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INSECTICIDES IN FOODSTUFFS

See **III / CARBAMATE INSECTICIDES IN FOODSTUFFS: CHROMATOGRAPHY AND IMMUNOASSAY**

ION ANALYSIS

Capillary Electrophoresis

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Introduction and Scope

Before the advent of capillary electrophoresis (CE), some impressive separations of inorganic species had been achieved by other electrophoretic methods, such as the separation of lanthanoids by paper electrophoresis using complexation with 2-hydroxyisobutyric acid (HIBA). With the introduction of capillary electrophoresis in 1981 by Jorgenson and Lukacs, separations of inorganic anions and cations started to appear quite early, but only sporadically until the late-1980s. There were several reasons for this. First, CE was a very new analytical technique and some time was needed for the theoretical background and instrumentation to mature. The second factor was competition with alternative analytical techniques, mainly spectroscopic methods (atomic absorption spectrometry, inductively coupled plasma spectrometry), in the area of determinations of metals, and ion chromatography in the area of separation of inorganic anions. Third, the main potential of CE was seen to be in the separation of biopolymers and biologically active compounds including drugs, with inorganic analysis being regarded as a relatively minor application area of CE. Although the last two factors remain valid, CE has matured to the stage where applications are sought in all areas of analysis, including inorganic analysis. This development has been characterized by more research oriented towards solving the practical requirements of inorganic analysis. The important developments in inorganic analysis by CE are summarized in **Table 1**.

Anions and cations migrate in opposite directions when placed in an electric field. Typical commercial

Table 1 Some important developments in inorganic analysis by CE

Time	*Development*
1967	First separation of cations (Br^{3+} Cu^{2+}) by free solution electrophoresis (0.1 mol L^{-1} lactate) in a 3 mm axially rotating tube
1974	Separation of alkali metal cations in 200–500 mm Pyrex capillaries with potentiometric detection
1979	Separation of inorganic anions using 200 mm PTFE capillary and conductometric detection
1983	Separation of Cu^{2+}, Fe^{3+} using a simple acetic acid electrolyte and direct photometric detection at 254 nm
1987	Indirect detection of anions
1989	Use of pre-capillary formed PAR complexes for separation of Co^{II}, Cr^{III}, Ni^{II}, Fe^{III} MEC (electrolyte containing SDS, pH 8, 0.1 mmol L^{-1} PAR) and using direct detection in visible
1990	Indirect photometric detection of anions using chromate electrolyte at pH 8 and reversed EOF
1990	Separation of rare earths metals and Li, Na, K, Mg using HIBA and indirect UV detection using an electrolyte of 0.03 mol L^{-1} creatinine-HAc pH 4.8, 4 mmol L^{-1} HIBA
1995	Hyphenation with ICP-MS: separation and detection of Sr, Cu, Fe^{III}, Fe^{II}, Cr, As, Sn^{II}, Sn^{IV}
1997	ITP-CE online: separation of Fe^{III} as pre-capillary formed EDTA complex; BGE: 25 mmol L^{-1} MES + 10 mmol L^{-1} bis-tris-propane, pH 6.6; leading: 10 mmol L^{-1} HCl + 20 mmol L^{-1} L-histidine + 0.1% HPMC, pH 6.0; terminating: 5 mmol L^{-1} MES

MEC, micellar electrochromatography

CE instruments have one point of sample introduction and one point of detection, so the choice must be made as to the polarity of the electrodes placed at the injection and detection ends of the capillary in order to establish the direction of movement of ions. Further, the electroosmotic flow (EOF) caused by the application of the separation potential will sweep the ions either towards or away from the detector, according to the polarity of the electrodes and the surface charge on the capillary. While it is theoretically possible to design a CE system in which a high EOF flowing towards the detector is established, causing both anions and cations to flow towards the detector, the electrophoretic mobilities of most inorganic anions and cations are too high to allow this approach to be applied. It is therefore more common for inorganic anions and cations to be analysed separately and for their electrophoretic movement to be in the same direction as the EOF (i.e. towards the detector). This is termed 'co-electroosmotic' separation.

Separation of Inorganic Anions

Separation Strategy

The electrophoretic mobilities of inorganic anions are quite large in comparison to those of most organic anions (**Figure 1**). Consequently, the normal instrumental arrangement is to assign the negative side of the separation voltage to the electrode at the injection end (so that the electrophoretic migration of the analyte anions is towards the positive electrode at the detection end). Moreover, since the normal flow of EOF in a fused silica capillary is towards the cathode (i.e. in the direction opposite to the electrophoretic migration of anions in the above case), it is normally necessary to suppress or reverse the EOF to give a rapid co-electroosmotic separation. Such EOF reversal can be achieved by dynamic modification of the fused silica capillary inner wall by adsorption of cationic compounds or by permanent (covalent bonding) of cationic groups onto the fused silica capillary inner wall to provide an overall positive charge on the wall. The dynamic modification method is used most commonly and can be achieved by adsorption of suitable cationic surfactants (such as C_{12} to C_{16} alkyltrimethylammonium salts) or of large molecules such as cationic polymers (e.g. polybrene, poly(N,N,N′,N′-tetramethyl-N-trimethylenehexamethylenediammonium dibromide) by adding these to the background electrolyte (BGE) or by flushing the capillary between the runs. This is illustrated schematically in **Figure 2**.

After introduction of the sample into the capillary and application of the separation voltage, the anions migrate towards the cathode at the detection end in the order of their effective electrophoretic mobilities, which means that the anions migrating fastest will be detected first. The flow of the bulk BGE driven by the EOF towards the detector (co-EOF separation) allows anions of low electrophoretic mobility to be carried to the detector point more rapidly than would occur based only on their electrophoretic mobility in case of a suppressed-EOF separation. Therefore typical co-EOF separations of anions are characterized by rapid analysis times, as illustrated in **Figure 3**.

Separation Selectivity

The electrophoretic mobilities of inorganic anions cover a wide range from about 30×10^{-9} $m^2\,V^{-1}\,s^{-1}$ to over 100×10^{-9} $m^2\,V^{-1}\,s^{-1}$ (Figure 1). Inspection

