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Review

Recent developments in ion chromatography

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Abstract

This paper summarizes how ion chromatography is now a multimode technique suitable for solving analytical problems in all areas of interest. Current and more recent applications will be overviewed within the new trends.

Keywords: Reviews; Chip technology; Miniaturization; Stationary phases, LC

Contents

1. Introduction

Ion chromatography (IC) can be defined as the widest expression of analytical chemistry, since IC represents both a tool for solving analytical problems in all areas of interest and, basically, equilibria, the core of analytical chemistry, which are the key factors for a separation. Equilibria play the principal role in IC and can be modulated as a function of the nature of the analytes, as such or modified, stationary phases and eluents. Through the choice of stationary phase and eluent composition the selectivity can be modulated, but the eluent must also meet the requirements of the detection system. In fact, advances in IC are strictly related to both good and highly selective separation and extremely low detection limits. In 1992, it was stated that ''Much of the practice of ion chromatography (IC) today is well-established science transformed into easily practiced technology; little art remains'' [1]. This declaration reminds us of the question put, each year, by jazz connoisseurs ''is jazz dead?'' But the answer is that they, and in a similar way the scientists working in the field of IC, continue to maintain this tradition and each year new theoretical and practical developments result from the research activities devoted to IC improvement.

More than 10 years ago, capillary electrophoresis (CE) appeared as a promising substitute for IC, mainly because of its higher speed of separation, but now IC remains the major analytical technique, and

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not only for inorganic species. IC offers greater sensitivity and analytical ruggedness; for example, by column switching and enrichment trap columns, detection of ppb or ppt concentrations can easily be achieved.

Ion exchange [2–6] moved to ion chromatography (IC) with Small [7] and later IC also increased its significance [8–12] when ion chromatography, mainly based on the ion-exchange mechanism, included ion-pair/ion-interaction chromatography, ion-exclusion chromatography [13], chelation-ion chromatography [14] and electrostatic chromatography [15]. Classic applications of IC are strictly associated with suppressed conductometric detection, and its evolution from suppressor beds to continuous electrochemically regenerated devices [16–22], and this has recently been summarized by the new concept *ion reflux* [23]. In the new IC method the water is the ''pumped phase'' and continuous eluent generation, ion separation, and suppression all occur within one ion-exchange bed. The concept of separation in IC has changed over time and not only are well-defined selectively separated peaks the goal of the technique but, in some cases, also a group, matrix removal is of interest, for example for the coupling of IC with new kinds of detection methods. Detection plays a relevant role for analytical purposes, and comprehensive reviews on detection techniques, coupled to IC, including atmospheric pressure ionization (API) MS [24,25], inductively coupled plasma (ICP) atomic emission spectrometry (AES) and ICP-MS [26], have recently been presented. Here, the discussion will mainly focus on the state of the art and advances in new stationary phases in studies on related equilibria [27], more recent applications of IC and the new trend, miniaturization [28].

2. Materials and applications

Examples of the evolution of stationary phases (both polymeric and inorganic exchangers) are considered below, since, as underlined above, the improvements in IC selectivity have mainly been obtained by developing new stationary phases and analytical methods.

The stationary phases used in IC have been polystyrene–divinylbenzene (PS–DVB) core particles surrounded by a monolayer of charged latex particles [8–12]. The use of a substrate based on ethylene–vinylbenzene (EVB) crosslinked with 55% divinylbenzene (DVB) resulted in solvent-compatible materials. Latex is usually vinylbenzyl chloride–divinylbenzene (VBC–DVB) or PS–DVB crosslinked for anion or cation exchange by incorporating different functional groups. Methacrylate-based latex anion resins are also produced, where glycidylmethacrylate (GMA) monomers are crosslinked with ethylene glycol dimethacrylate (EDM). Anion exchangers contain alkanol or alkyl quaternary amine functional groups and cation exchangers contain sulfonic, carboxylic, carboxylic/phosphonic, carboxylic/phosphonic/crown ether functional groups [27].

Differences in selectivity between VBC–DVB and GMA–EDM anion exchangers are exhibited mainly for more polarizable anions such as iodide or thiocyanate. Fig. 1 shows the suitability of a meth-

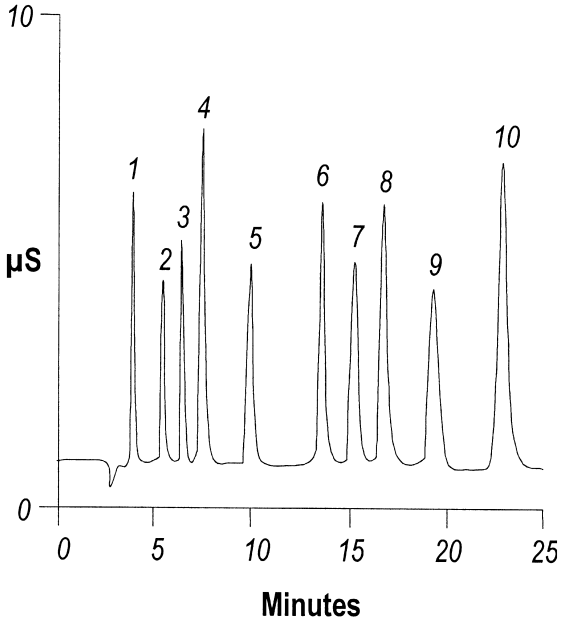

Fig. 1. Separation of oxyhalides and inorganic anions on a methacrylate-based, latex agglomerate. Column, IonPac AS9 HC; eluent, 9 m*M* sodium carbonate; flow-rate, 1.0 ml/min; detection, suppressed conductivity; injection volume, 25 μl. Solutes: 1=fluoride, 2=chloride, 3=bromate, 4=chlorite, 5=nitrite, 6=bromite, 7=chlorate, 8=nitrate, 9=phosphate, 10=sulfate. From Ref. [27] with permission.

