the determination of urinary oxalate [32], phosphate, sulfate, and oxalate [31], as well as for the simultaneous determination of sulfate, nitrite, bromide, and nitrate [37] have also been reported.

2.2.3. Analysis of citrate and isocitrate

Due to its strong affinity for ion-exchange resins, the determination of urinary citrate levels via suppressed IC techniques is most efficiently performed using an exchange column of short length. Singh and Nancollas [53] developed IC conditions for the

accurate determination of urinary citrate. The citrate peak, which eluted at 8 min, was clearly separated from the other matrix anions (chloride, phosphate, sulfate, and oxalate) which co-eluted about 2 min after injection. Confirmation of the citrate peak was obtained by treating the sample with citrate lyase prior to injection. The suppressed IC conditions used included a Dionex AG3 analytical column with 8.0 m*M* Na_2CO_3 as eluent at a flow-rate of 2.2 ml min^{-1}. Run on a Dionex QIC IC, the analyses were performed by injecting 100 μl of 50- or 100-fold diluted urine samples. The minimum amount of urinary citrate that could be detected by this method was 0.05 mmol l^{-1}.

Singh et al. [54] also developed a suppressed IC method for the simultaneous determination of citrate and isocitrate in urine. Accuracy of this method was assessed by the method of standard addition. Detection limits for each anion were found to be sufficient for the determination of citrate and isocitrate in a wide variety of urine samples obtained from male and female human subjects, without any sample preconcentration. Ogawa et al. [55] have also reported on the determination of urinary citrate.

2.2.4. Analysis of sodium (Na^+), ammonium (NH_4^+) and potassium (K^+)

The determination of the total concentrations of sodium, potassium, and ammonium is required for correct urinary speciation calculations, as these three cations form stable ion pairs with anions, such as oxalate, sulfate, and phosphate. Sodium, potassium, and ammonium, together with major anions, such as chloride, sulfate, and phosphate, also constitute a major part of the ionic strength of urine. Samples were diluted before injection into the IC. Small et al. [1], in the very first paper on IC, demonstrated the application of technique for the determination of urinary Na^+, K^+, and NH_4^+ ions. Anderson [25] later also demonstrated the use of suppressed IC for the determination of these ions in urine. Since then there has been a major improvement in the determination of these species by IC, especially in total analysis time, accuracy, and precision.

2.2.5. Analysis of magnesium (Mg^{2+}) and calcium (Ca^{2+})

Although IC methods for the determination of small concentrations of magnesium and calcium in the presence of a large concentration of sodium chloride are well developed, there is no report on the analysis of urinary magnesium and calcium levels by IC. The IC methods typically developed to analyze the concentrations of the two alkaline earth cations in plasma [25,28–30] and water [56] may be extended for their quantification in a urine sample.

2.2.6. Analysis of other ions

2.2.6.1. Determination of bromide

Using amperometric detection, Abuku et al. [42] developed an IC method for the determination of urinary bromide. Calibration curves were obtained in the range of 0.05–5 mg l^{-1} bromide and the coefficient of variation was calculated in the range of 1.3–9.4%, with a recovery of over 93% of added bromide. The method was found to compare well with quantification using spectrophotometry. Moore et al. [57] used an IC method for the determination of bromide in the urine of Greyhound dogs. The urine samples obtained from 103 racing Greyhounds resulted in a mean of 3.6±2.72 mg l^{-1} bromide. Urine samples from a Greyhound to which 2 g KBr was administered, yielded a diurnal pattern with a half-life of 7.6 days.

2.2.6.2. Analysis of nitrite, bromide, nitrate and thiocyanate

Michigami et al. [37,58] developed an IC method with ultraviolet detection for the simultaneous determination of nitrite, bromide, nitrate, and sulfate using an ODS (octadecyl silica) column dynamically coated with cetylpyridinium chloride. The eluent was comprised of a 1.0 mmol l^{-1} citrate solution in 2.5% methanol at pH 6.5. The results of thiocyanate quantification by IC compared well with those obtained using a spectrophotometric method based on thiocyanate complexation with Fe^{3+}. The method was employed for the determination of thiocyanate in the urine of smokers and non-smokers [58]. The data indicated that the urinary thiocyanate level of smokers was significantly higher than that of non-smokers.