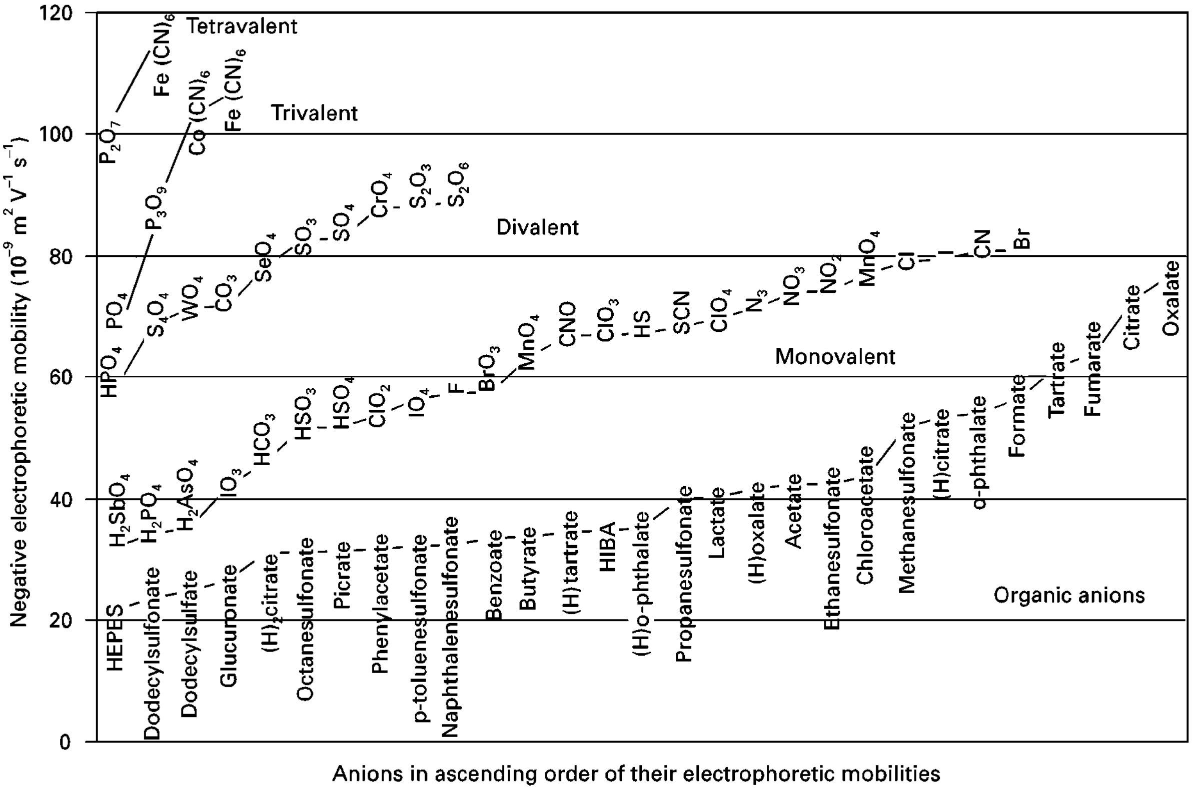

Figure 1 Electrophoretic mobilities of inorganic and some organic anions in ascending order of their mobilities. Key: (H), protonated form; $(H)_2$, diprotonated form, charges left out for simplicity. Electrophoretic mobilities were calculated from tabulated ionic conductances or taken from published values.

of Figure 1 shows that most inorganic anions have a unique value of electrophoretic mobility and should be straightforward to separate without the need to optimize the separation selectivity. However, in some cases electrophoretic mobilities of ions are similar and steps need to be taken to manipulate selectivity in order to achieve a separation. The approaches available to achieve this goal are summarized in **Table 2.**

Probably the most universal tool for manipulating selectivity is to alter the solvation of the analyte anion

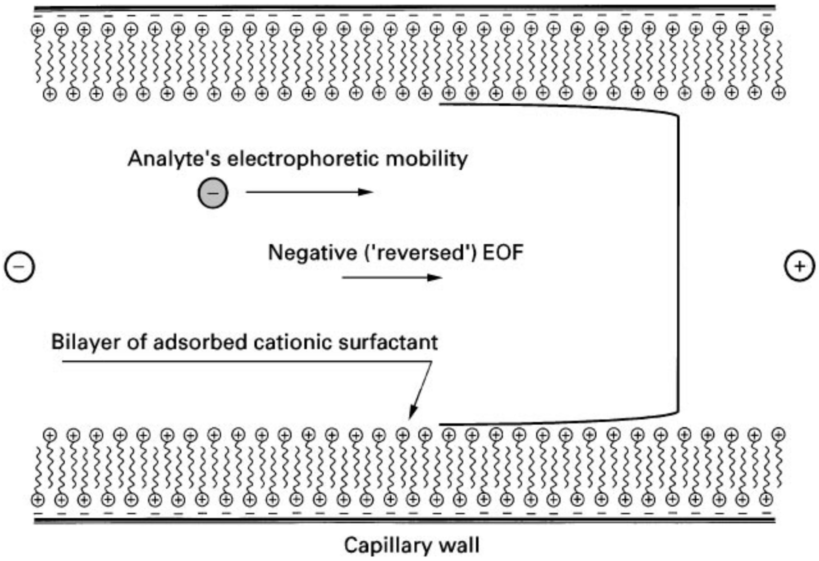

Figure 2 Schematic representation of co-electroosmotic separation of anions using EOF reversal.

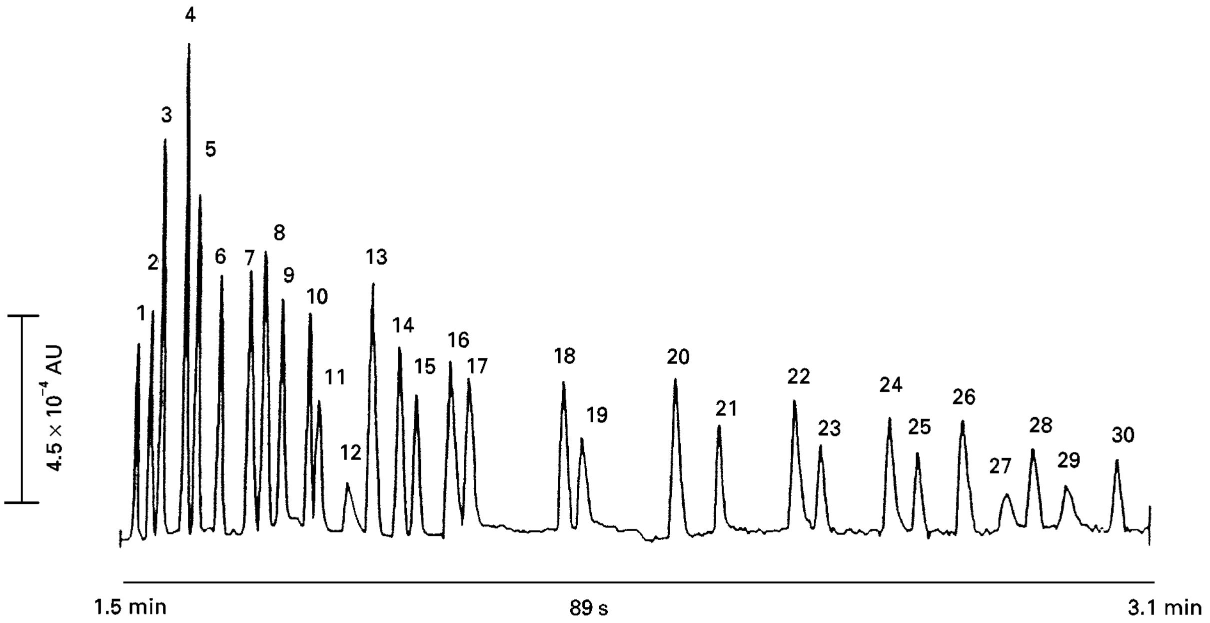

Figure 3 Separation of 30 anions using chromate BGE. Conditions: capillary, fused silica 75 μm i.d., 0.600 m length, 0.527 m to detector; BGE, 5 mmol L^{-1} chromate, 0.5 mmol L^{-1} tetradecyltrimethylammonium bromide (TTAB), pH 8.0; separation voltage, − 30 kV; detection, indirect at 254 nm; injection, electrokinetic at 1 kV for 15 s; sample, 0.3–1.7 ppm of each anion. Peak identification: 1, thiosulfate; 2, bromide; 3, chloride; 4, sulfate; 5, nitrite; 6, nitrate; 7, molybdate; 8, azide; 9, tungstate; 10, monofluorophosphate; 11, chlorate; 12, citrate; 13, fluoride; 14, formate; 15, phosphate; 16, phosphite; 17, chlorite; 18, galactarate; 19, carbonate; 20, acetate; 21, ethanesulfonate; 22, propionate; 23, propanesulfonate; 24, butyrate; 25, butanesulfonate; 26, valerate; 27, benzoate; 28, L-glutamate; 29, pentanesulfonate; 30, D-glucanate. (Reproduced with permission from Jones WR and Jandik P (1991) Controlled changes of selectivity in the separation of ions by capillary electrophoresis. *Journal of Chromatography* 546: 445–458.)