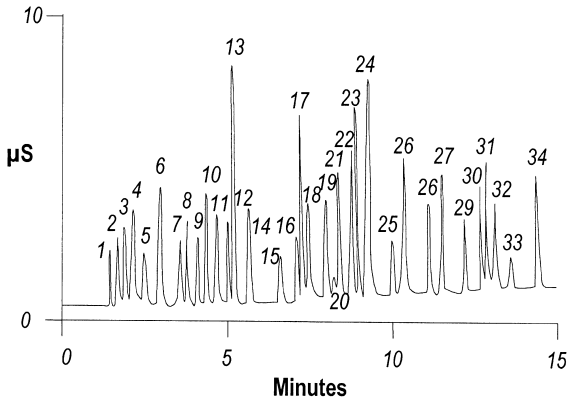

Fig. 2. Gradient separation of inorganic and organic acid anions on an hydroxide selective stationary phase. Column, IonPac AS11; eluent, DI water/sodium hydroxide gradient; flow-rate, 2.0 ml/min; detection, suppressed conductivity. Solutes: 1=isopropylethylphosphonate, 2=quinate, 3=fluoride, 4=acetate, 5=propionate, 6=formate, 7=methanesulfonate, 8=pyruvate, 9=chlorite, 10=valerate, 11=monochloroacetate, 12=bromate, 13=chloride, 14=nitrite, 15=trifluoroacetate, 16=bromide, 17=nitrate, 18=chlorate, 19=selenite, 20=carbonate, 21=malonate, 22=maleate, 23=sulfate, 24=oxalate, 25=ketomalonate, 26=tungstate, 27=phthalate, 28=phosphate, 29=chromate, 30=citrate, 31=tricarballylate, 32=isocitrate, 33=*cis*-aconitate, 34=*trans*-aconitate. From Ref. [27] with permission.

acrylate-based column for the separation of inorganic anions and oxyhalides. This column (Dionex IonPac AS9 HC) is a solvent-compatible, 55% crosslinked, superporous EVB–DVB core particle coated with a glycidoxyethyl methacrylate-based latex [29]. An additional feature of this kind of column is the improved resolution of fluoride from the water dip.

Selectivity variations for anion exchangers have been obtained by modifying the structure of the ion-exchange sites. Both alkylamine (AA) and alkanolamine (AO) functionalized resins have been produced, and some of the latter are *hydroxide selective*. These resins are particularly suitable for hydroxide ion-based eluents coupled with suppressed conductivity detection. Fig. 2, which shows the separation of 34 inorganic and organic acid anions, illustrates an example of the behavior of these materials [27]. The effect of the ion-exchange site and eluent modifiers has also been reported by Bruzzoniti et al. [30] in a study of the chromatographic behavior of divalent carboxylic acids on alkyl amine stationary phases containing zero, one or two hydroxyl groups. The eluent temperature is another parameter affecting the selectivity. Column temperature variations, resulting in enhancement of the separation of monovalent and divalent cations [31], have more recently been demonstrated to be suitable for altering the selectivity order for anion groups [32].

The evolution of stationary phases in the IC of anions can be summarized by a comparison of the performance of a series of columns devoted to environmental water analysis. A new anion-exchange stationary phase, namely IonPac AS14A (Dionex), has recently been developed by using a new block-grafting technique [33]. This column is suitable for a large pH range and, due to the higher capacity, also for moderate- to high-ionic strength samples. The characteristics of this and similar columns are given in Table 1.

Table 1
Comparison of the characteristics of anion-exchange columns

Column type[a]	Particle		Column capacity (μequiv./column)	Hydrophobicity	Latex		pH stability
	Diameter (μm)	Porosity			Diameter (nm)	Crosslinking (%)	
AS4A-SC (4 mm I.D.)	13	Micro	20	Medium–low	90	0.5	0–14
AS14 (4 mm I.D.)	9	Macro	65	Medium–high	Grafted	Grafted	2–11
AS14A (4 mm I.D.)	7	Macro	120	Medium–high	Block-grafted	Block-grafted	0–14
AS14A (3 mm I.D.)	5.5	Macro	30	Medium–high	Block-grafted	Block-grafted	0–14

[a] Resin crosslinking, 55%; functional group, quaternary amine; solvent compatibility, 0–100%.

