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Review

Enhancement of selectivity in capillary electrophoretic separations of metals and ligands through complex formation

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Abstract

A significant improvement of separation selectivity can be achieved in capillary electrophoresis by exploiting the formation of complexes which modify the analyte electrophoretic mobilities. Many organic compounds with electron-donor properties can form weak charge transfer complexes with suitable metals. These types of complexing equilibria can be exploited in the separation of both species involved in complex formation: metals and organic compounds. The first part of this article will review the use of complexation reactions to enhance separation selectivity in the analysis of metals whereas the second part will consider addition of metal ions to the background electrolyte to achieve separation of organic ligands. © 1998 Elsevier Science B.V.

Keywords: Reviews; Selectivity; Complexation; Enantiomer separation; Metal cations; Organic acids; Peptides; Carbohydrates; Oligonucleotides

Contents

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1. Introduction

Capillary zone electrophoresis (CZE) is a highly efficient separation method that comprises all types of electrophoresis in fused-silica capillaries of less than 100 μm I.D. The separation principle of CZE is based on the differential electrophoretic mobility of charged compounds. Several factors such as pH, conductivity and ionic strength of the background electrolyte (BGE) have significant effects on migration behavior in capillary electrophoresis (CE) [1,2].

Improvement of separation selectivity is of fundamental importance in separation science. The increase in selectivity has been achieved through the addition of various additives to BGE. The application of CE to uncharged compounds required the development of electrokinetic chromatography [3]. Micellar electrokinetic chromatography (MEKC) is now a well established technique that allows electrophoretic separations of uncharged compounds, by combining an electrophoretic separation process with a partitioning process [4,5]. Several other kinds of complexations have been also used in order to maximize separation selectivity in CZE. An example is the separation of racemates through the addition of cyclodextrins to the background electrolyte (Ref. [6] and references therein).

This article will give an overview of a particular means of selectivity manipulation that involves formation of weak charge transfer complexes between organic compounds with electron donor properties and suitable metals. Formation of these complexes is generally rapid and the relatively weak complexes are in equilibrium with the non-complexed components. This type of complex formation is widely exploited in chromatography to achieve selectivity. The use of metals in chromatographic separations has been extensively reviewed by Guha and Janak [7].

Complexation equilibria with a wide variety of anionic complexing reagents added to the background electrolyte have been exploited in the separation of metal ions by CE [8–16]. An overview of the metal analysis is given in Refs. [17,18]. The equivalent ionic conductivity of most metal cations and, consequently, their electrophoretic mobilities are too close to provide selective separations simply based on the migration behavior of free cations. The separation of most multivalent metals requires the addition of an appropriate complexing agent to the BGE and the great utility of this approach is that different ligands can provide selectivity for different classes of metals.

Another promising approach is given by the addition of a suitable metal to the BGE for selective complexation with particular entities. The species involved in complexation equilibria with metals undergo a change in charge distribution that leads to a significant variation of their electrophoretic mobility.

This review will give a critical literature survey of complexation reactions between metal ions and organic molecules to enhance selectivity in CZE. The most important parameters affecting complexation equilibria, i.e., ligand type and concentration, and electrolyte pH will be discussed with the aim of orienting the reader on the development of CE procedures suitable for practical solutions. The applicability of this means of selectivity manipulation is well documented in the literature, the data presented are collected from the literature since the beginning of 1985.

2. Separation of metal ions

Reliable and rapid techniques are needed to determine metal ions in medicines, soil samples, drinking water, etc. A number of techniques such as various atomic spectroscopic methods [19], electrochemical methods and ion chromatography are available today for the analysis of metal ions [20–24].

CE is becoming a routine technique for the analysis of metals owing to its relative simplicity, high efficiency and separation speed. Sensitive detection of metals in CE is attained by indirect UV absorbance, the most straightforward method to solve the problem of the weak UV absorption of most metals [25]. Moreover, the detection of metal ions can be accomplished through the formation of UV-active complexes of the same ions [26].

One of the problems in the analysis of inorganic ions by CE is that, in some cation groups, the individual cations have almost the same mobility due to their similar size and identical charge. Divalent transition metals and lanthanide cations are exam-

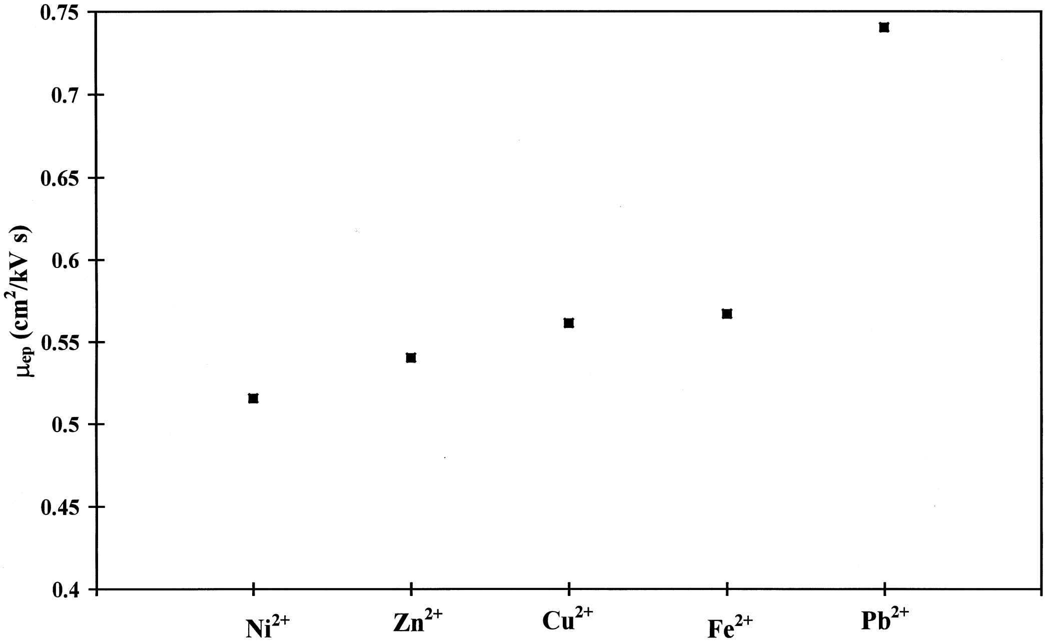

Fig. 1. Electrophoretic mobilities of metal ions (from Ref. [37], with permission).

ples. As reported in Fig. 1, the mobility of five different transition metals is identical; the only exception is represented by lead which has a higher mobility due to its lower degree of hydration [27]. The electrophoretic mobility of ions in an electric field is not only influenced by their charge, mass and physical dimensions but also by interactions with buffer components. In cases where the mobilities of the free cations are very similar, the introduction of additional chemical interactions into the separation system is necessary. The addition of a water soluble complexing reagent to the electrolyte buffer is used to modify the mobility of metal ions to a discriminating extent as the ions that are complexed to a greater degree migrate more slowly than those that have a lower fraction in the complex form. This is a result of the effective charge on the complexed metal being reduced.