by adding organic solvents. For those anions that exhibit protonation equilibria in the pH range used in CE, substantial selectivity changes can be achieved by pH variations. The effective mobility of the analyte is defined as the weighted average of all the mobilities of each of the forms, which for an acid H_nA undergoing protonation equilibria is

$$\bar{\mu}_{\mathrm{A}} = \sum_{i=0}^{n} \mu_i \alpha_i \qquad [1]$$

Table 2 Equilibria utilized for governing the separation selectivity

Anions/cations	*Source of separation selectivity*	*Examples of BGE additives*
Anions	Changes of effective mobility by utilizing protonation equilibria	pH optimization
	Changes of effective mobility by changes in solvation of the anions	Organic solvents
	Ion association of anions capable of hydrophobic interactions (e.g. I^-, SCN^-), with amphiphilic cations	Hexadecyltrimethylammonium
	Ion exchange interactions with polycationic molecules	Polybrene, polyethyleneimine
Cations	Complexation with an auxiliary ligand in partial complexation mode	HIBA, lactate, etc.
	Influence of the metal on the pK_a of a ligand group (total complexation mode)	PAR
	Dissociation of bound water molecules to mixed hydroxo–ligand –metal complexes (total complexation mode)	EDTA, CDTA and analogues
	Ion association of anionic complexes with amphiphilic cations, e.g. TBA^+ or hexamethonium (total complexation mode)	DHABS, CN^-
	IEEC (ion exchange electrochromatography), e.g. with poly(diallyldimethylammonium chloride), (total complexation mode)	EDTA, Quin2
	Changes of effective mobility by changes in solvation of the cations	Organic solvents

HIBA, 2-hydroxyisobutyric acid; PAR, pyridylazoresorcinol; EDTA, ethylenediaminetetraacetic acid; CDTA, trans-1,2-diamino-cyclohexane-N,N,N′,N′-tetraacetic acid; DHABS, 2,2′-dihydroxyazobenzene-5,5′-disulfonate; Quin, 2,8-amino-2-[(2-aminomethyl-phenoxy)methyl]-6-methoxyquinoline-N,N,N′,N′-tetraacetic acid.

where μ_i is mobility and α_i is the fraction of the anion ion existing in form *i*.

For this approach to be successful, the BGE must be well buffered to avoid pH inhomogeneity within the migrating sample zone. Finally, ion exchange-type interactions between the analyte anions and some cationic water-soluble polymers may also be used to manipulate separation selectivity.

Detection of Anions

Direct and indirect photometric detection Most commercial CE instruments are equipped with a photometric detector and therefore direct and indirect photometric detection are the most commonly used detection methods in CE. Probably the most simple and robust detection technique in terms of baseline stability and lack of system peaks is direct photometric detection. Unfortunately this can be applied only to a few inorganic anions, such as iodate, bromide, thiocyanate, nitrate, nitrite, and some others.

Indirect detection has the prime advantage of being universal in its applicability and is the most frequently applied detection mode for CE separations of both inorganic anions and cations. An absorbing co-ion (commonly referred to as the probe ion) is added to the BGE and the detector monitors a suitable absorbing wavelength of the probe. Migrating bands of analytes displace the probe from the BGE and indirect detection is possible due to the resulting decrease in absorbance. The limit of detection (LOD) of a nonabsorbing analyte ion when detected by this process is given by:

$$c_{\mathrm{LOD}} = \frac{c_{\mathrm{P}}}{R \times D} = \frac{c_{\mathrm{P}} \times N_{\mathrm{BL}}}{R \times A} = \frac{N_{\mathrm{BL}}}{R \times \varepsilon \times l} \qquad [2]$$

where c_{LOD} is the concentration LOD, c_{P} is concentration of the probe co-ion in the BGE, R is the transfer (or displacement) ratio (that is, the average number of probe co-ions displaced by one analyte ion), D is the dynamic reserve (the ratio of absorbance caused by the probe in the BGE to baseline noise), N_{BL} is the baseline noise, A is the absorbance caused by the probe in the BGE, ε is the molar absorptivity of the probe, and l is the effective pathlength.

The factors influencing detection sensitivity in indirect photometric detection are summarized in **Table 3**. As a general rule, the BGE should be well buffered to ensure optimal reproducibility, but at the same time the buffer must not add any competing co-ions to the BGE so that the probe ion is the only species displaced by the analyte (maximizing *R*) and detection sensitivity is therefore maximized. For indirect detection of anions it has been shown that this can be achieved either by using a buffering counter ion (i.e. one having the opposite charge sign to the probe) or by using buffering species of extremely low mobility, such as buffering ampholytes employed at a pH close to their p*I*. A further consideration in maximizing detection sensitivity is to ensure that the probe and the analyte have similar mobilities. **Figure 4** shows the electrophoretic mobilities of some inorganic anions and some anionic probes. Typical concentration detection limits for indirect detection are in the low μmol L^{-1} region, although sub-μmol L^{-1} detection limits have been achieved by using highly absorbing dyes as probes.

Other detection methods Electrochemical detection methods, such as conductometric, amperometric and potentiometric detection methods, can be used in CE of inorganic anions with the first being the most successful. End-capillary detection, in which the detection electrodes are placed at the capillary outlet, has proved to be the optimal configuration. Commercial instrumentation for end-capillary conductometric detection has been introduced and is a useful approach for highly conductive analyte ions separated in a low conductivity BGE, such as 2-(N-

Table 3 Factors influencing sensitivity of indirect photometric detection

Factor	*Methods of achievement*
Minimal analyte peak width	Matching the mobilities of the analyte and the probe co-ion(s); minimizing solute–wall interactions
Maximal transfer ratio (*R*)	Matching the mobilities of analyte and probe co-ion(s); choice of BGEs without further co-ions which can compete with the probe (especially of similar mobilities)
Minimal baseline noise (N_{BL})	Minimal detector noise (usually by ensuring that the background absorbance is not too high); minimal 'chemical noise' (i.e. minimal solute–wall interactions of the analytes and/or probe)
Maximal absorptivity (ε) of the probe	Correct choice of the probe
Maximal effective pathlength (ℓ)	Use of larger rather than smaller i.d. capillaries, use of extended pathlength capillary or z-cell