The IonPac AS4A was the first column specified in US Environmental Protection Agency (EPA) Method 300.0 for the monitoring of inorganic anions in drinking waters [34], but the AS14 A is characterized by a better overall peak selectivity and an improved separation of fluoride from the column void volume. A representative statistical evaluation of two hydroxide-selective microbore columns has been made for the quantitation of anionic species in ultrapure hydrogen peroxide (30%, w/w) [35]. The study involved the IonPac AS11 (core: EVB cross-linked with 55% DVB, with an anion-exchange latex electrostatically bonded to the surface) and the IonPac AS15, with a core similar to the AS11 but with an anion-exchange layer grafted to the surface. Fig. 3 compares the chromatograms obtained with each column for 30 ppb peroxide-based standards of anions.

Much effort has been devoted to developing new methods for the determination of bromate, since it is classified as a probable carcinogen and is found as a disinfection by-product in public water after ozonization treatment. The methods of the EPA for the analysis of bromate [36] are characterized by a minimum detection limit (MDL) range between 20 and 0.12 μg/l. Delcomyn et al. [37], coupling an IC separation (AS9-HC analytical column) with conductivity and UV-post-column reaction (PCR) detection, achieved practical quantitation limits of 0.05 and 0.10 μg/l, respectively, for the oxyhalides bromate and chlorite. Simultaneous separation of five arsenic species [As(III), As(V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine] as well as of redox species of arsenic and selenium [As(III), As(V), Se(IV) and Se(VI)] has been achieved by IC (analytical column: IonPac AS11) and ICP-MS determination [38]. The method, suitable for the analysis of groundwater and extracts of contaminated soils, is characterized by a detection limit of 4 μg/l for selenium species and 0.4–0.8 μg/l for arsenic species. Dudoit and Pergantis [39], by coupling IC (analytical column: AS14) in series with conductivity detection and ICP-MS, achieved the determination of nine species (chloride, nitrate, sulfate, bromide, iodide, bromate, iodate, arsenite, arsenate) in drinking water. A complete chromatographic separation was not achieved, but co-elution problems were solved by the use of two detectors and detection limits matched the requirements for water for human consumption.

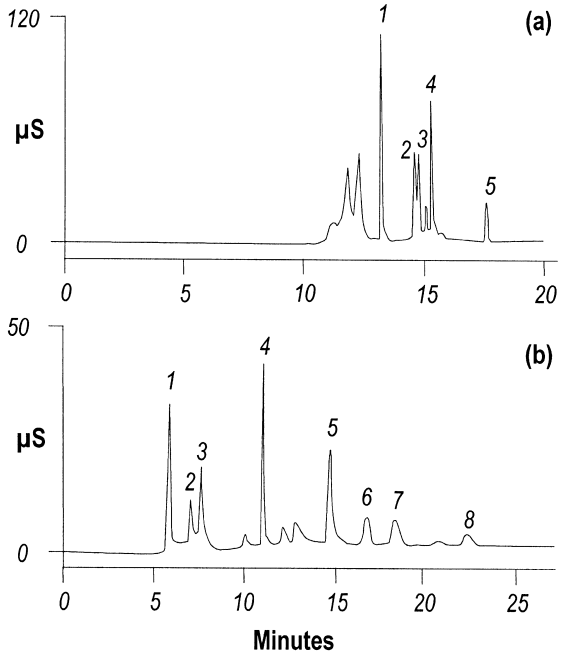

Fig. 3. Example chromatograms of 30 ppb peroxide-based standards on: (a) AS11 column and (b) AS15 column. Peak identities (AS11): 1=chloride, 2=bromide, 3=nitrate, 4=sulfate, 5=phosphate; (AS15): 1=fluoride, 2=acetate, 3=formate, 4=chloride, 5=sulfate, 6=bromide, 7=nitrate, 8=phosphate. From Ref. [35] with permission.

The Hamilton PRP-X 100 (Merck), a styrene–divinylbenzene-based copolymer derivatized with trimethylammonium, has also been extensively used in speciation studies in foods [40] and surface waters [41]. Heitkemper at al. [40] determined total and speciated arsenic in rice by IC–ICP-MS, comparing several procedures for the extraction and performance of three anion-exchange columns, namely a Waters IC-Pak Anion HR, a Dionex AS7+AG7 and a Hamilton PRP-X100. A rapid determination (9 min) of As(III), As(V), MMA and DMA was achieved by Roig-Navarro et al. [41] using IC (analytical column: Hamilton PRP-X 100) coupled with ICP-MS (Fig. 4). The method, which is suitable for the analysis of surface waters, provided detection limits lower than 0.05 μg/l for arsenic species and 5.5 μg/l for Cr(VI). The speciation of essential elements in human serum, based on the separation of