2.2.6.3. Analysis of thiosulfate

A suppressed IC method for the determination of urinary thiosulfate has also been reported [59]. The authors describe a column-switching technique which allows for sensitive and accurate detection. The method provides interference-free detection, with a sensitivity of less than 0.2 nmol per 24 h urine, allowing for the detection of thiosulfate in the study of sulfur metabolism.

2.2.6.4. Analysis of arsenic and related compounds

The coupling of inductively-coupled plasma-mass spectrometry (ICP-MS) with IC has been used successfully to determine arsenic compounds such as arsenious acid, monomethylarsinic acid, dimethylarsinic acid, trimethylarsine oxide, arsenobetaine, and disodium arsenate [60]. The authors report detection limits in excess of 0.045 μg l^{-1} and relative standard deviations better than 5% when using two Excelpak ICS-A35 columns packed with a polymer-based hydrophilic anion-exchange resin and an eluent consisting of 10 mmol l^{-1} tartaric acid adjusted to pH 3.5. A similar method has been reported by Sheppard et al. [61] for the determination of As^{3+}, As^{5+}, dimethylarsinic acid, and methylarsonic acid. A single separator column was used in conjunction with ICP-MS. These authors reported that sensitivity could be improved in two ways: (1) by reducing the mass spectral interferent $ArCl^{+}$ via chromatographic resolution of the chloride from the negatively charged arsenic species; and/or (2) by using an He–Ar mixed gas plasma as the ionization source. It should be noted that the intensity of the $ArCl^{+}$ interference was increased with the use of the mixed gas plasma.

2.2.6.5. Determination of organic acids and bicarbonate (HCO_3^-)

For the accurate determination of the anions of weak organic acids such as vanillylmandelate and carbonate, ion-exclusion chromatography is, by far, the method of choice. By using water and HCl or octanesulfonic acid (1–1.5 mmol l^{-1} at 0.8–1.0 ml min^{-1}) as eluent, these anions can be separated on an ion-exclusion column containing a high-capacity sulfonated ion-exchange resin. Rich et al. [39] used IC coupled to ion-exclusion chromatography for the determination of urinary vanillylmandelate. Vanillylmandelic acid was extracted from diluted urine by the use of an anion-exchange resin. After elution from resin, the urine extract was chromatographed on an ion-exclusion column. Peaks were detected on a suppressed conductivity detection system. The results of urinary vanillylmandelic acid determination by IC compared well with those obtained by other standard methods.

Although the determination of urinary carbonate has not been reported to date, ion-exclusion chromatography using water as eluent may be the method of choice, as reported by Kreling and DeZwaan [38] for other biological fluids.

2.3. Other biological fluids

2.3.1. Analysis of saliva

Methods for the detection of selected analytes in human saliva [37,58,62] have been reported. Matsushita [62] has demonstrated that IC as an analytical tool can be applied to the analysis of saliva. The author employed a single column method for the simultaneous separation of inorganic anions and metallic cations using eluents containing chelating agents. EDTA, CDTA, and GEDTA were each tested for ease of use and speed of analysis. EDTA was found to be a more successful eluent than GEDTA or CDTA and resulted in successful separation. While conductimetric detection was successful for all of the 21 analytes determined, UV detection was found to have the lower detection limits.

2.3.2. Analysis of tear fluid

Buchberger and Rieger [22] reported the use of non-suppressed IC with indirect UV detection as a successful method for the determination of cations and anions 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 mmol l^{-1} cerium(III) sulfate solution. The tube was centrifuged at 800 *g* and 2 μl of this solution were further diluted with 50 μl of 0.05 mmol l^{-1} cerium(III) sulfate solution for the determination of sodium and potassium by cation chromatography. The remaining solution was used for the determination of magnesium and calcium also

by cation chromatography. The eluate of this injection (Ca and Mg) after 10 s 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 the determination of anions. Anion chromatography was carried on a 50×6-mm I.D. Polyspher IC AN column. The mobile phase, a 0.5 mmol l^{-1} potassium hydrogenphthalate (containing 53 mmol l^{-1} ethylene glycol)–2-propanol (1000:27), was used at 2 ml min^{-1}. Peaks were detected by indirect UV detection at 260 nm. A 100×3.2-mm I.D. Polyspher IC CA column (Merck) was used for cations determination. Eluent, containing 0.05 mmol l^{-1} cerium(III) sulfate solution (prepared from cerium(III) sulfate octahydrate) was run at 1 ml min^{-1} and peaks were detected by indirect UV detection at 254 nm. The limits of detection (all in mg l^{-1}) were about 0.07, 0.12, 0.25 and 0.6, respectively, for sodium, potassium, magnesium and calcium.