3. Factors influencing complexation equilibria

3.1. Ligand concentration

The optimum ligand concentration in the separation buffer can be calculated if the stability constants of the complexes of different metals with the ligand are known [28,29]. The optimum concentration is the ligand concentration at which the complexes have the highest mobility differences and therefore the highest resolution.

The separation of rare earth ions, for instance, is a challenging problem due to their similar chemical and physicochemical behavior. In the particular case of rare earth ions the addition of a monovalent ligand to the buffer leads to the formation of the following complexes [28]:

$$M^{3+} + L^- \rightleftarrows ML^{2+} \qquad (1)$$

$$\beta_1 = \frac{[ML^{2+}]}{[M^{3+}][L^-]} \qquad (2)$$

$$M^{3+} + 2L^- \rightleftarrows ML_2^+ \qquad (3)$$

$$\beta_2 = \frac{[ML_2^+]}{[M^{3+}][L^-]^2} \qquad (4)$$

$$M^{3+} + 3L^- \rightleftarrows ML_3 \qquad (5)$$

$$\beta_3 = \frac{[ML_3]}{[M^{3+}][L^-]^3} \tag{6}$$

$$M^{3+} + 4L^- \rightleftarrows ML_4^- \tag{7}$$

$$\beta_4 = \frac{[ML_4^-]}{[M^{3+}][L^-]^4} \tag{8}$$

Due to the fast equilibration between the different complexes it is possible to describe the system as a single complex $ML_{\bar{n}}^{(3-\bar{n})+}$ with the average number of ligands $\bar{n}$ that is equivalent to the average degree of complexation and that can be calculated as follows:

$$\bar{n} = \frac{\beta_1[L^-] + 2\beta_2[L^-]^2 + ... + m\beta_m[L^-]^m}{1 + \beta_1[L^-] + \beta_2[L^-]^2 + ...\beta_m[L^-]^m} \tag{9}$$

Eq. (9) shows that the degree of complexation depends on the concentration of the free ligand. If the stability constants of different metal complexes with the same ligands are known, it is possible to calculate the average degree of complexation $\bar{n}$. Table 1 shows the fraction of free (α_M) and complexed (α_{MLn}) rare earth metal ions and average number of ligands $\bar{n}$ in a solution containing α-hydroxyisobutyric acid (HIBA). The distribution of chemical species shown in Table 1 indicates that the predominant species in the solution are the free metal ion (M^{3+}), the 1:1 (ML^{2+}), the 2:1 (ML_2^+) and the 3:1 (ML_3) complexes whereas the 4:1 complex is only present as a small fraction. The separation between different ions arises from the difference of their average degree of complexation $\bar{n}$, which depends on the stability constants of the metal ions with the selected ligand. As an example, Fig. 2 shows overall stability constants of the rare earth complexes with acetate, lactate and HIBA taken from the literature [30,31]. Complexation with acetate fails to separate these ions. Only HIBA and lactate complexes can be separated by CE due to the high difference in their stability constants [28]. Fig. 3 shows the dependency of migration times of a group of alkali, alkaline earth and transition metals from the HIBA concentration [32]. The effects of an increased ligand concentration are pronounced with transition metals that are more efficiently resolved when the concentration of the HIBA is increased. Since alkali metals do not form complexes with HIBA the increase in migration time observed as the HIBA concentration is increased must arise from the decrease in electroosmotic flow (EOF) due to ionic strength variations.

Table 1

Fractions of free (α_M) and complexed (α_{MLn}) rare earth metal ions and average number of ligands ($\bar{n}$) in a 4 m*M* HIBA electrolyte solution at pH 4.3

Metal	α_{M}^{3+}	α_{ML}^{2+}	α_{ML2}^{+}	α_{ML3}	α_{ML4}^{-}	$\bar{n}$
La	0.578	0.360	0.612			0.482
Ce	0.448	0.496	0.052	0.004		0.612
Pr	0.407	0.496	0.093	0.005	0.000	0.697
Nd	0.333	0.572	0.085	0.010	0.000	0.772
Sm	0.296	0.520	0.170	0.013	0.001	0.903
Gd	0.250	0.481	0.244	0.024	0.001	1.045
Tb	0.181	0.470	0.307	0.040	0.002	1.212
Dy	0.141	0.384	0.387	0.084	0.004	1.426
Ho	0.122	0.365	0.413	0.093	0.006	1.494
Er	0.097	0.309	0.472	0.112	0.010	1.629
Tm	0.079	0.309	0.473	0.123	0.016	1.686
Yb	0.070	0.296	0.431	0.169	0.033	1.797
Lu	0.047	0.222	0.514	1.171	0.045	1.946

Reprinted from Ref. [8], with permission.

3.2. Influence of pH

In most of the cases the ligand is an ionizable compound, therefore Its concentration depends on the pH as follows [28]:

$$[HL] \rightleftarrows [H^+] + [L^-] \tag{10}$$

$$K_s = \frac{[H^+] + [L^-]}{[HL]} \tag{11}$$

the concentration of ligand $[L^-]$ is determined by:

$$K_s = \frac{[H^+] + [L^-]}{C_s - [L^-]} \tag{12}$$

and

$$[L^-] = \frac{K_s C_s}{[H^+] + K_s} \tag{13}$$

where C_s is the total ligand concentration.