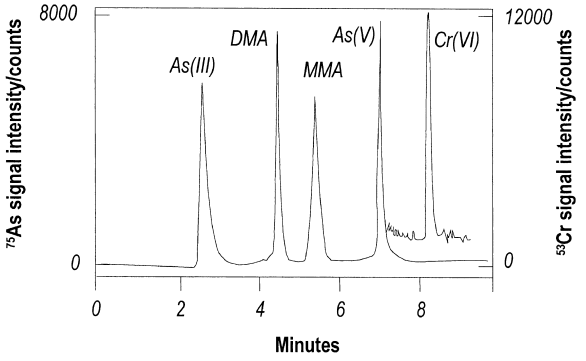

Fig. 4. HPLC–ICP-MS chromatograms of a standard mixture obtained using a Hamilton PRP-X100 column. The concentration of all arsenic species was 3.4 μg l^{-1} (as As) and 200 μg l^{-1} for Cr(VI). Mobile phases: (As) 4 m*M* NH_4HCO_3, 2% methanol (pH 8.0); (Cr) 0.3 m*M* NH_4HCO_3, 2% methanol (pH 8.0). From Ref. [41] with permission.

proteins on a strong, hydrophilic polyether resin-based anion exchanger (Mono-Q HR 5/5, Pharmacia LKB), was achieved by Muñiz et al. [42]. The separation was coupled to post-column isotope dilution analysis with double focusing ICP-MS, and speciation of metal biomolecules for Fe, Cu and Zn resulted in quantitative recoveries for Fe and Zn.

Spherical polystyrene–divinylbenzene beads with a chemically attached hydrophilic surface and derivatized with diethylaminoethyl (DEAE) functional groups (e.g. Vydac 301 VHP575) proved to be suitable for the separation of proteins and related compounds. Lesignoli et al. [43] used this kind of stationary phase in the HPIC analysis of single- or double-stranded oligonucleotides using complementary peptide nucleic acid (PNA) probes, introducing the first approach to the use of PNA in HPLC for the detection of a specific gene sequence (Fig. 5).

The cation exchangers used in IC were sulfonated PS–DVB resins. The main difficulty that had to be overcome was the greatly different selectivities of these resins for monovalent and divalent cations. As mentioned above, alternatively to classic sulfonic cation-exchange sites, weak acid functionalities, carboxylic (IonPac CS12, CS14) [44,45], carboxylic and phosphonic (IonPac CS12A) [46,31] or carboxylic/phosphonic/crown ether (IonPac CS15) [47], have been used for the new cation exchangers. The different selectivities achieved with IonPac 12A and IonPac CS15, due to the presence of the 18-

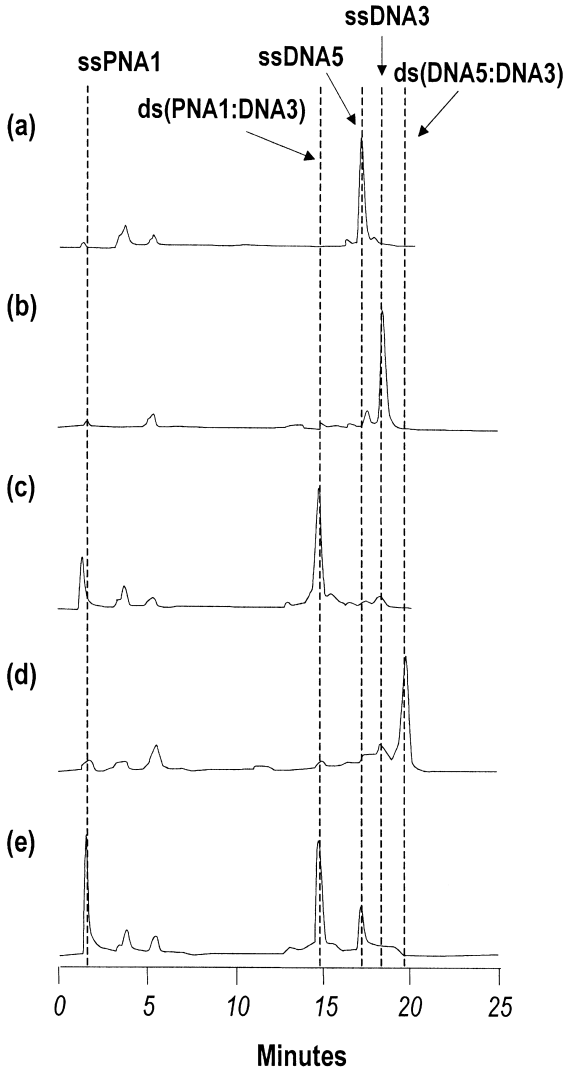

Fig. 5. Ion-exchange chromatograms at 35 °C of: (a) DNA 5; (b) DNA 3; (c) PNA 1-DNA 3; (d) DNA 3-DNA 5; (e) PNA 1 added to solution (d). Column, Vydac 301 VHP575; detection, UV (260 nm); sample, 150 pmol of each strand; elution, binary linear gradient: from 100% A (0.05 *M* Tris–HCl in water, pH 8) to 100% B (0.05 *M* Tris–HCl, 0.5 *M* NaCl in water, pH 8). From Ref. [43] with permission.

crown-6 ether group permanently attached to the macroporous substrate beads of the latter column, resulted in improved separations. A combination of these columns enabled the quantitation of common inorganic cations in samples at a 1000:1 sodium-to-ammonium concentration ratio and the separation of