Salas-Auvert et al. [35] have also reported the application of IC method for the analysis of human and animal tear fluid for chloride, nitrate, sulfate, and phosphate.

2.3.3. Analysis of sweat

Cole and Landry [19] described an IC method for the analysis of sulfate in sweat. Two adsorbent filter discs (after sulfate decontamination) were removed from their weighing bottles and placed under a Parafilm covering that was then carefully taped to the arm. After 45 min, the sweat-laden discs were returned to the weighing bottle and total mass was recorded. Mass of collected sweat was determined after the subtraction of dry mass (in the bottle). If the mass of collected sweat was less than 50 mg, it was discarded; when it was less than 100 mg, it was pooled with the matching sample from the opposite forearm. Four ml of 1.0 mmol l^{-1} sodium hydroxide were added to the weighing bottles (containing the discs) for the elution of sulfate and other ionic species of sweat. Elution was carried out over at least 10 min at room temperature, with occasional agitation of the discs in the solution. The sodium hydroxide eluate was decanted and spun at 1000 *g* for 5 min to remove any cellulose fibers loosened from the discs. An aliquot of the supernatant was immediately injected into a Dionex D-10 ion chromatograph equipped with two 50×4-mm guard columns, a 250×4 mm anion separator column and a fixed-bed 100×9 mm s1 suppressor column in series. The mean concentration of sulfate in sweat from 22 volunteers was 80±3 μmol l^{-1} (n=39; range 35–128 μmol l^{-1}), while matching serum sulfate concentration was 299±25 μmol l^{-1}.

3. Ion chemistry

In this section we have included the applications of IC used in the understanding of ion chemistry of biological fluids. Specifically, it deals with the physical chemistry aspects related to nephrolithiasis and dental research, such as speciation, driving force for crystals formation and crystallization, and pathophysiology.

3.1. IC application in nephrolithiasis research

3.1.1. Driving force for mineralization

The formation of kidney stones within the urinary tract has become increasingly important despite substantial efforts made by researchers and clinicians worldwide over the years. Uroliths are most commonly composed of calcium oxalate mineral phases; however, mixed stones comprised of calcium phosphate, as well as oxalate phases, are also frequently found. No single factor has been identified as key to separate patients with idiopathic stone disease from normal subjects. The results of both clinical and non-clinical studies suggest that the likelihood of stone formation can be related much more readily to the free ionic concentrations of stone-forming ions present in urine, i.e. to the supersaturation of urine with respect to calcium oxalate and calcium phosphate phases. Therefore an estimation of the driving force for mineralization can provide a diagnostic tool for such clinical conditions. The calculation of the mineralization driving force typically requires the measurement of the total concentrations of ions, such as chloride, phosphate, sulfate, citrate, carbonate, sodium, ammonium, potassium, hydronium, magnesium, and calcium. By correcting the calcium, oxalate, and phosphate ion concentrations with respect to the formation of important ion pairs [21,63], the free concentrations of these ions can be esti-

mated. These values, in turn, can then be used for the calculation of the true driving force (dG) for the biomineralization processes of calcium oxalate and/or calcium phosphate crystal growth, as expressed by Eq. (1) and Eq. (2).

$$dG = -\Delta G \quad (1)$$

$$-\Delta G = (RT/n) \ln SS \quad (2)$$

where R is the gas constant, T = temperature in Kelvin, n = number of ions in a molecule of the mineral phase, and SS is the supersaturation ratio. Several speciation (computational) programs for the accurate calculation of urinary supersaturation with respect to calcium oxalate and phosphate phases have been reported [64]. Since IC is able to provide rapid and accurate determinations of all ions required for the supersaturation calculations (with the exception of hydronium ion quantification), the technique can be and has been employed as a major tool aiding nephrolithiasis research.

3.1.2. Crystallization studies of calcium phosphate and oxalate phases

The factors important for renal stone formation may be studied in vitro using crystallization methods [48,65–68]. These kinetic studies require a rapid and reliable method for the analysis of oxalate, phosphate and calcium levels in crystallization media. IC has been established as the method of choice for such applications because of its simultaneous detection of several ions with little or no sample preparation.