When the pH is increased the concentration of the ligand is also increased and consequently the degree of association is likewise increased. However, the most dramatic effects deriving from an increase of electrolyte pHs are related to EOF variation. At

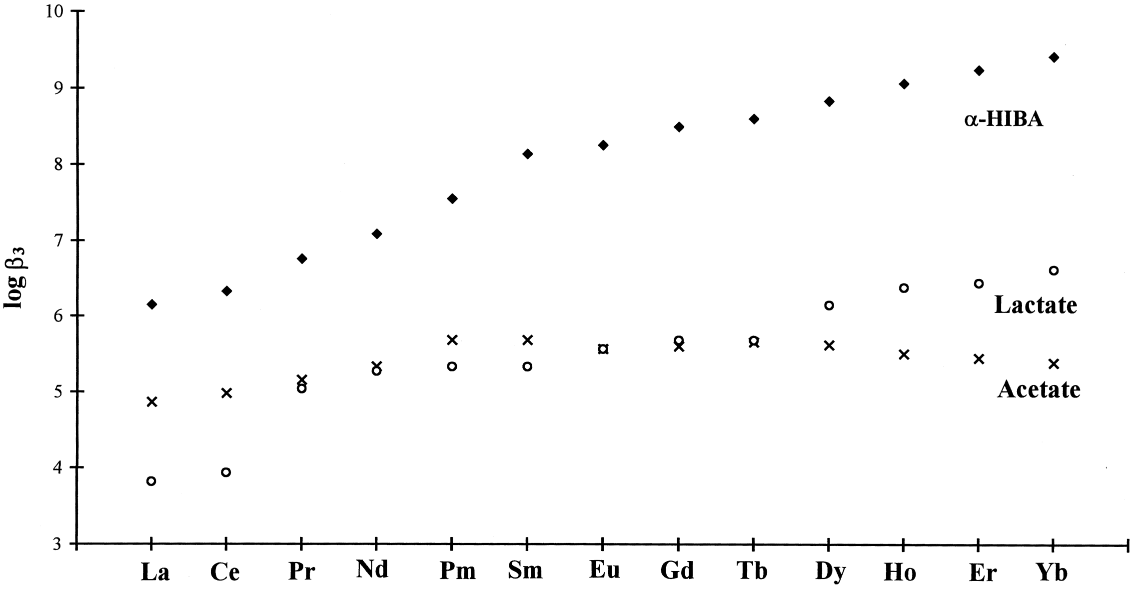

Fig. 2. Overall stability constants of the rare earth complexes (from Ref. [28] with permission).

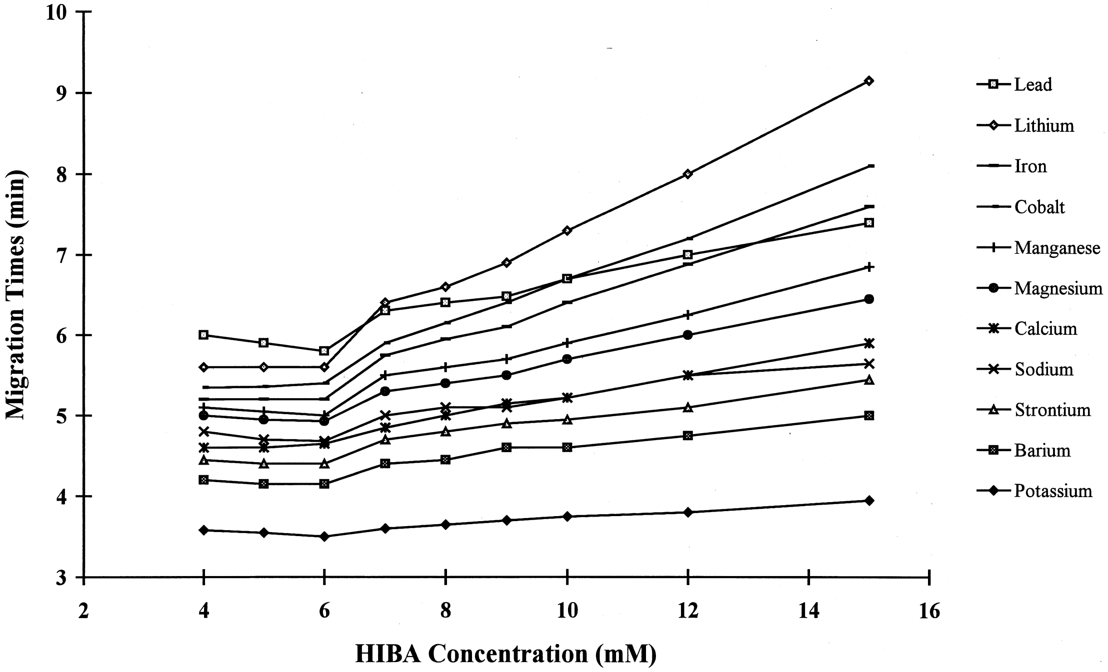

Fig. 3. Effect of HIBA concentration on the migration times of a group of alkali, alkaline earth and transition metals (from Ref. [32], with permission).

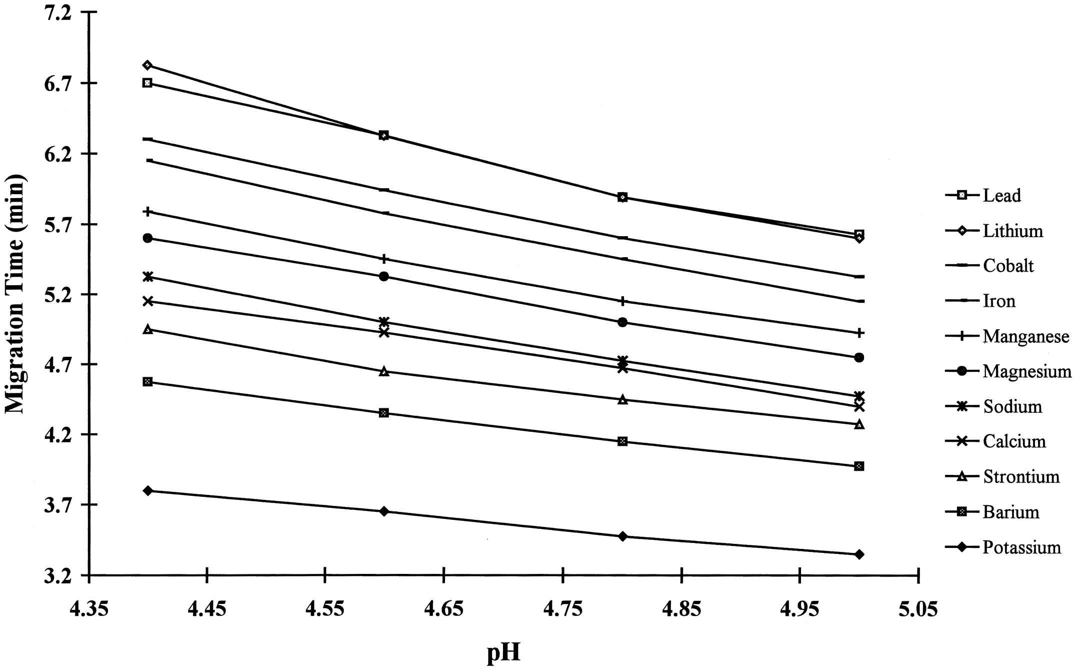

Fig. 4. Effect of pH on the migration times of various inorganic metal cations. Electrolyte buffer: 5 m*M* UV-cat-1, 6.5 m*M* HIBA. The natural pH was 4.4 and the pH was raised with *N*,*N*-diethylaminoethanol (from Ref. [32], with permission).

higher pHs additional dissociation of silanols occurs that leads to an increase of EOF, which in turn accelerates analyte migration. Fig. 4 shows the dependency of electrophoretic mobility of rare earth HIBA complexes on pH. As the pH increases the migration time decreases due to the increase of EOF. The pH is also affecting the complexing equilibrium and this effect become evident by comparing the slopes of migration times. The slopes of curves corresponding to metals that do not undergo complexation are expected to reflect only the change of EOF with pH.