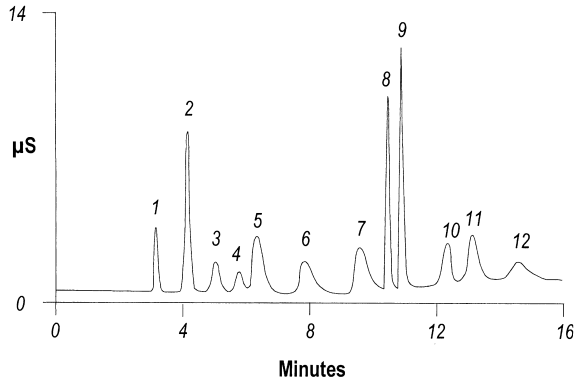

Fig. 6. Power industry amine additives and common inorganic cations quantitated with the IonPac CS15 column. Eluent, 9 m*M* methanesulfonic acid (MSA)+0.7% methyl ethyl ketone (MEK), gradient from 6 to 9 min to 27 m*M* MSA+10% MEK; flow-rate, 1.0 ml/min; temperature, 25 °C; suppressor, recycled mode. Peaks: 1=Li^+, 2=Na^+, 3=2-diethylaminoethanol, 4=morpholine, 5=ethanolamine, 6=NH_4^+, 7=5-amino-1-pentanol, 8=Mg^{2+}, 9=Ca^{2+}, 10=3-dimethylaminopropylamine, 11=K^+, 12=cyclohexylamine. From Ref. [48] with permission.

commonly used corrosion inhibitors of power industry water was achieved by IonPac CS15 [48] (Fig. 6). The drawback of the latter column is that it requires an eluent containing organic solvent. More recently, a new high-capacity carboxylated column (IonPac CS16) was developed which does not require an eluent based on an organic solvent [49]. The separation of mono- and divalent cations plus ammonium is achieved by working isocratically at 65 °C and the only marked advantage of this higher-capacity column is that resolution between the pairs sodium and ammonium and calcium and strontium is significantly improved. The above-mentioned polymers in some cases (IonPac CS5A [27]) bear both quaternary ammonium and sulphonate functional groups. This kind of column has been designed to separate a broad range of metals by anion- and cation-exchange chromatography using weak (oxalic acid) or strong (pyridine-2,6-dicarboxylic acid, PDCA) chelating agents. Historically, the most attractive application, chelation ion chromatography, was the coupling of IonPac CS5 to an on-line chelation and preconcentration procedure for the determination of trace transition and rare-earth elements in seawater, and digested biological, botanical and geological samples [50]. Other recent applications are the simultaneous determination of heavy and transition metals in biochemical samples and nitrate/phosphate fertilizer solutions [51,52]; comprehensive reviews have been published in the field of metal and metal species determination by IC [53,54].

Silica- and alumina-based ion exchangers have also been widely used, and an attempt has been made to develop new stationary phases. The advantages of quaternary ammonium-modified, reversed-phase columns for the separation of anions were first investigated by Cassidy and Elchuk [55]. The so-called *Shomburg column* [56], based on a silica gel coated with poly(butadiene–maleic acid) (PBMA) copolymer, was successfully used for the separation and determination of mono- and divalent cations in non-suppressed IC. The cation-exchange properties of an unmodified silica gel (Develosil 30-5) have been studied by Ohta et al. [57], obtaining the simultaneous separation of Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} in environmental samples. They also demonstrated the dependence of the cation-exchange efficiency on Al, present as an impurity in silica gels [58]. On this basis they developed a procedure for the separation of mono- and divalent cations based on a silica gel modified by coating with Al [59]. Highly selective separation and sensitive determination, both for mono- and divalent cations, has been obtained by silica gels modified with aluminium and zirconium [60] (Fig. 7a and b). Acidic eluents have been used and the resolution of monovalent cations was enhanced, in some cases, by adding 15-crown-5 (1,3,7,10,13-pentaoxacyclopentadecane). These synthesized materials have also shown good applicability for the separation of aliphatic and benzenecarboxylic acids in the ion-exclusion mode. The efficiency of silica-modified ion-exchange materials can be illustrated by the following example. Grard et al. [61] analyzed mixtures of sulfobutyl-ether-β-cyclodextrins (SBE-β-CDs) by separation on a low-capacity anion exchanger (quaternary amine) based on 10 μm, large-pore silica (Vydac 302 IC), coupling IC to evaporative light scattering detection. The technique gave a characteristic fingerprint depicting the mixture complexity and the average degree of substitution for each mixture. This is extremely significant, since SBE-β-CDs, in addition