3.2. IC application in dental research

The formation of tartar, as well as that of tooth enamel, can be correlated to the driving forces (or supersaturation indices) for the precipitation of dicalcium phosphate dihydrate (DCPD) and fluoroapatite, respectively, in human saliva. IC is the established method for the determination of the ions required to calculate the free concentrations of the ionic constituents of DCPD and fluoroapatite [69]. IC can also be used as a tool to study effectively the kinetics of DCPD and enamel formation in vitro.

3.3. Pathophysiology

IC has been useful in elucidating oxalate pathophysiology, especially in understanding the renal handling of oxalate [32]. The technique has proved reliable for assessing the levels of plasma oxalate in ultrafiltrates. IC has also been used for the screening of citrate and D-isocitrate [54] in different groups of human subjects. Citrate and/or D-isocitrate have been identified as the chelators of calcium [70] and inhibitors of calcium oxalate and calcium phosphate crystal growth [71–74] and, therefore, their concentrations in urine can be correlated with the potential for kidney stone formation.

Everett et al. [43] described a versatile method for the simultaneous measurement of nitrite and nitrate anions in both plasma and isolated tumor models based on anion-exchange chromatography with spectrophotometric detection at 214 nm. In the authors' view, the analysis of nitric oxide-derived nitrite and nitrate ions in biological fluids represents a proven strategy for determining nitric oxide participation in a diverse range of physiological and pathophysiological processes in vivo.

4. Critical comments and concluding remarks

Based on the results presented in this review, the accurate analysis of desired analytes in biological fluids can be carried out by IC. Detectors for various ions present in concentrations ranging from trace to major are varied and include conductivity, UV–Vis spectrophotometry, and amperometry. Ion-exclusion chromatography has been reported as the method of choice for the determination of carbonate and other important organic acids. In virtually all of the IC methods, sample preparation consisted mostly of dilution. In only one case, NaCl was removed for the determination of oxalate when detection was carried out using suppressed conductivity. For the analysis of blood, plasma and serum protein removal was required more frequently; however, one paper reported the determination of analytes without its removal [75].

IC also offers applications in research and clinical studies related to the understanding of ion chemistry of various biological fluids. It can be used as a

diagnostic tool in both clinical and research applications. The determination of ion concentrations of certain analytes in blood or urine can be used as an important assessment for a number of clinical conditions either to diagnose disease or to monitor the success of a treatment or therapy. Together with speciation computational methods it can be used to determine supersaturation index for the formation of principal kidney stone minerals, such as the calcium oxalates and calcium phosphates, as well as indications for the clinical conditions of hypercalciuria and hyperoxaluria. It can also be used to calculate the driving force for the formation of tartar and enamel in control patients, as well as those prone to caries.

The technique fulfils several requirements of a reliable microanalytical method by providing sufficient speed, automation, ease of use, accuracy and sensitivity. Detection limits normally are not an issue as most major cations and anions in various biological fluids are present at mg l^{-1} or higher concentrations. The only loss in sensitivity during simultaneous determination of major cations or major anions come from the sample dilution required to get baseline resolution of analyte peaks. For example, as can be seen in Fig. 1, ammonium ion peak height became quite small after 400 times dilution, although its concentration in undiluted urine was about 15 mmol l^{-1}. Sensitivity for various ionic species, which can be determined by IC, in pure solutions is normally quite good as many ions can be detected at nanogram or lower levels.

The main limitation of IC concerning sensitivity (in the analysis of biological fluids) is the achievement of the baseline resolution of a small (trace)-concentration ion peak in the presence of large-concentration ion peak. For example, in a urine sample it is sometime difficult to achieve baseline resolution of a small-concentration oxalate ion peak (corresponding to a concentration of ~40 mg l^{-1}) in the presence of a large-concentration sulfate ion peak (~4000 mg l^{-1}). This limitation significantly reduces the detection limit of urinary oxalate. In pure solution, oxalate can be quantified by IC down to a concentration of 10 μg l^{-1} without any sample preconcentration. In normal urine, however, the detection limit is only 2–4 ppm. Sample dilution becomes necessary for baseline resolution of the two