3.3. Influence of ligand type

Weak, medium or strong complexing agents can be used to modulate selectivity in CE.

Table 2 shows the stability constants of metal

Table 2
Stability constants of different metal complexes

	HIBA			HQS^-		EDTA
	Log β_1	Log β_2	Log β_3	Log β_1	Log β_2	Log β_3
Cu^{2+}	2.74[a]	4.34[a]	4.38[a]	12.50[b]	23.10[b]	18.80[d]
Zn^{2+}	1.72[a]	3.01[a]	3.40[a]	8.40[b]	15.10[b]	16.50[d]
Pb^{2+}				8.53[b]	16.13[b]	18.04[d]
Ni^{2+}	1.67[a]	2.80[a]	2.84[a]	10.00[b]	18.10[b]	18.62[d]
Fe^{2+}				8.40[b]	15.10[b]	14.32[d]
Fe^{3+}				11.6[c]	22.8[c]	25.10[d]

[a] Ref. [33].
[b] Ref. [34].
[c] Ref. [35].
[d] Ref. [36].

complexes with different ligands [33–36]. Weak complexing agents of the type HIBA have to be added in large quantities to the separation buffer to modify the mobility of different cations. The different complexes, having similar stability constants coexist in solution and can be described as $ML_{\bar{n}}$ where $\bar{n}$ is the average number of ligands [37].

Complexation of divalent cations with a medium complexing agent, e.g., 8-hydroxyquinoline-5-sulfonic acid (HQS^-), leads to a complete separation only when HQS^- is used at stoichiometric or excessive concentrations [38]. The complexes exist predominantly in the ML_2^{2-} form.

Stronger complexing agents, e.g., EDTA, form stable metal complexes due to their high stability constants. Even at a very low concentration of ligand nearly all metal ions are completely complexed by L^{4-} and only ML^{2-} complexes are present in the solution [39,40]. These types of complexing agents are used in the pre-capillary complexation approach in which metal ions are converted into strongly charged complexes. In the case of transition metals, anionic complexes with similar charge are formed, therefore the separation selectivity is very poor. However, EDTA can be used to manipulate the selectivity under conditions of incomplete complexation [39].

4. Influence of complexation equilibria on electrophoretic mobility

The apparent mobility of metal ions is given by the sum of their electrophoretic mobility and the EOF. In the presence of a complex forming equilibrium the electrophoretic mobility of a complex can be calculated form the mobilities of the different complexes and their proportion to the equilibrium.

$$\mu = \mu_{eo} + \chi_{M^{3+}} \mu_{M^{3+}} + \chi_{ML^{2+}} \mu_{ML^{2+}} + \chi_{ML_2^+} \mu_{ML_2^+} + \chi_{ML_3} \mu_{ML_3} + \chi_{ML_4^-} \mu_{ML_4^-} \qquad (14)$$

The electrophoretic mobility of a metal complex decreases when increasing the complexation degree $\bar{n}$. The free cations move along the capillary at a rate proportional to their ionic mobility, whereas the complexed ions move at lower speeds. The equilibrium kinetics between free metal cations and complexed species must be very rapid to maintain tight electrophoretic zones. If the equilibrium is too slow the various chemical species of an analyte would migrate at different velocities, therefore broadening the electrophoretic zones. The affinity of the ligand for an ion should also be carefully considered. The larger the formation constant, K, the lower the apparent charge on the cation and thus the slower the mobility of the cation is expected to be. If the fraction of metal in the free cation is too small it may take too much time for an analyte to emerge from the column with negative consequences on the separation efficiency. On the contrary, if the metal ions are not sufficiently complexed their mobility may be too similar to achieve a complete resolution.

5. Addition of weak complexing agents

5.1. Mono-, di-, triprotic and hydroxycarboxylic acids

Transition metal cations, alkali and alkaline earth metal cations have similar electrophoretic mobilities. The separation of these cations requires the addition of a complexing agent able to selectively decrease their electrophoretic mobility by in situ complexation.

The role of complexing agents (acetic, glycolic, lactic, HIBA, oxalic, malonic, malic, tartaric, succinic and citric acid) and the influence of pH on separation selectivity have been extensively investigated [11]. The various acids were added in concentration ranges of 0.1 to 6.4 m*M* to an imidazole buffer which served as both the carrier electrolyte and for indirect UV detection. Imidazole was chosen as the carrier electrolyte as its mobility matches that of most of the analytes. The four monoprotic complexing agents acetic, glycolic, lactic and HIBA affect the separation of the six ions K^+, Ba^{2+}, Ca^{2+}, Na^+, Mg^{2+}, Li^{2+} contained in the test mixture in a similar way. Separation into six peaks was obtained in the presence of all monoprotic acids and the pH did not influence the migration order. However, each acid provides optimal resolution in a different pH interval. HIBA and lactic acid, offering a wider applicable pH range with good separation and high efficiency, should be preferred as complexing agents over other monoprotic acids.

The effects of diprotic acids were more complex and the migration order of the six ions was considerably different. The pH substantially influenced the separation and the migration order. Diprotic oxalic, malonic and succinic acids provided a wider migration time-span with a wider peak to peak separation time and are also good choices in the separation of divalent cations. Fig. 5 shows a comparison of the migration times of the six model ions in the presence of different complexing agents. The separation was carried out in the pH range where optimum resolution could be obtained.

Lee and Lin [12] analyzed a mixture of 19 metal ions (Li, Na, K, Cs, Mg, Ca, Sr, Ba, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ag, Al and Pb) (charges are omitted for simplicity) by CE, using glycolic acid as the complexing agent in different electrolyte systems at pH 4.0. With the exception of Ag^{+} and Al^{3+} all other ions could be separated in less than 15 min.