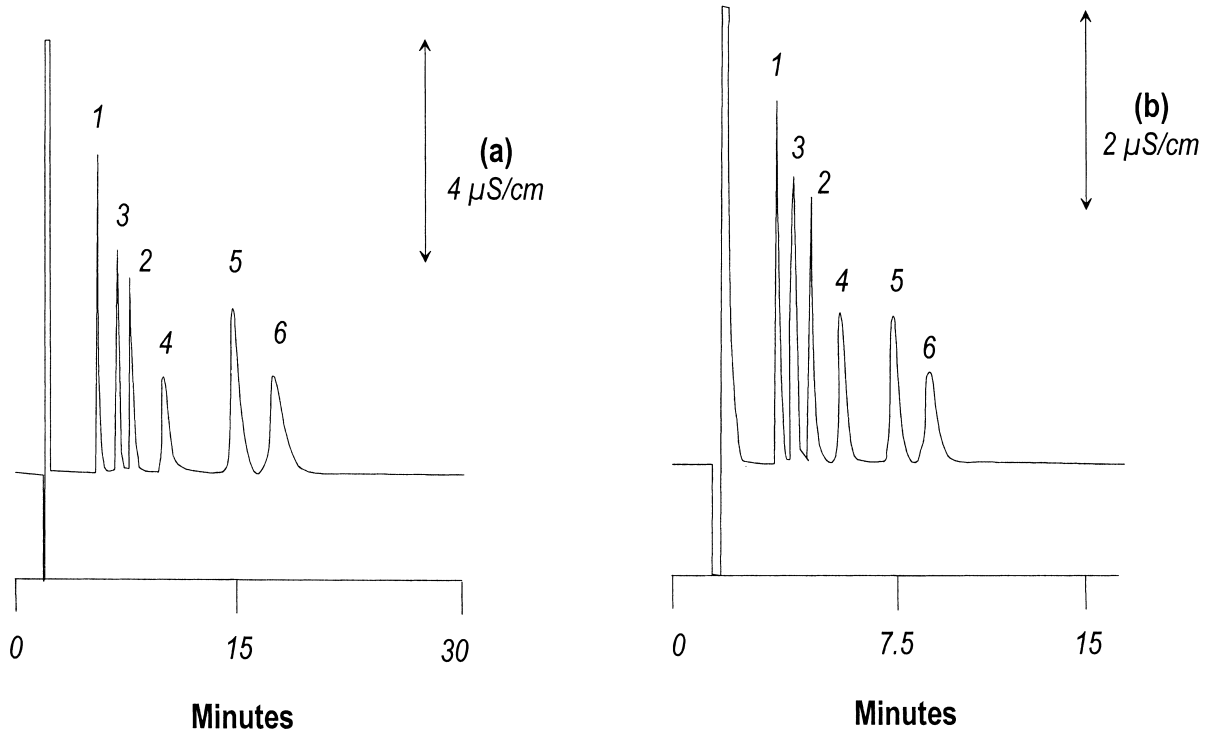

Fig. 7. Chromatograms of common mono- and divalent cations on (a) Al-silica and (b) Zr-silica gel columns after elution with: (a) 2 m*M* nitric acid–2 m*M* 15-crown-5 and (b) 10 m*M* tartaric acid–10 m*M* 15-crown-5. Detection: indirect conductivity. Peaks: 1=Li^+, 2=Na^+, 3=NH_4^+, 4=K^+, 5=Mg^{2+}, 6=Ca^{2+}. From Ref. [60] with permission.

to drug formulations, are also widely used as chiral selectors for enantiomeric separations in CE.

Among the silica-based materials, mesoporous silica and aluminosilicate have also been prepared using a surfactant-based supramolecular approach [62–64]. This procedure can lead to ordered molecular sieves (M41S) with different arrays of mesopores, characterized by pore dimensions ranging from 20 to 100 Å. The possibility of absorbing hydrophobic [65] and both hydrophobic and ionic analytes [66] has been investigated. More recently, a series of mesoporous materials was synthesized and their retention of haloacetic acids was evaluated. The analyte affinity order for mesoporous silica was the same as the chromatographic selectivity in an anion exchanger. These results provide the basis for the potential applications of mesopores as a stationary phase for IC separations [67].

Studies on less traditional ion exchangers concern the production of anion exchangers by modifying hydrophobized silica or other matrices with both aliphatic and aromatic ‘‘ionones’’ (polyelectrolyte complexes obtained by heterogeneous interaction between anion-active compounds and polycations). The efficiency of this kind of material has been demonstrated in the separation of inorganic anions and sulphonic acids [68–70]. ODS reversed-phase columns have also been coated dynamically with zwitterionic detergents [71,72]. These stationary phases contain both anionic and cationic functional groups and, in this way, both repulsive and attractive electrostatic interactions are exerted on the charged analytes. The analytes are eluted as a pair of ions and the mechanism is more complicated than in pure ion exchange [72]. This particular method, called electrostatic ion chromatography (EIC), enables the use of water for elution and, under these conditions, conductivity detection results in extremely low de-

tection limits for the simultaneous determination of inorganic cations and anions. EIC has also been performed using hydroxide solutions as mobile phase and suppressed conductivity detection [73], showing high sensitivity for inorganic anions SO_4^{2-}, F^-, Cl^-, NO_2^-, Br^-, NO_3^-, ClO_3^-, I^-. This approach, in contrast to the above-mentioned EIC elution mode, leads to the control of retention times by varying the concentration of the eluent and a single peak is obtained for each analyte. More recently, to overcome the problem arising from the limited stability of silica-based materials at pH <2 or >7, a covalently bonded polymeric zwitterionic stationary phase has been synthesized [74]. Inorganic cations and anions have been separated on this polymer-based zwitterionic stationary phase using an aqueous solution of perchloric acid or perchlorate, but the separation mechanism differs from that usually shown by the zwitterionic materials mentioned above.