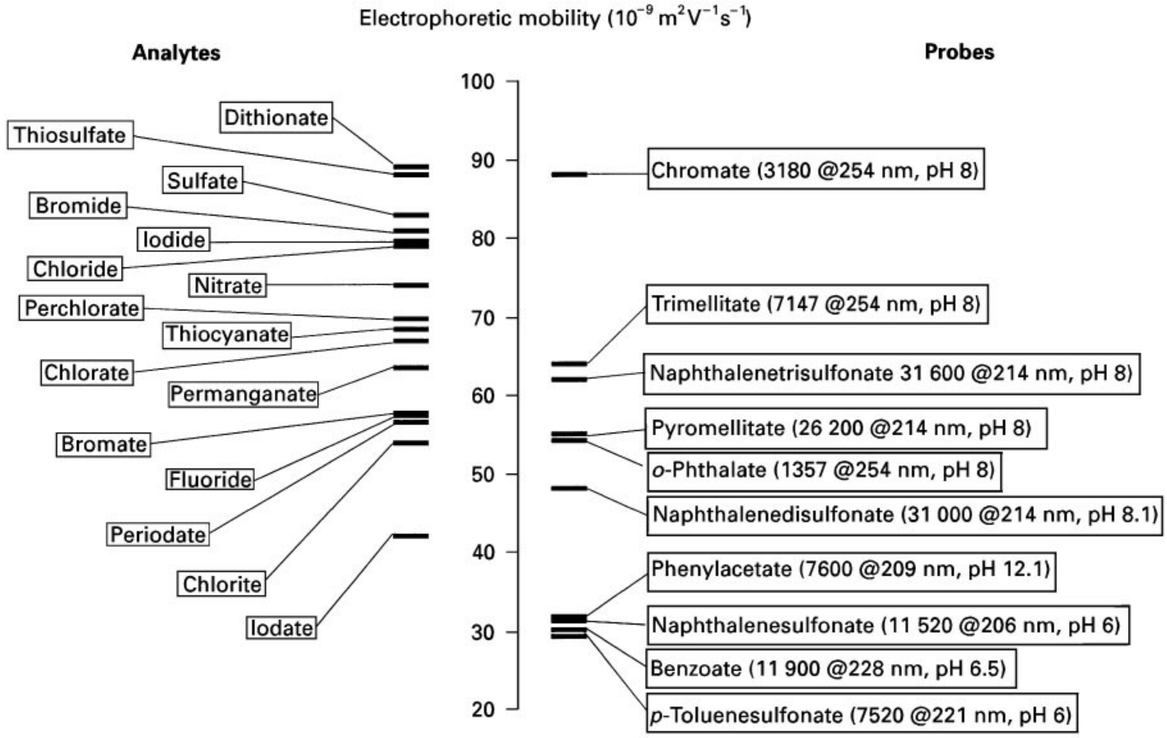

Figure 4 Matching of electrophoretic mobilities of some inorganic anionic analytes and probes. Given in brackets are molar absorptivity (L mol^{-1} cm^{-1}) at a wavelength (nm), and optionally a pH. (Data taken from Doble P and Haddad PR (1998) Indirect photometric detection of anions in capillary electrophoresis. *Journal of Chromatography A* 834: 189–212.)

cyclohexylamino)ethanesulfonic acid (CHES) buffer. The detection sensitivity is best for the most mobile anions and typical LODs are in the low μmol L^{-1} range. End-capillary amperometric detection based on oxidation of anions such as nitrite, iodide, thiocyanate, azide or sulfite on gold, platinum or carbon fibre electrodes has been applied, with LODs down to the low nmol L^{-1} region. Finally, potentiometric detection using liquid membrane, solid-state coated wire or metallic copper electrodes has been applied to a range of anions. Concentration detection limits in the μmol L^{-1} range have been achieved.

The use of information-rich detection techniques, such as mass spectrometry (MS), provides additional information that can be of advantage for analyte identification or to enhance the separation selectivity. The intriguing task of coupling CE to ICP-MS has been solved with the design of special nebulizers, such as the direct injection nebulizer, which introduces 100% of the sample to the plasma and does not cause any detectable peak broadening. Despite the disadvantages of high running costs and equipment complexity, CE interfaced to inductively coupled plasma mass spectrometry (CE-ICP-MS) has been successfully applied to speciation studies, such as of inorganic and organic species of selenium or arsenic, for which LODs in the low ppt range have been reported.

Method Optimization

An important factor in the attainment of a robust CE method, delivering reproducible migration times and possessing some matrix tolerance, is correct buffering of BGEs. **Figure 5** shows the difference in tolerance towards an alkaline sample matrix for a buffered BGE and an unbuffered BGE, from which it can be seen that buffering is essential in the analysis of samples of this type.

With appropriate knowledge of the underlying principles of CE, such as methods for governing the separation selectivity or maximizing the sensitivity of indirect detection, methods for the separation of a limited number of analytes can often be developed without use of computer-based optimization procedures. In the case of inorganic anions the most frequently used BGE is sodium chromate (pH 8.0) containing a low concentration of a cationic surfactant such as tetradecyltrimethylammonium bromide for reversal of the EOF. The chromate ion acts as the probe for indirect photometric detection and has an electrophoretic mobility that is similar to those of many inorganic anions. Sensitive indirect

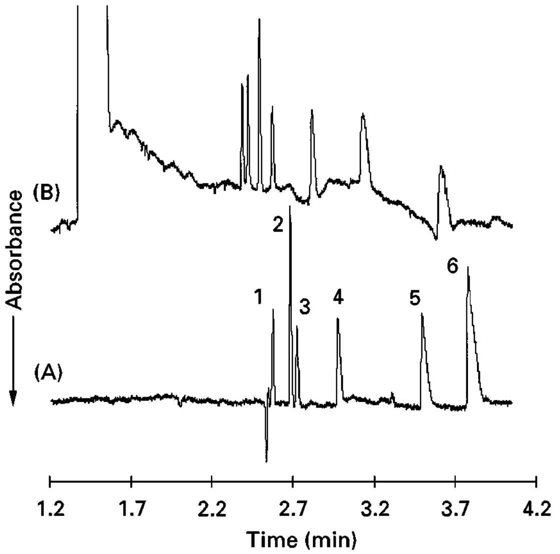

Figure 5 Tolerance to alkaline matrix illustrated using buffered (A), and unbuffered (B) chromate electrolytes. Conditions: capillary, fused silica 75 μm i.d., 0.600 m length, 0.500 m to detector; BGE, 5 mmol L^{-1} chromic trioxide, 20 mmol L^{-1} Tris, 0.5 mmol L^{-1} TTAB, pH 8.5 (a) or 5 mmol L^{-1} sodium chromate, 0.5 mmol L^{-1} TTAB, pH 8.5 (b); separation voltage, − 20 kV; detection, indirect at 254 nm; injection, hydrostatic at 100 mm for 10 s; temperature, 25°C; sample, 0.1 mmol L^{-1} of each anion in 50 mmol L^{-1} sodium hydroxide. Peak identification: 1, chloride; 2, sulfate; 3, nitrate; 4, chlorate; 5, phosphate; 6, carbonate. (Reproduced with permission from Doble P, Macka M, Andersson P and Haddad PR (1997) Buffered chromate electrolytes for separation and indirect absorbance detection of inorganic anions in capillary electrophoresis. *Analytical Communications* 34: 351–353.)

photometric detection at 254 nm can be achieved. Recently, buffered chromate electrolytes have been introduced using counterionic (cationic) buffers such as Tris or diethanolamine (Figure 5).

Sample Introduction and Sample Pretreatment

Sample introduction by electromigration methods is known to be matrix-dependent, therefore hydrostatic/hydrodynamic sample introduction is typically used. If electromigration injection is to be employed, matrix effects should be examined and standard addition rather than an external standard method should be used for calibration. Field-amplified stacking effects can be used for samples of lower ionic strength than the BGE, leading to lower concentration detection limits.

Real samples often require the application of simple procedures such as extraction, filtration to remove particular matter, or dilution, prior to the CE step and an online or at-line combination of such procedure(s) with CE is desirable. A dialysis/flow-injection analysis (FIA) sample clean-up system coupled online to a CE allowed analysis of a number of anions in a variety of samples with complex matrixes such as milk, juice, slurries or liquors from the pulp and paper industry. In some cases, matrix removal (sample clean-up) combined with preconcentration of the analyte(s) may be necessary. Online isotachophoresis (ITP)-CE systems are capable of analysing anionic analytes in complex matrixes, but commercial instrumentation is not available widely. A general rule for the applicability of CITP-CE for sample clean-up is that the mobility of the analyte(s) should differ from the matrix ion(s) to be removed.