Shi and Fritz [8] optimized the conditions to separate alkali, alkaline earth, transition metal and lanthanide cations by CZE. Hydroxyisobutyric, phtalic, tartaric, lactic, malonic and succinic acid were used as complexing agents. Of these, HIBA, phtalic, tartaric and lactic acid were the most promising. The achievement of a good separation required a careful optimization of the concentration of complexing agents, UV-indirect co-ion, methanol and pH. A separation of 27 alkali, alkaline earth, transition and rare earth metal ions was obtained in 6 min using lactic acid as the complexing agent and 4-methylbenzylamine as the indirect detection coion in the presence of 5% methanol at pH 4.25. The addition of methanol, up to 20% to the separation buffer was found to increase transit times and resolution. Methanol acts on the separation by reducing EOF. In addition its dielectric constant is lower than that of the water. Complexation reactions and formation of high degree complexes can be promoted in a low dielectric constant medium thus providing additional possibilities for improving the separation.

A complexing reagent commonly used in complexation reactions with lanthanides [41,42] is HIBA. HIBA was introduced in 1981 as a complexing agent

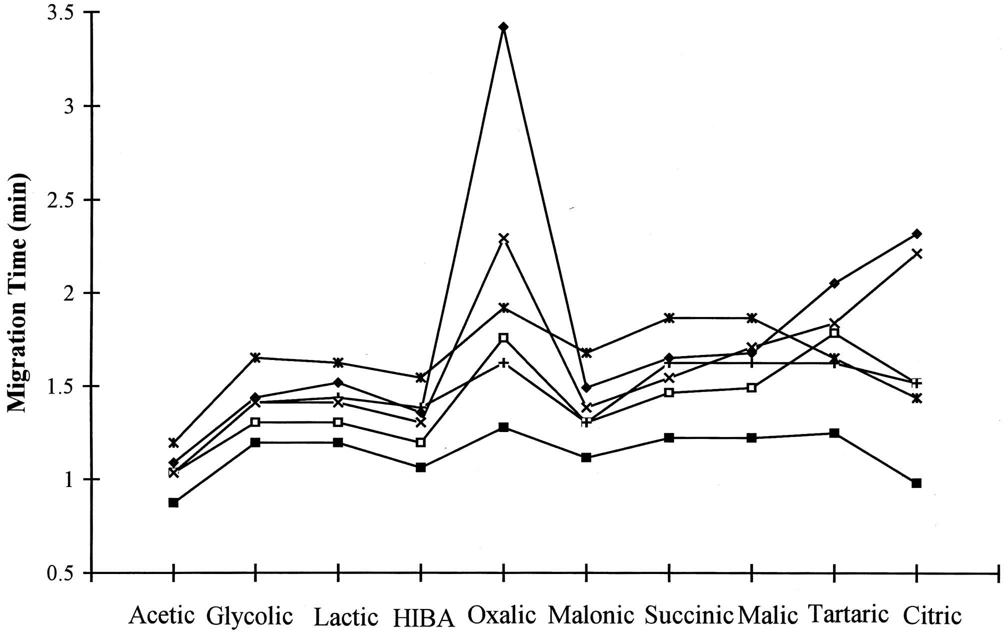

Fig. 5. Comparison of migration times of six ions in the presence of different complexing agents in the pH range where optimum separation could be obtained: in acetic acid at pH 6.0, in succinic and citric acid at pH 4.5, in malic acid at pH 3.7, in all the other acids at pH 4.0. The pH was adjusted with 1 *M* solution of the complexing acid. ■ = K^{+}, + = Na^{+}, * = Li^{2+}, ○ = Ba^{2+}, × = Ca^{2+}, ♦ = Mg^{2+} (from Ref. [11], with permission).

in isotachophoretic separations of lanthanide cations [43] and is today widely used in the separation of metal ions by CE [44–49,9,13]. This complexing reagent is commonly used due to its solubility and transparency in the UV-absorbing electrolytes [32,9,50,51]. Using HIBA as the complexing agent a mixture of nineteen alkali, alkaline earth cations and lanthanide metal cations (less than 10 ppm of each) was separated in 2 min [32] in a buffer containing a proprietary highly UV absorbing amine, UV-cat-1.

An electrolyte buffer containing 5 m*M* UV-cat-1, 8 m*M* HIBA was used to analyze an orange juice complex acidic matrix [32]. The analysis required an increase in the concentration of HIBA to enhance the resolution between Ca^{2+} and Na^+ and the use of indirect detection at 185 nm. Similar conditions were used in the analysis of trace amounts (less than 5 ppm) of Ni^{2+} and Zn^{2+} in a sample containing 5% phosphoric acid, 5% nitric acid and 5% Na^+ [26]. HIBA was used at a concentration of 8% to enhance the resolution of Zn^{2+} and Ni^{2+} from Na^+. Koberda et al. [52] addressed the problem of determining Na^+, K^+, Ca^{2+} and Mg^{2+} in parenteral solutions containing a large amount of Na^+. The high concentration of Na^+ caused an overlap between Na^+ and the other cations. Again the resolution was maximized by increasing the concentration of HIBA. An increase in the concentration of the complexing agent reduces EOF resulting in a net increase in the migration time of all the cations. In addition, the mobility of Mg^{2+} and Ca^{2+} decreases due to a higher level of interaction with HIBA. A carrier electrolyte containing 30 m*M* HIBA proved to be the best compromise between peak separation and baseline noise caused by the increase in buffer conductivity and running current.

Chen and Cassidy [14] optimized pH, HIBA concentration, type of UV-absorbing co-ion and the nature of the capillary surface for the simultaneous separation of 26 metal ions including three alkaline, three alkaline earth, six transition metals and 12 lanthanides (Fig. 6). The authors proposed to use aromatic amines with an amino group connected to a benzene ring via an alkyl chain with pK_a values larger than 7 or quaternary ammonium salts for UV indirect detection. Only the ionic fraction of the UV-adsorbing co-ion contributes to detection whereas the neutral fraction affects the linearity range. The best indirect reagent was *N*,*N*-dimethylbenzylamine (DBA) used at a concentration of 6 m*M*. A 75-μm I.D. capillary and an operative pH of 5 were chosen to optimize *S*/*N* ratio and resolution. The use of surfactants to manipulate EOF was also investigated. Triton-X 100 (0.2 m*M*) gave the best combination of resolution and column efficiency.