Another feature of IC is chelation ion chromatography, suitable for the separation of metals in samples of high ionic strength such as concentrated brines or seawater and some comments on this are warrented. Jones and coworkers investigated this field in order to improve the detectability of both alkali earth and heavy metals, and they showed that the chelating ability of Xylenol Orange and Chrome Azurol S sorbed onto PS–DVB substrates changes markedly with different substrates [75]. Kantipuly et al. [76] illustrated the extensive range of chemically bonded chelating resins available for the separation and concentration of trace metals, among the many combinations of chelating ligands and supporting materials. Various kinds of stationary phases have been reviewed by Jones and Nesterenko [77] and Paull and Haddad [14]. The most recent trend involves dynamic and permanent coating of an appropriate substrate with a chelating agent. Either a polymeric or an octadecyl silica reversed-phase substrate is coupled with hydrophobic metallochromic ligands. A polystyrene–divinylbenzene reversed-phase column precoated with Methylthymol Blue (MTB) was found to be suitable for samples at ionic strength up to 1.0 *M* NaCl and was highly selective for UO_2^{2+} [78]. Large-volume injection and an eluent step gradient procedure enabled the determination of UO_2^{2+} without interference, at μg/l concentration, in a saline lake sample. Paull et al. [79], using MTB in the dynamic mode, obtained the separation of transition and heavy metals with different selectivity and improved peak shapes in comparison to the permanent coating mode. More recently, Bashir and Paull [80], using an iminodiacetic acid-functionalized silica column (IDA silica gel, BioChemMack, Moscow, Russia), developed a highly selective and sensitive method for the determination of beryllium in water samples. The method, successfully applied to the analysis of various kinds of water samples and a certified reference freshwater sample [US National Institute of Standards and Technology (NIST) 1640], is characterized by a detection limit of 4 μg Be(II)/l (Fig. 8).

The synergic effect of the nature of the stationary phase and eluent composition for the separation of analytes has been widely demonstrated and ion-exchange, ion-pair, ion-exclusion and electrostatic

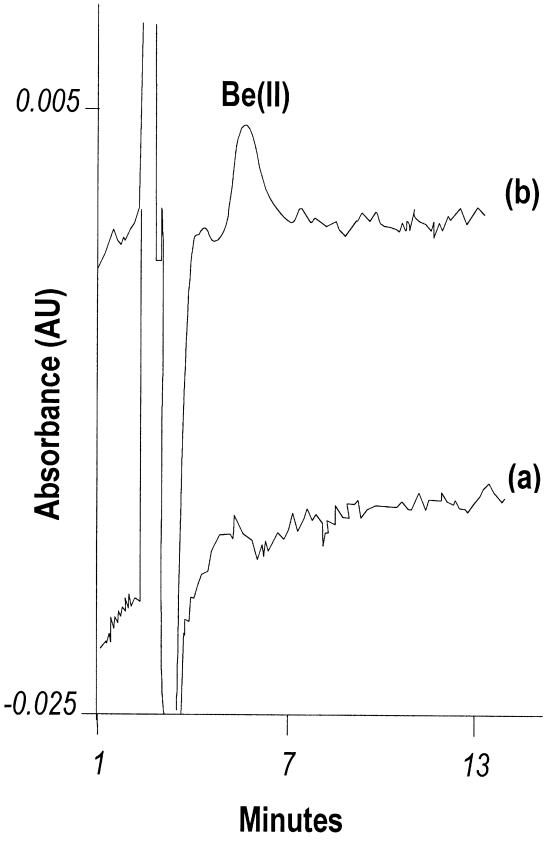

Fig. 8. Overlaid chromatograms of (a) simulated seawater and (b) simulated seawater spiked with 0.04 mg/l Be(II). Eluent conditions: 0.4 *M* KNO_3, pH 3.0. From Ref. [80] with permission.

ion chromatography mechanisms can be used in a single or multi-mode approach. In this respect, a recent study by Doyle et al. [81] must be mentioned. They developed a multicomponent mobile phase utilizing an ion-exchange, ion-exclusion and ion-pairing mechanism on an anion-exchange column (Waters IC-Pak Anion HR) for the separation of anions in the residue from low explosive. The novel aspect is that each component of the mobile phase acts independently in solution.

As shown above, both inorganic materials and synthetic polymers have been developed and used for IC applications. In addition to the quoted and traditional literature, references to more recent products are available in the reports of the 2000 and 2001 Pittsburg Conferences in *LC–GC* [82,83] where, for the 2000 conference, three cation-exchange columns, six anion columns and a mixed-mode column based on titania, and for the 2001 conference, five cation-exchange columns and six anion columns, are presented (some are mentioned above).

3. New perspectives and challenges

The above-mentioned developments in IC, in studies on the coupling of more sensitive detectors to chromatographic systems, represent a well-defined and constant trend in separation science. In fact, studies, materials and devices made to improve IC were and are the basis of other techniques. A development which is formally new in separation and preconcentration techniques is miniaturization.