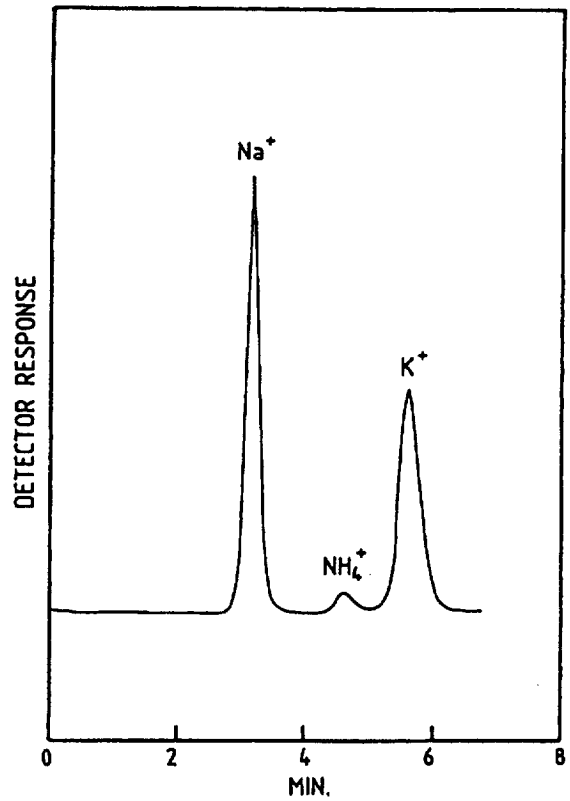

Fig. 1. Ion chromatogram showing the peaks of sodium, ammonium, and potassium in a 400 times diluted urine sample. Dionex 2120i ion chromatograph; injection volume, 250 μl; detector sensitivity, 10 μS full scale; analytical column, Dionex 2 AG-4 columns; eluent, 0.005 mol l^{-1} HCl; flow-rate, 2.0 ml min^{-1}; Peak integrator, Dionex 4217 (Singh and Abbas, unpublished work).

anion peaks containing small and large concentrations. Fortunately, in most cases, when urinary oxalate concentration is low, the concentrations of other ions, such as sulfate, are also low and IC may be successfully used to determine urinary oxalate levels [48]. In cases where high sulfate concentration interferes, the determination of oxalate may be carried out by removing the sulfate ions as barium sulfate precipitate from the sample as reported by Toyota [51]. However, care should be taken when using this procedure, as some oxalate may be lost as solid barium oxalate because barium oxalate is only

sparingly soluble. The loss of oxalate (if any) can be easily determined using the procedure for oxalate determination in synthetic solutions containing known concentrations of oxalate and sulfate. The determination of oxalate by using such a procedure may be more accurately carried out if added barium concentration does not exceed urinary sulfate concentration and that only a partial sulfate removal is carried out. Sulfate removal by barium sulfate precipitation method may also be achieved by passing the sample through a cation-exchange column, in the barium form, which would selectively remove sulfate ions without affecting the oxalate concentration. The determination of small or trace concentration of oxalate may also be performed by the selective concentration of oxalate on alumina [50]. During this preconcentration step, however, a large percentage (up to 80%) of the urinary sulfate is co-adsorbed onto the alumina, thus limiting the method's efficiency. The removal of chloride can be most efficiently achieved by passing the sample through a cation-exchange column in the silver form [25].

With the development of improved separator columns and IC instrumentation, rapid analysis of many urinary anions can be carried out simultaneously, as shown in Fig. 2. For common cations and anions, rapid IC analysis can be obtained by developing methods for their simultaneous determination. The simultaneous determination of cations and anions in biological solutions is not only advantageous but in some cases it is necessary. For example, some biological samples, such as tear fluid, are insufficient for two analyses, one for the cations and one for the anions. In order to meet such a requirement, two types of approach have been used. Approach one exploits changes in instruments, such as the use of two or more cation- and anion-separation columns [76–80] or a single mixed-bed ion-exchange column [81]. The other approach is based upon the development of suitable mobile phases, such as the use of a complexing agent as the mobile phase [62,82,83]. According to this approach, simultaneous determinations of major anions, such as chloride and sulfate, along with cations such as magnesium and calcium, in biological fluids (such as blood, urine, saliva, and sweat) may be carried out by suppressed or single-column IC with ethylene-