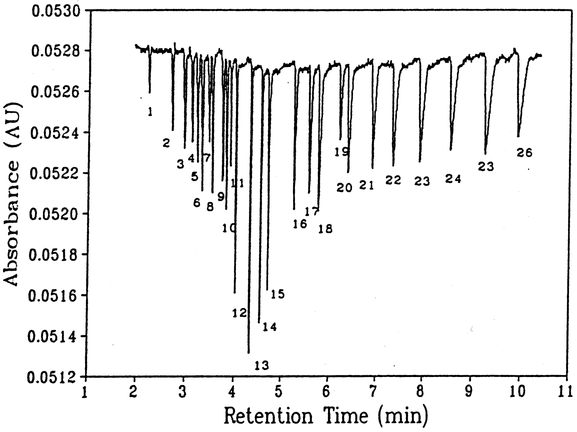

Fig. 6. Separation of 26 metal ions in an uncoated capillary. Electrolyte, 6 m*M* *N*,*N*-dimethylbenzylamine (DBA) and 4.2 m*M* HIBA at pH 5.0 adjusted with acetic acid and containing 0.2 m*M* of Triton-X (TX-100), $1=K^+$, $2=Ba^{2+}$, $3=Ca^{2+}$, $4=Na^+$, $6=Mn^{2+}$, $7=Fe^{2+}$, $8=Co^{2+}$, $9=Ni^{2+}$, $10=Zn^{2+}$, $11=Li^{2+}$, $12=La^{3+}$, $13=Ce^{3+}$, $14=Pr^{3+}$, $15=Nd^{3+}$, $16=Sm^{3+}$, $17=Eu^{3+}$, $18=Gd^{3+}$, $19=Cu^{2+}$, $20=Tb^{3+}$, $21=Dy^{3+}$, $22=Ho^{3+}$, $23=Er^{3+}$, $24=Tm^{3+}$, $25=Yb^{3+}$, $26=Lu^{3+}$ (from Ref. [14], with permission).

Lanthanide separations were obtained [53] on C_{18} and C_1 capillaries with an electrolyte buffer containing 4 m*M* HIBA, 9 m*M* benzylamine as UV-absorbing co-ion and 20 m*M* acetic acid. The C_{18} phase showed a slightly enhanced separation of Eu(III) and Gd(III).

5.2. 18-Crown-6

A special complexation mechanism is the formation of inclusion complexes by using 18-crown-6. The formation of inclusion complexes with 18-crown-6 is often used in the separation of K^+/NH_4^+ or Sr^{2+}/Ba^{2+}. The mobility of the other cations is not significantly affected. The effect of 18-crown-6 on the cations can be attributed to the formation of an inclusion complex whose stability depends on the fit between the ionic diameter of the cation and the cavity. 18-Crown-6 strongly binds K^+ due its specific cyclic structure, therefore it is often added to BGE to moderate the mobility of K^+ and used in a mixture with other complexing agents.

A series of mono-, di- and trivalent cations were separated in a BGE composed of imidazole, HIBA, 18-crown-6 and methanol [13]. The effects of the system parameters on migration and EOF were investigated using a one-variable-at-a-time optimization approach and modeling the electrophoretic behavior of the inorganic cations as a function of HIBA, 18-crown-6 and methanol concentration.

Francois et al. [54] studied selectivity by varying the concentration of two complexing agents, lactic acid and 18-crown-6 added to a 10 m*M* imidazole buffer of pH 4.5. The addition of 5 m*M* lactic acid allowed resolution of Mn(II), iron(II), cobalt(II), cadmium(II), nickel(II) and zinc(II) cations. The addition of 18-crown-6 was necessary to separate Na^+ from Pb^{2+} and NH_4^+ from K^+.

The separation of ammonium and potassium in mixtures with other alkaline and alkaline earth metals was carried out in a copper(II)-based electrolyte system containing 18-crown-6 [55]. The mobility of cupric ion, at pH 3 matches that of many inorganic compounds. In addition, its spectral characteristics are suitable for UV detection.

Bachmann et al. [56] separated ammonium, alkali and alkaline earth metals by adding 18-crown-6 to a 500 μ*M* cerium(III) sulphate carrier electrolyte. The choice of the appropriate cerium counter ion offers the possibility of optimize peak symmetry either of the highly mobile ions or of the less mobile ions. Detection limits in the 0.1–0.3 μ*M* range were achieved with fluorescence detection and high-performance liquid chromatography (HPLC).

6. Addition of multidentate chelating reagents

An alternative approach to separate metals by CE is the complete complexation of metals through the addition of strong complexing agents to the sample followed by separation of metal complexes [57,58]. This approach offers the advantage of detecting metal ions with high sensitivity by direct UV detection and to eliminate interference from complex sample matrices. The addition of a chelating agent, 8-hydroxyquinolinesulfonic acid (HQSH) to the mobile phase was proposed by Swaile and Sepaniak [59]. The anionic form of this reagent HQS^- forms, in aqueous solutions, fluorescent complexes with a number of ions. The use of these types of complexes for detection is well-known in chromatography [60,61]. In CZE the formation of these complexes allows sensitive detection of metals by laser-based fluorimetry. In addition, by manipulating buffer parameters such as the HQS^- concentration and pH, it is possible to modify separation selectivity. The system has been optimized in the separation of a test mixture containing Ca(II), Mg(II) and Zn(II). All the parameters that affect complexation influenced both detectability and CZE efficiency. A slightly alkaline electrophoresis buffer containing 1–5 m*M* of HQS^- resulted in the best detectability. Detection limits in the part per billion range were achieved.

Timerbaev et al. [62] used HQS^- to separate transition and alkaline earth metals as pre-column formed chelates with direct UV detection.

Iki et al. [58] separated 4-(2-pyridylazo)resorcinol (PAR) from its chelates of Co(III), Fe(II), Ni(II) and V(V) by CE with a spectrophotometric detection achieving detection limits of $2 \cdot 10^{-7}$ *M*. The authors state that only Co(III), Fe(II), Ni(II) and V(V) chelates are sufficiently stable to survive during electrophoresis. When 10^{-4} *M* of PAR is added to the buffer peaks corresponding to Cd(II), Mn(II) and Zn(II) were seen on the electropherogram.

In a recent article Krokhin et al. [63] obtained a highly efficient separation of metal–EDTA complexes using so-called kinetic differentiation (KD) mode CE and ion-exchange electrokinetic chromatography. The name KD derives from (KD)–HPLC and is due to the fact that a column behaves as if it is able to differentiate the kinetic stability of different chelates and to elute only the inert ones. The EDTA complexes which are singly or doubly negatively charged are discriminated through the interaction with a polycationic polymer added to the buffer with the aim of improving the selectivity.