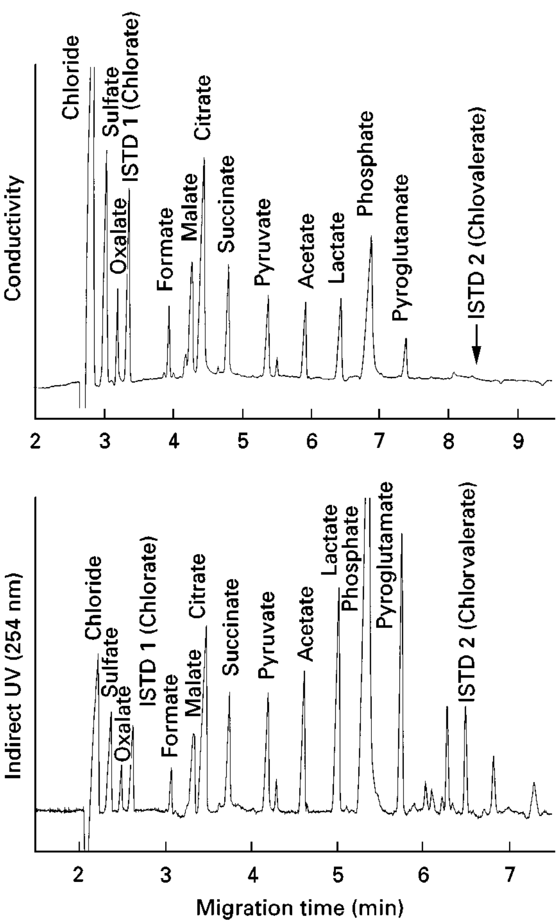

Figure 6 Separation of inorganic and anions and carboxylic acids in a beer sample using simultaneous nonsuppressed conductivity and indirect conductivity detection. Conditions: capillary, fused silica 50 μm i.d., 0.600 m length to end-capillary conductivity detector, 0.480 m to photometric detector; BGE, 7.5 mmol L^{-1} 4-aminobenzoic acid, 0.12 mmol L^{-1} TTAB, pH 5.75; separation voltage, − 30 kV; detection, indirect at 254 nm; injection, hydrodynamic at 25 mbar for 12 s; sample, 10 × diluted stout. (Reproduced with permission from Klampfl CW and Katzmayr MU (1998) Determination of low-molecular-mass anionic compounds in beverage samples using capillary zone electrophoresis with simultaneous indirect ultra-violet and conductivity detection. *Journal of Chromatography A* 822: 117–123.)

Applications

A high proportion of the real samples analysed for inorganic anions by CE have been water samples (drinking water, mineral water, river water, ground water, well water, etc.), with fruit juices and beverages being another common type of sample. The fact that CE is not tolerant to high ionic strength samples is reflected in the infrequent application of CE to samples such as seawater, unless considerable dilution of the sample is undertaken. **Figure 6** shows a successful application of CE to the determination of inorganic anions using simultaneous indirect photometric and conductometric detection. This example also illustrates the fact that real samples usually require separation of inorganic anions from organic anions.

Cations

Separation Strategy

As for inorganic anions, most inorganic cations have electrophoretic mobilities that are too high to permit counter-EOF separation, so these species are usually separated co-electroosmotically. In this case the electrode with positive polarity is placed at the injection side and when a bare fused silica capillary is used, the EOF is towards the detection side (cathode). Under these conditions the migration order is such that the analyte with the highest positive electrophoretic mobility migrates first and the analyte having the lowest positive mobility migrates last.

In contrast to the case for inorganic anions, many inorganic cations exhibit very similar mobilities, such as the whole group of rare earth metals or numerous transition metal ions (**Figure** 7). Therefore additional sources of separation selectivity to those available for anions are needed in order to separate these species. The main approach used is the addition of an auxiliary ligand to the BGE in order partially to complex the analyte cations (**Figure** 8). Provided the degree of complexation is different for each analyte cation, the effective charge and hence the effective mobility of each analyte will be unique and separation should be possible. These auxiliary ligands can be divided qualitatively into two groups according to the thermodynamic stability of the complexes formed with the analyte cations. **Table 4** summarizes the important characteristics of CE separation of metal ions using either weakly or strongly complexing auxiliary ligands.

Weakly complexing auxiliary ligands (such as HIBA or 19-crown-6) usually serve the sole purpose

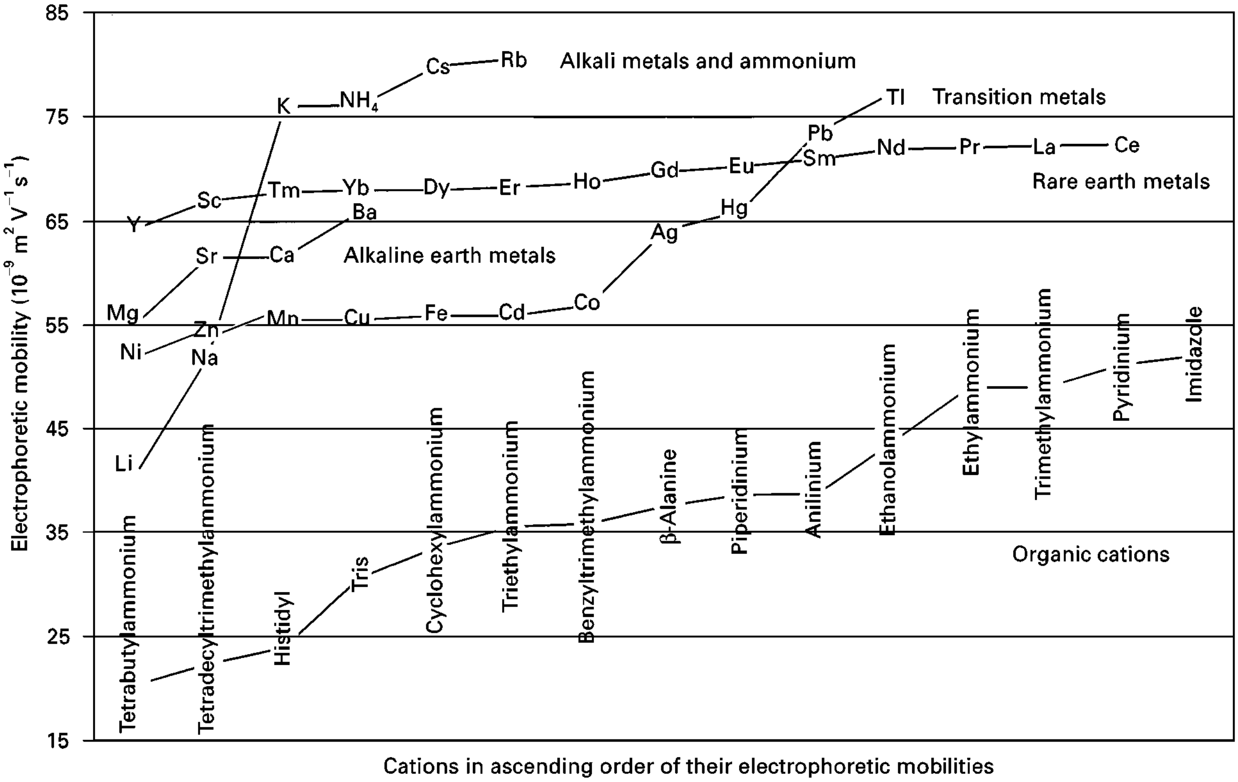

Figure 7 Electrophoretic mobilities of metal ions and some organic cations in ascending order to their mobilities. Ammonium was included with the alkaline metals because of its frequent importance in analysis of metal ions. Charges of metal cations have been left out for simplicity; transition metal ions divalent apart from monovalent Ag and Tl; organic cations all bear a charge of + 1.