An open tubular column liquid chromatograph in silicon has been described by Manz et al. [84]. A review on the miniaturization for the different types of separation techniques has been published by Luque de Castro and Gámiz-Garcia [85]. They describe micro-scale liquid chromatography (LC) on the basis of available micro columns (0.2–0.5 mm I.D.), packed and open tubular columns (0.05–0.2 and 0.005–0.01 mm I.D., respectively) and flow-rates down to nano-LC (10–1000 nl min^{-1}). The concept of micromachined systems on glass chips was also introduced, but only for capillary electrophoresis. Liquid nanochromatography (nano-LC columns, 75 μm I.D.) was coupled to electrospray ionization (ESI) MS [86] and a NanoFlow LC–ESI-MS system was used to analyze DNA adducts [87]. Colocated monolith support structure (COMOSS) cubes micromachined into a quartz wafer, covered by a second thermally bound wafer, were proposed as a packed bed for chromatographic applications by He and Regnier [88]. However, as in other papers devoted to the micromachined column, separation was achieved by working in capillary electrochromatography mode.

Developments in separation methods on microfabricated devices have recently been reviewed by Kutter [89]. Again, it must be pointed out that the majority of papers dealing with the miniaturization of separation techniques on-chip refer to CE applications.

Advances in this field, with respect to the main subject of this paper, are represented by the work performed by Kang et al. [90] and Murry et al. [91].

The first paper [90] describes the fabrication of a miniaturized ion-exchange separator, with integrated electrical conductivity detection, by micro-electromechanical systems (MEMS) technology. The separator, fabricated on a silicon chip, is characterized by eight channels (each 50 μm wide, 250 μm deep and 3 cm long) etched on a (110) silicon wafer. Bonded-phase chemistry was used for developing the anion-exchange capacity due to polyethyleneimine immobilization. The behavior of this instrument has been characterized by the separation of bromide, chloride, nitrate and sulfate ions.

The second paper [91] deals with the ion-chromatography separation of inorganic anions on-chip. Micro-separation channels were obtained on a silicon wafer by standard photolithography, wet and dry chemical etching, and anodic bonding techniques. The silicon wafer was sealed with a Pyrex cover plate. The separation channels on-chip were coated with quaternary ammonium latex particles (AS5A) and Fig. 9 compares the separation achieved using a 3.5 m×50 μm I.D. capillary column (coated with AS5A latex) and a silicon chip connected to a 34 cm coated capillary. The ion-exchange behavior of the on-chip system, checked by the separation of thiourea, NO_2^- and NO_3^-, was linear for the peak area and concentration in the range from 5 μ*M* to 1 m*M* and detection limits of 0.5 μ*M* were obtained. It is of relevance to note that IC on-chip has been achieved by following two different approaches. The most important advantages of miniaturization are that this kind of instrument requires a small volume of sample

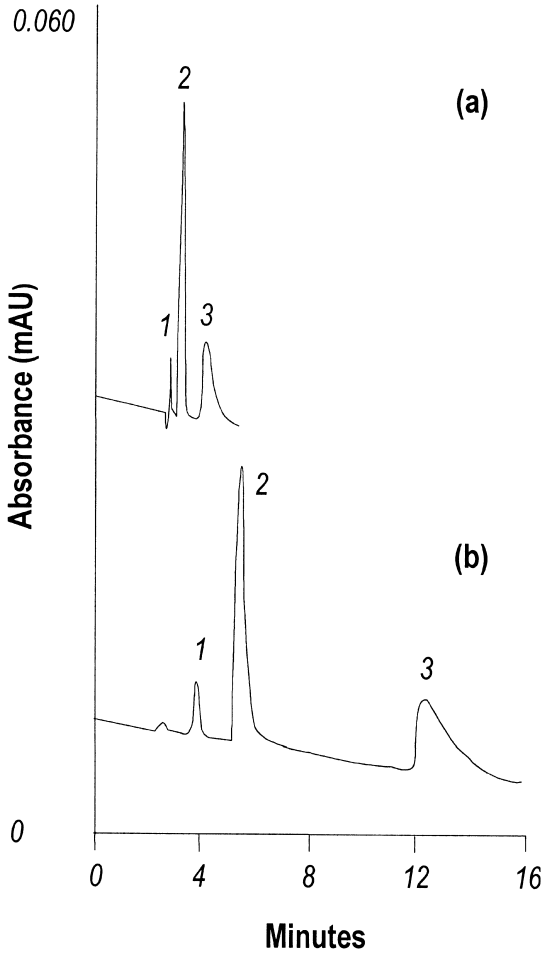

Fig. 9. (a) Separation of inorganic anions using a 3.5 m×50 μm I.D. capillary column coated with anion-exchange AS5A latex particles. Flow-rate 2.6 μl/min. (b) Separation of inorganic anions using a silicon chip and a 34 cm coated connecting capillary. Flow-rate 150 nl/min. Eluent (a,b): 1 m*M* KCl. Peaks: 1= thiourea, 2=NO_3^-, 3=I^-. From Ref. [91] with permission.

and eluents, and analysis times are significantly reduced. This means that they are well suited both for traditional analysis and emerging fields of research such as genomic and proteomic analysis [92].

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