An interesting approach that combines the advantages of chelation reaction with the simplicity of the on-column approach was proposed by Haumann and Bachmann [64] who carried out a chelation reaction by mixing the zones of separated analytes during the electrophoretic migration on a column. Weak complexing agents such as tartaric acid or HIBA were added to the buffer to support the separation of the metal ions. The idea is to inject the sample at the anodic end of the capillary (where the detector is placed), and a plug of EDTA solution at the opposite side (cathodic end). When metal ions migrate though the EDTA-zone complexation takes place and the negatively charged metal–EDTA complexes can be separated by electrophoresis. This method can be applied to different chelates on the condition that a fast reaction occurs during contact time of the zones and the chelates are stable enough after leaving the reaction zone to reach the detector. Regan et al. [65] compared the separation of PAR CO^{2+}, Cu^{2+}, Fe^{2+} and Zn^{2+} chelates obtained with and without addition of PAR to the electrophoretic buffer. Some of the metal–Par chelates, e.g., Zn^{2+} and Cu^{2+} are unstable and required the addition of a low concentration of chelating agent ($1\cdot10^{-4}$ M) to the running buffer. Resolution and efficiency were greatly improved by carrying out electrophoresis in the presence of PAR. The same authors investigate a sample staking technique to increase detection limits. For Co^{2+}, Fe^{2+} and Zn^{2+} detection limits of $1\cdot10^{-8}$ M were achieved.

7. Separation of organic molecules through the addition of inorganic agents to the electrolyte buffer

7.1. Organic acids

Changes of relative mobility through complexation equilibria have mainly been applied to the separation of metal ions. The complementary principle that involves the addition of cations to the running buffer when separating anions has been applied only to a limited extent. In general the use of running buffers containing metal cations in CZE leads to a significant reduction in efficiency and reproducibility [66,67]. Metals are adsorbed onto the silica surface, thus lowering the zeta potential and reducing electroosmotic velocity [68,69]. In addition, complexation of the analytes with the cations adsorbed on the wall induces peak broadening and reduction in efficiency [70]. In many cases alternative approaches have been adopted to employ metal ions in the separation of negatively charged species. The use of metals in micelle-containing buffers overcomes these problems since the micelles provide a competitive surface for the interaction with the metal ions [70]. Another approach is to shield silanols by adding an EOF modifier to the separation system [71].

The addition of alkaline earth cations to the running buffer effects the separation of formate and tartrate. Ca^{2+} (0.2–0.6 mM) added to the running buffer induces a selective retardation of the tartrate. Lalljie et al. [72] optimized the conditions for the quantitative determination of formate, tartrate, malate, succinate and glycolate in chicory root extract. An EOF modifier together with reverse polarity were adopted to accelerate the movement of ions towards the detector.

By using copper sulfate as the complexing agent Wiley [73] devised a rapid and sensitive method to analyze formulated detergents. The copper complexes were detected by direct UV detection at 254 nm with a detection limit of 0.01 μg/ml which allowed measurement of trace levels. In a similar approach Harvey [71] analyzed traces of polyaminipolycarboxilic acids through the formation of copper(II) complexes. The addition of divalent copper ions caused an alteration of the Stern layer structure resulting in a reduction or even in a reversal of the EOF. In the paper [71] it is mentioned that particular attention must be paid to column pretreatment and buffer equilibration in order to achieve reproducible results. Lack of reproducibility deriving from variability of EOF with copper-containing electroseparation buffers was also reported by other authors [68,69]. Micellar separations were investigated to increase separation reproducibility. A baseline separation of a mixture of five polyaminopolycarboxylic acids was obtained in both CTAB and SDS buffers containing $Cu(OAc)_2$. The addition of CTAB led to a reversal of EOF resulting in shorter migration times for the negatively charged copper complexes [71].

Some metallochromic ligands – e.g., arsenazo I, arsenazo III, PAR, xylenol orange and Chrome azurol S – that are widely used as chelating agents able to form stable metal complexes, were separated by CE and MEKC [66]. A complex buffer system containing Ca^{2+} or Zn^{2+} in excess over EDTA and citric acid at a concentration higher than that of metal ions provided an efficient separation of some of the analytes.

The effects produced by direct addition of divalent cations on the electrophoretic mobility of aromatic and aliphatic acids were extensively investigated by Chiari et al. [74]. Fig. 7 shows the electrophoretic mobilities of several organic acids in a pH 5.5 MES buffer containing different cations. Among the others, Cu^{2+} significantly affects the migration behavior of a larger number of analytes. Ni^{2+} was also found to moderately affect selectivity leading to a retardation of phthalic acid, nicotinic acid and *N*-acryloylglycine. In this investigation a coated capillary was used to exclude the influence of wall effects.

7.2. Enantiomers

The use of optically active copper(II) complexes is well-known in chromatography [75]. Gassmann et al. [76] resolved a number of racemic mixtures of dansyl amino acids by CE. The separation is based on the diastereomeric interaction between DL-amino acids and a copper(II) complex of L-histidine contained in the electrolyte buffer. The use of a laser-fluorescence detector allowed detection of fentomole amounts of the racemic mixtures. The electrophoretic mobility of the free amino acid is higher than that of the amino acid bound to Cu(II)–L-histidine. The amino acid fraction involved in the formation a ternary mixed chelate complex exhibits a lower mobility due to the positive charge carried by the Cu(II)–L-histidine complex. Gozel et al. [69] applied CE to the chiral resolution of a racemic mixture of 14 dansylated amino acids using a Cu(II)–aspartame complex. In an aspartame containing BGE the addition of Cu(II) induces chelation of the metal ion through the formation of a six-membered ring formed by the Cu(II), the α-amino and the β-carboxy groups of the aspartame. During electrophoresis, amino acids replace one of the residues of the $Cu(aspartame)_2$ complex forming a ternary complex. Separation of amino acid enantiomers occurs since additional hydrophobic interactions between the aspartame phenylalanine residue and the amino acid lateral chains induce slight differences in the stability constants of each enantiomeric form of the amino acid. The use of a micellar electrolyte solution adds another dimension to the separation and positively