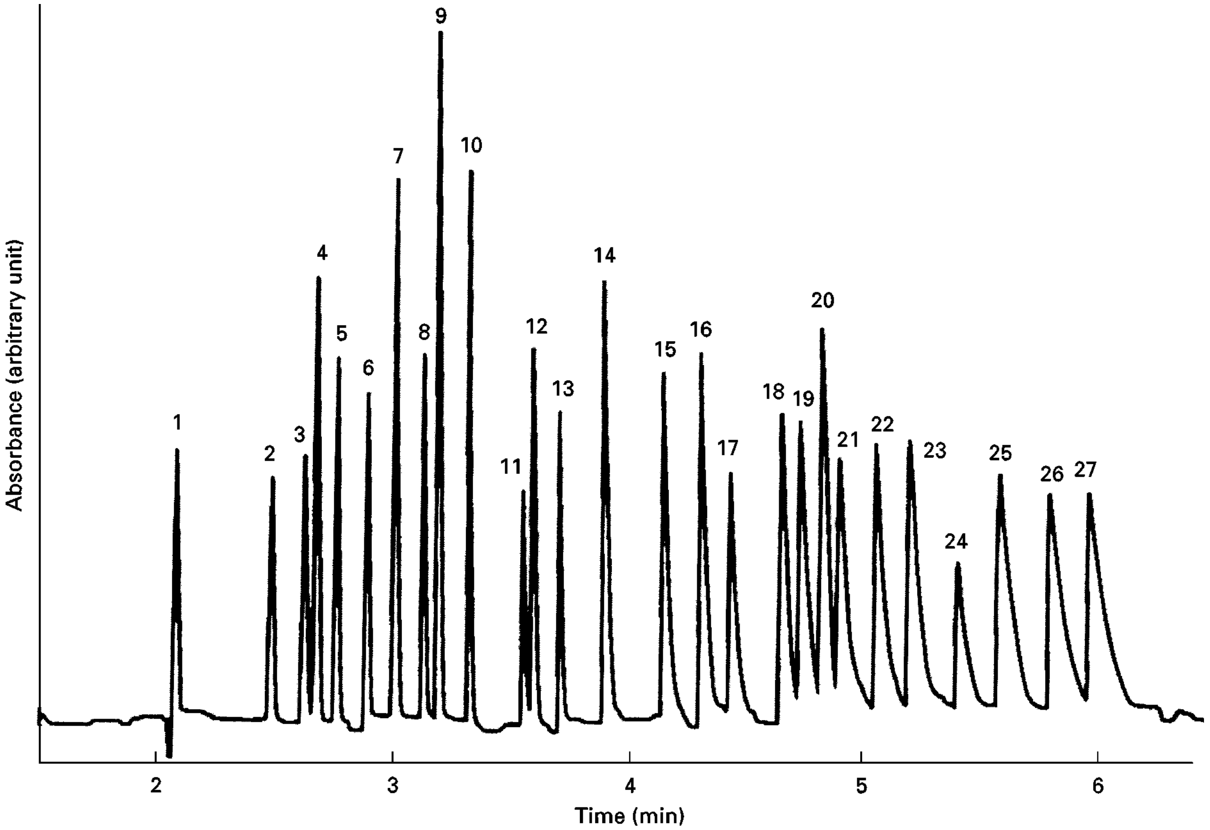

Figure 8 Separation of 27 alkali, alkaline earth, transition and rare earth metal ions in a single run using lactate as auxiliary ligand. Conditions: capillary, fused silica 75 μm i.d., 0.600 m length, 0.527 m to detector; BGE, 15 mmol L^{-1} lactic acid, 8 mmol L^{-1} 4-methylbenzylamine, 5% methanol, pH 4.25; separation voltage, 30 kV; detection, indirect at 214 nm; injection, hydrostatic at 100 mm for 30 s; sample, 1–5 ppm or each metal. Peak identification: 1, K$^+$; 2, Ba^{2+}; 3, Sr^{2+}; 4, Na$^+$; 5, Ca^{2+}; 6, Mg^{2+}; 7, Mn^{2+}; 8, Cd^{2+}; 9, Li$^+$; 10, Co^{2+}; 11, Pb^{2+}; 12, Ni^{2+}; 13, Zn^{2+}; 14, La^{3+}; 15, Ce^{3+}; 16, Pr^{3+}; 17, Nd^{3+}; 18, Sm^{3+}; 19, Gd^{3+}; 20, Cu^{2+}; 21, Tb^{3+}; 22, Dy^{3+}; 23, Ho^{3+}; 24, Er^{3+}; 25, Tm^{3+}; 26, Yb^{3+}; 27, Lu^{3+}. (Reproduced with permission from Shi YC and Fritz JS (1993) Separation of metal ions by capillary electrophoresis with a complexing electrolyte. *Journal of Chromatography* 640: 473–479.)

of manipulating the separation selectivity and normally form part of the BGE into which samples containing free metal ions are injected. That is, *on-capillary complexation* is utilized (Figure 8). Since a substantial part of the metal exists as an uncomplexed cation, indirect absorption detection using a cationic absorbing probe contained within the BGE is normally applied. Rapid complexation equilibria are necessary to enable manipulation of the selectivity of separation by varying parameters of the BGE such as the concentration of the auxiliary ligand and pH (Figure 8).

A completely different strategy is to use ligands forming stable, typically anionic complexes,

Table 4 Typical method properties according to the stability of the complex formed betweeen the auxiliary ligand and the metal ion analyte

Thermodynamic stability of the metal complex with the auxiliary ligand	*Low*	*High*
Typical examples of the auxiliary ligand	HIBA, citric acid, lactic acid	CN$^-$, polydentate ligands such as EDTA and its analogues, metallochromic ligands
Degree of metal ion complexation	Partial	Total
Method of complex formation	On-capillary	Pre-capillary, often with on-capillary also used
Method of photometric detection	Indirect	Direct
Ease of selectivity manipulation	Straightforward	More difficult
Polarity of separation voltage on injection side	+	+, −

which are then separated as anions using the same approaches discussed earlier. Auxiliary ligands such as ethylenediaminetetraacetic acid (EDTA) or 2-pyridylazoresorcinol (PAR) may be used for this purpose and are usually added to the sample (that is, *precapillary complexation* is used). Often the BGE also contains a low concentration of the ligand in order to prevent the decomposition of less stable complexes. Most (or all) of the metal is complexed, so that detection using direct photometry is usually applicable. The complexation equilibria can be slow, but this then makes it difficult to alter the selectivity of separation and to retain good peak shapes. These separations are carried out in bare fused silica capillaries, fused silica capillaries with reversed EOF, or using other capillary wall chemistries. Depending on the charge of the metal complexes, the separation can be performed in either the co- or counter-electroosmotic modes, with either positive or negative separation potential applied on the injection side.

Metal ions are known to adsorb onto the surface of silica particles through interaction with silanol groups. In the case of polyvalent metal cations, this adsorption can be considered to be irreversible. Although the surface density of silanols on fused silica is lower by approximately an order of magnitude than for porous silica, it is well documented in CE using bare fused silica capillaries that metal ions present in the BGE (even as an impurity) can adsorb onto the capillary wall. The extent of adsorption is often reflected in changes to the EOF, with both suppression and reversal of EOF having been demonstrated. Despite these facts, coated capillaries have found relatively little use in separations of metal ions. In bare fused silica capillaries, the risk of adsorption of metal ions is counteracted by typically weakly acidic and complexing electrolytes.

Separation Selectivity

The most powerful and straightforward tool governing separation selectivity is control of the degree of complexation with the auxiliary ligand. For a metal ion present in several forms that are in equilibrium with rapid kinetics of interchange between the forms, the effective mobility of the analyte is given by the weighted average of the mobilities of each of the forms. For a metal ion M migrating in a BGE containing a ligand L forming complexes ML, ML_2, …, ML_i, the effective mobility of the metal can then be expressed as:

$$\bar{\mu}_{\mathrm{M}} = \sum_{i=0}^{n} \mu_i \alpha_i = \frac{\sum_{i=0}^{n} \mu_i [\mathrm{ML}_i]}{\sum_{i=0}^{n} [\mathrm{ML}_i]} = \frac{\sum_{i=0}^{n} \mu_i \beta_i [\mathrm{L}]^i}{\sum_{i=0}^{n} \beta_i [\mathrm{L}]^i} \qquad [3]$$

where μ_i is mobility and α_i is the fraction of metal ion existing in form i, $[\mathrm{ML}_i]$ is the concentration of the complex ML_i, [L] is the concentration of the form of ligand forming the complex and β is the overall stability constant ($\mathrm{ML}_0 = \mathrm{M}$, $\beta_0 = 1$).