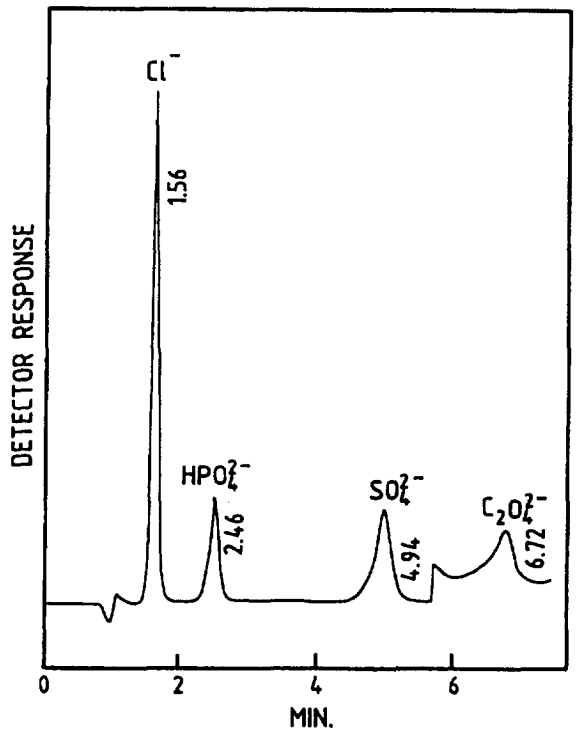

Fig. 2. Ion chromatogram showing the peaks of chloride, phosphate, sulfate and oxalate in a 200 times diluted urine sample. Dionex 2120i ion chromatograph; injection volume, 250 μl; detector sensitivity, 1000 μS full scale (for first three peaks) and 3 μS full scale (for oxalate peak); analytical column, Dionex AG-4 and AS-4; eluent, a mixture of 3.0 mmol l^{-1} $NaHCO_3$ and 2.4 mmol l^{-1} Na_2CO_3; flow-rate, 2.0 ml min^{-1}; Peak integrator, Dionex 4217 (Singh and Abbas, unpublished work).

diaminetetraacetic acid (EDTA) as eluent [82]. By interacting with EDTA, magnesium and calcium form anionic complexes and can be determined as anions simultaneously with chloride, phosphate and sulfate. Small sized samples can also be analyzed by using an approach similar to Buchberger and Rieger [22], as can be seen in Fig. 3.

Finally, we would like to emphasize (as also mentioned earlier in the Section 1) that while analytes routinely determined in biological matrices are not generally considered toxic themselves, the presence of dangerous bacteria in many biological solutions effectively makes them hazardous materials. Therefore, all normal safety procedures should

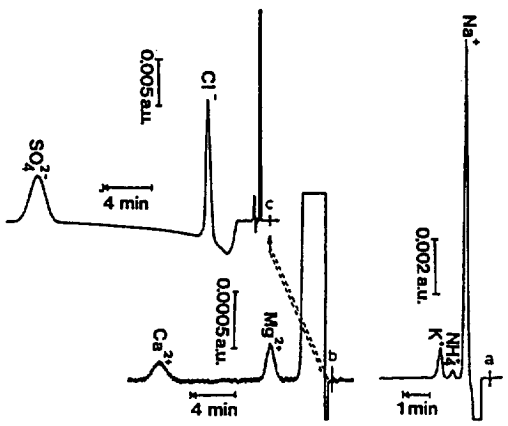

Fig. 3. Chromatogram of a tear fluid sample containing 2.62 g l^{-1} of sodium, 206 mg l^{-1} of potassium, 12.5 mg l^{-1} of magnesium, 25 mg l^{-1} of calcium and 3.94 g l^{-1} of chloride. (a) Sample diluted 1:416; (b) sample diluted 1:16; (c) anion chromatogram of the fraction collected from cation chromatography. Conditions for cation chromatography: column, Polyspher IC CA (100×3.2 mm I.D.); eluent, 0.05 m*M* cerium(III) sulfate solution; flow-rate, 1 ml min^{-1}; indirect UV detection at 254 nm; attenuation: 0.02 a.u.f.s. Conditions for anion chromatography: column, Polyspher IC AN (50×3.2 mm I.D.); eluent, 0.5 m*M* potassium hydrogenphthalate (containing 53 m*M* ethyleneglycol)-2-propanol (1000:27); flow-rate, 1 ml min^{-1}; indirect UV detection at 260 nm (reproduced from Ref. [22]).

be followed during the handling of various biological fluids.

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