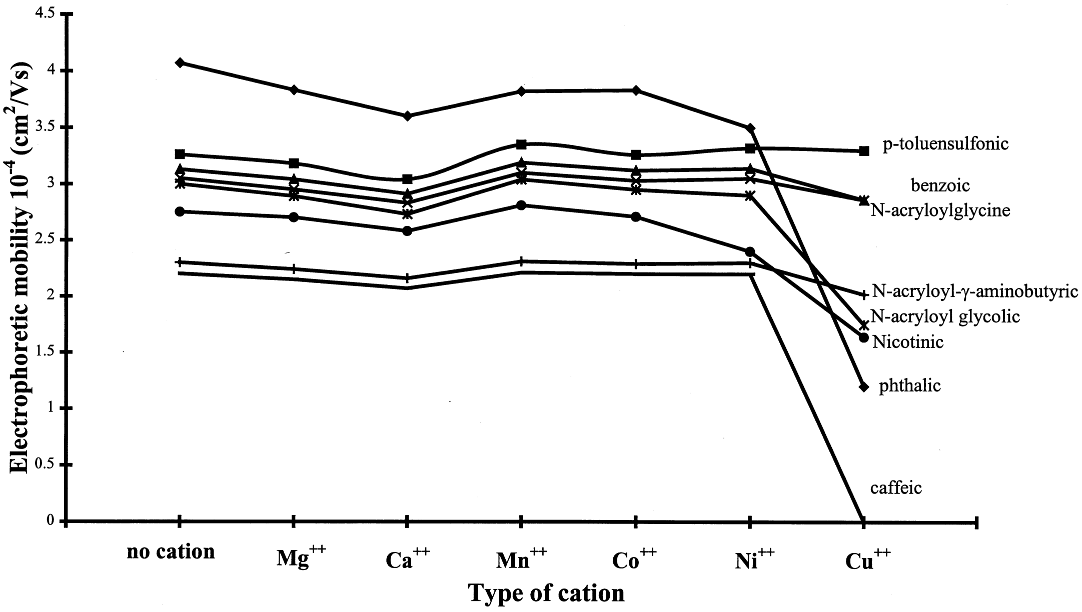

Fig. 7. Influence of various cations on the electrophoretic mobility of organic acids (from Ref. [74], with permission).

affects the resolution. Chiral micelles chelate metal ions and produce a phase able to resolve dansylated amino acids [70].

7.3. Biopolymers

The potential of metal ion-containing buffers to enhance the resolution of peptides in CE was evaluated by Mosher [77] who investigated the effects of Zn(II) and Cu(II) added to BGE on the separation of dipeptides containing histidine. A complete resolution of two positional isomers, L-Ala–L-His and β-Ala–L-His was achieved in an acidic pH buffer supplemented with 20–30 m*M* Zn(II).

Metals are known to form complexes with oligonucleotides with either the phosphate groups and/or the bases [78,79]. Cohen et al. [70] employed metal–oligonucleotide complexation to manipulate selectivity of these species which are difficult to resolve due to small differences in their mobility as the number of bases increases. They found enhanced selectivity of oligonucleotides via differential complexation with metals added to a buffer system containing SDS micelles. The divalent metals used – Cu(II), Zn(II) and Mg(II) – are known to be electrostatically attracted by the surface of the micelles [80]. The increase in separation observed is a result of the complexation of the oligonucleotides with the metals attached to the surface of the micelles. The kinetics of this association–dissociation process are rapid enough to ensure an high peak efficiency. The addition of SDS micelles to the system reduces the adsorption of cations on the capillary surface since the metals are preferentially adsorbed by the micelles. The best results were obtained with Zn(II) whereas the use of Cu(II) provided good band spacing but broader peaks.

In the analysis of carbohydrates one of the main problems is represented by the lack of charge of these species. Carbohydrates can be converted in situ to ions through complexation with metals. The addition of a carbohydrate to an aqueous solution of an alkaline earth metal acetate causes complexation with the ion metal and the complexes can be separated from each other on the basis of their different complexation capabilities (Fig. 8) [81]. The metal cations are gradually adsorbed onto the wall thus gradually reducing EOF and even reversing the direction of EOF. The reversal of the direction of EOF and a good separation of several pentose-1-phenyl-3-methyl-5-pyrazolone (PMP)-derivatives was observed with carriers containing Ca^{2+}, Ba^{2+}, Sr^{2+} and Mg^{2+}.

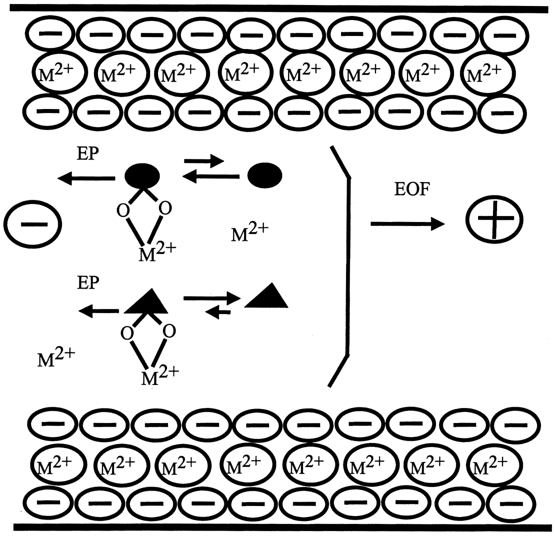

Fig. 8. Illustration of the separation mechanism of carbohydrates as in situ formed chelates by zone electrophoresis. EOF = Electroosmotic flow, EP = electrophoresis, M^{2+} = alkaline earth metal ion (from Ref. [81], with permission).

8. Conclusions

CE offers many possibilities for the separation of metal ions using mobility modifications of the ions through complexation during the sample preparation step or the separation process itself. Depending on the spectroscopic properties of the ligand direct or indirect detection is used.

Complexation reactions of metal ions with organic compounds contained in the BGE represent a powerful means of selectivity manipulation. Many of the complexes are formed by the weak charge-transfer interaction of the compounds acting as electron donors and metal ions acting as electron acceptor. The rates of formation of the complexes are generally very rapid and the properties of the complexes are suitable for CE analysis. A number of metal ions have been separated by CZE in a background electrolyte containing a wide variety of complexing agents. Most of the ligands are mono-, di- or trivalent organic acids. The ligand optimal concentration depends on the stability constants of the complexes and influences both the average degree of complexation ($\bar{n}$) and the metal electrophoretic mobility. The pH of the BGE is also a crucial parameter determining the degree of ionization of the complexing acid. pH variations influence the separation in two ways: (i) by altering the degree of ionization of the silanols on the wall and consequently EOF and (ii) by affecting complexing equilibria.

Complexation equilibria with metal ions added to the running buffer produce changes in the ionization of organic compounds with significant changes in their electrophoretic mobility. Addition of inorganic cations to the BGE with the aim of increasing selectivity in the analysis of organic compounds has been applied to a limited extent due to the reduction in efficiency caused by the addition of cationic species to the BGE. In most of the cases the use of metal cations to modulate selectivity required the use of micelle-containing buffers or the addition of EOF modifiers to prevent adsorption of cations onto the wall which would have detrimental effects on separation efficiency and reproducibility.

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