A different situation occurs when utilizing auxiliary complexing ligands that form strong complexes with the metal ions. Since most of the metal ion is complexed under all BGE conditions, the complex formation/dissociation cannot be used to govern the separation selectivity as in the case of weakly complexing ligands. Apart from minor factors influencing the selectivity, such as solvation changes in various media, there are very few means to bring about a change in the charge/mass value of the analytes (and consequently a substantial change in selectivity). These include: (1) dissociation of functional groups on the ligand exhibiting protonation equilibria; (2) exchange of the remaining water molecules on the metal ion coordination sites not saturated by the ligand; and (3) ion association or ion exchange equilibria in the BGE solution (Table 4). The separation selectivity therefore varies considerably with the nature of the ligand used.

Detection

Direct and indirect photometric detection As with inorganic anions, direct and indirect photometric detection are the most commonly used detection methods in the separation of metal ions, being employed in about 85% of publications. Since most hydrated metal ions do not absorb at all, or have only weak absorption bands in the UV region above 185 nm, direct detection is normally possible only when the metal ions are complexed with an auxiliary ligand. Thus the auxiliary ligand, which in earlier discussion has been shown to play a crucial role in the separation of metal ions, also enhances the detectability of these species. Metallochromic ligands form highly absorbing ($\varepsilon \sim 10^5\ \mathrm{L\ mol^{-1}\ cm^{-1}}$) coloured complexes with metal ions. When these complexes are stable and the separation results in well-shaped peaks, very good detection sensitivity can be obtained. For example, determination of transition metals in the form of complexes with PAR achieved concentration LODs in the order of $10^{-7}\ \mathrm{mol\ L^{-1}}$ or absolute LODs at fmol levels. Even lower LODs ($80\ \mathrm{nmol\ L^{-1}}$ or 0.5 fmol for Zn) have been obtained for porphinate complexes ($\varepsilon \sim 10^5\ \mathrm{L\ mol^{-1}\ cm^{-1}}$), but the detection method is less universal because different metal ions exhibit a range of absorption maxima and absorptivities.

The underlying principles for indirect photometric detection of inorganic cations are the same as for inorganic anions (see above). **Figure 9** illustrates matching of electrophoretic mobilities of some metal cations as analytes with some cationic probes.

Other detection methods Conductometric, amperometric and potentiometric detection methods can be utilized for inorganic cations and the same principles discussed apply. End-capillary conductometric detection has been used for a range of cations using low-conductive histidine-MES BGEs with similar success to anion analysis (LODs are in low μmol L^{-1} range). End-capillary amperometric detection utilizing reduction of metal ions such as thallium, lead, cadmium, etc., and inorganic and organic mercury compounds on gold, platinum or mercury films, has been applied with low nmol L^{-1} range LODs. Potentiometric detection can also be used for metal ions and, as for anions, the solid-state electrodes show greatest promise.

CE coupled with electrospray mass spectrometry has achieved detection of about 30 metal ions by positive ion MS. Although chemical noise does not allow detection below ppb levels, LODs are generally below those obtained for indirect photometric detection. Impressive LODs have been obtained by CE-ICP-MS, e.g. of 0.06 ppb for Sr^{II} (8 fg or 90 amol).

Method Optimization

The separation of up to 30 metal ions in one run using weakly complexing auxiliary ligands is a challenging analytical task and optimization of the BGE composition often requires the use of computer methods based on thermodynamic complexation models or the use of artificial neural networks. The BGEs used most frequently for metal ion analysis would normally utilize indirect photometric detection and a typical BGE contains a weakly complexing auxiliary ligand (such as HIBA) for governing separation selectivity for most transition metal ions and rare earth metals. It often also contains a crown-ether as a second auxiliary ligand to separate ammonium from potassium (see Figure 7) and an indirect detection cationic probe (such as imidazole, benzylamine, or creatinine) at pH ~ 4.5–5.

While most authors have demonstrated the application of their developed separation to a real

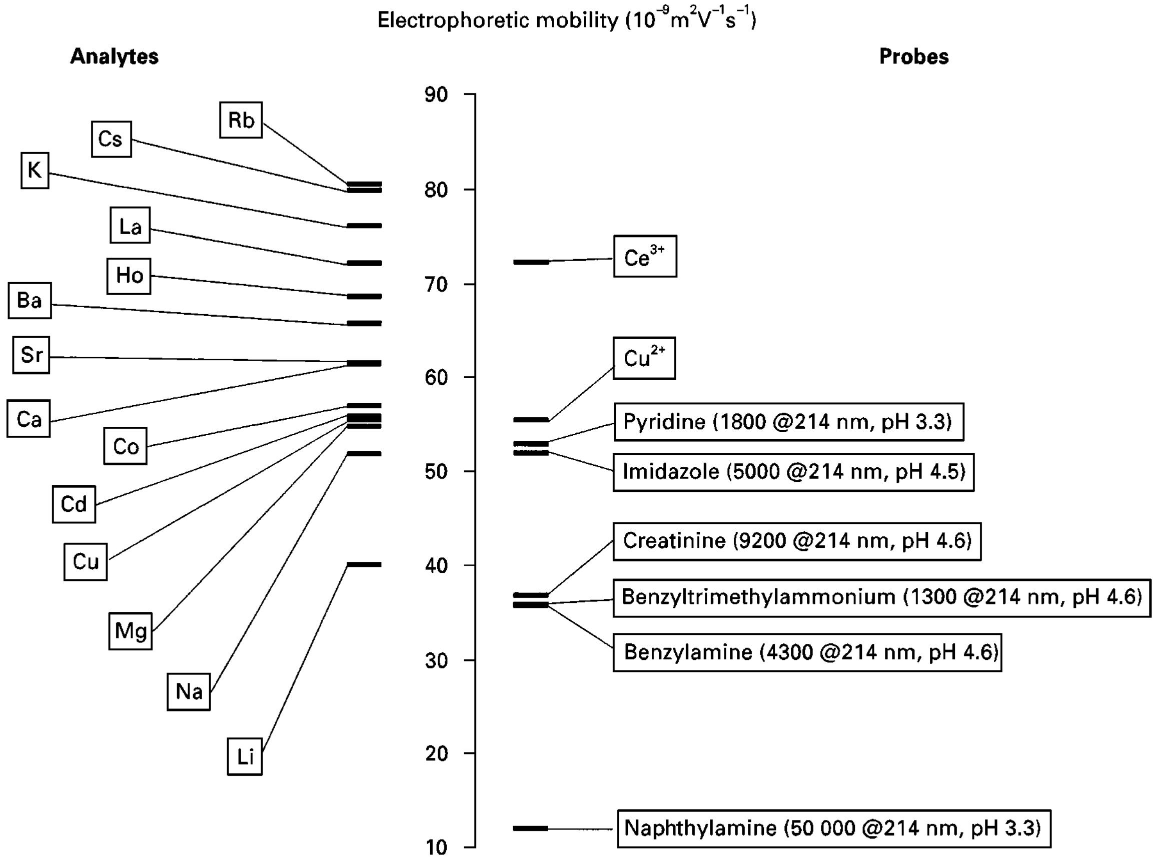

Figure 9 Matching of electrophoretic mobilities of some metal ion analytes and probes. Given in brackets are molar absorptivity ($L\ mol^{-1}\ cm^{-1}$) at a wavelength (nm), and optionally a pH. (Source of data: Beck W and Engelhardt H (1992) *Chromatographia* 313; Chen M and Cassidy RM (1993) Separation of metal ions by capillary electrophoresis. *Journal of Chromatography* 640: 425–431.)

sample of some kind, there have been relatively few studies on aspects of method validation, such as precision and accuracy. Typical precision for migration times, peak areas and peak heights show a relative standard deviation ranging from 3 to 10%.

Sample Introduction and Sample Pretreatment

In addition to the principles discussed earlier for anions, stacking effects can be achieved in the separation of metal ions using on-capillary complex formation in which oppositely migrating metal cations and ligand anions converge and react at the boundary between the injected sample plug and the BGE. A necessary condition to utilize the stacking by on-capillary complexation is fast complex formation. This approach was first demonstrated for four divalent metal complexes with PAR in which a plug of 1 mmol L^{-1} PAR was first injected into the capillary are then electromigration injection from a sample without added ligand was performed. This method with an optimized stacking procedure gave detection limits in the range of 10 nmol L^{-1}.

Ion exchange and chelating resins may be used to preconcentrate metal ions prior to CE determination in an offline configuration. Ion exchange materials are less successful for the selective preconcentration of heavy metals than chelating (iminodiacetate or dithiocarbamate) resins, or by the formation of metal chelates with an excess of the reagent, followed by adsorption of the chelate on a hydrophobic column.

Applications

As with anions, many of the real samples analysed have been various water samples, fruit juices and beverages and food. An example of an unusual application of CE to the analysis of high salinity samples is shown in **Figure 10**.

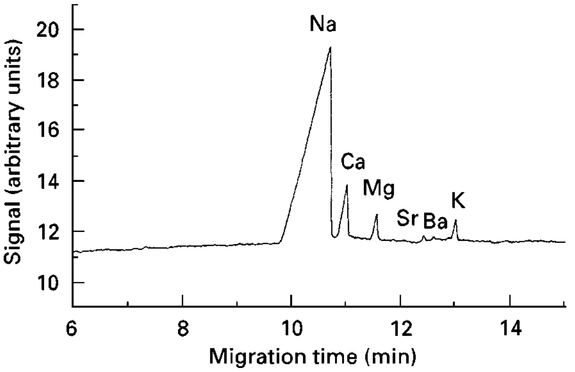

Figure 10 Separation of metal ions in a mixture of salt water and formation water using indirect photometric detection. Conditions: capillary, fused silica 75 μm i.d., 0.600 m length, 0.520 m to detector; BGE, 6.5 mmol L^{-1} HIBA, 5 mmol L^{-1} UVCAT-1 (Waters), 6.2 mmol L^{-1} 18-crown-6, 25% (v/v) methanol, apparent pH 4.8; separation voltage, 20 kV; detection, indirect at 185 nm; injection, hydrodynamic at 98 mm for 20 s; sample, diluted by a factor of 125. (Reproduced with permission from Tangen A, Lund W and Frederiksen RB (1997) Determination of sodium, potassium, magnesium and calcium ions in mixtures of sea water and formation water by capillary electrophoresis. *Journal of Chromatography A* 767: 311–317.)

Simultaneous Separations of Anions and Cations

Despite the potential advantages of reduced analysis time and costs that arise from analysing both anions and cations in one run, separate analysis of both groups is still much easier and more robust. The simple fact that anions and cations migrate in opposite directions in an electric field means that a simultaneous analysis cannot be realized with one point of sample introduction and one point of detection.

However, there are two approaches that have proved successful. The first uses a sample injection simultaneously in two capillaries operated with the same BGE, with both capillaries equipped with independent detectors located near their ends. A relatively easier approach uses just one capillary, but sample is injected at both ends and the detector is located approximately in the middle of the capillary. This approach has been applied to the analysis of a range of real samples; an example is given in **Figure 11**.

It should be noted that when applying indirect detection using both an anionic and a cationic probe, the probes should be mixed in their free acid and free base forms to avoid the presence of competing ions in the BGE.

Future Developments

Control of the separation selectivity will be an area of development in CE of inorganic species. Utilization of ion exchange-type interactions with pseudostationary phases, and particles of sub-μm size, can introduce new selectivity into a CE separation. It is instructive to examine the separation selectivities achieved by ion chromatography and CE under standard conditions, as revealed by the elution or migration order of common analytes. In the case of IC, anions are eluted in the following order of retention times: $F^- < Cl^- < NO_2^- < Br^- < NO_3^- < PO_4^{3-} < SO_4^{2-} < I^-$. However, the migration times of these species in co-EOF CE are $Br^- < Cl^- < SO_4^{2-} < NO_2^- < I^- < NO_3^- < F^- < PO_4^{3-}$. A similar pattern emerges when the same comparison is made for inorganic cations, for which the IC retention times follow the order: $Li^+ < Na^+ < NH_4^+ < K^+ < Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$, while migration times for co-EOF CE are

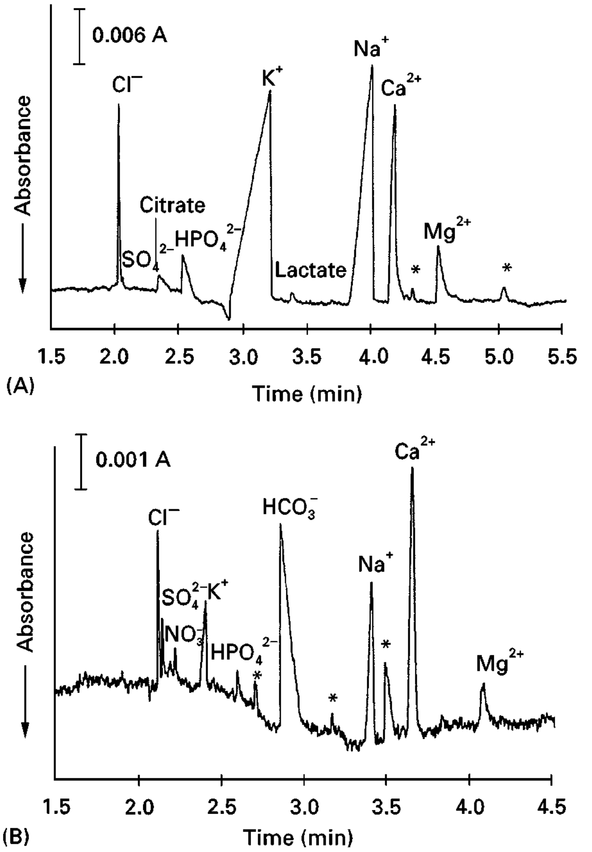

Figure 11 Simultaneous determination of inorganic anions and cations in milk (A) and mud (B) after offline dialysis. Conditions: capillary, fused silica 50 μm i.d., 0.500 m total length, 0.200 m to detection window from the anodic end; BGE, 6 mmol L^{-1} 4-aminopyridine, 2.7 mmol L^{-1} H_2CrO_4, 30 μmol L^{-1} CTAB, 2 mmol L^{-1} 18-crown-6, pH 8; separation voltage, 20 kV; detection, indirect at 262 nm; injection, hydrostatic at 50 mm for 10 s (cathode end) and 50 mm for 10 s (anode end) (A) or at 50 mm for 20 s (cathode end) and 100 mm for 40 s (anode end) (B); time between the injections, 60 s (A) or 30 s (B). (Reproduced with permission from Kuban P and Karlberg B (1998) Simultaneous determination of small cations and anions by capillary electrophoresis. *Analytical Chemistry* 70: 360–365.)

$NH_4^+ < K^+ < Ba^{2+} < Sr^{2+} < Ca^{2+} < Na^+ < Mg^{2+} < Li^+$. Complementary selectivities are again apparent. These selectivities suggest that a mixed-mode separation system in which the movement of analytes is influenced both by electromigration effects and ion exchange interactions might provide a means to manipulate selectivity in order to solve existing separation problems.

New developments can also be expected in the area of detection techniques. For instance, the use of highly absorbing cationic probes in carefully formulated BGEs that avoid competitive displacement has the potential to increase the sensitivity using indirect photometric detection. Further development of other detection techniques can be anticipated, especially those compatible with the capillary dimensions and suitable for on- or end-capillary use and which do not necessitate elaborate changes to the capillary.

Finally, more advances in the area of online sample treatment and/or preconcentration techniques are likely to occur as CE becomes more of a routine tool for the determination of inorganic species in complex samples.

Further Reading

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