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Journal of Chromatography A, 792 (1997) 495–518

JOURNAL OF  
CHROMATOGRAPHY A

## Review

# Strategies for selectivity control in capillary electrophoresis of metal species

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### Abstract

Capillary electrophoresis has recently attracted considerable attention as a promising analytical technique for metal ion separations. Significant advances that open new application areas for capillary electrophoresis in the analysis of metal species have occurred based on exploiting various auxiliary separation principles. These are mainly due to complexation, ion pairing, solvation and micellization interactions between metal analytes and electrolyte additives which alter the separation selectivity in a broad range. Likewise, many separation studies for metal ions have been concentrated on the use of pre-electrophoresis derivatisation methodology. Approaches suitable for manipulation of selectivity for different metal species including metal cations, metal complexes, metal oxoanions and organometallic compounds, are discussed, with special attention paid to the related electrophoretic system variables using illustrative examples. Discussion also presents guidelines for optimisation of separation conditions based on basic theoretical considerations. © 1997 Elsevier Science B.V.

*Keywords:* Reviews; Selectivity; Metal cations; Metal complexes

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## 1. Introduction

Since the early 1990s, metal ion separation is one of the most rapidly developing areas of capillary electrophoresis (CE) [1–4]. A study of current literature witnesses that metal ion analysis by CE has left the stage of initial evaluation and is on the way to being refined and established for routine use. On the other hand, not all the capabilities of a CE system on developing separation conditions and optimising chemical selectivity have been exploited to date. In this regard, we consider it worthwhile to address a special issue of how the separation selectivity can be adjusted for different metal species in CE. It is written with the primary intention to provide an extensive review on fundamental studies in this area. The information presented is also suitable as a guide to selection of separation conditions and method development.

The separation of metal species by CE is often complicated by the fact that for most metal ions or metal complexes, the electrophoretic mobilities are similar in magnitude. This is not surprising, given that the electrophoretic mobility,  $\mu_{\text{eff}}$ , is originally determined by the intrinsic solute parameters, effective charge and size. Assuming a metal species to be a spherical (Stokes') particle, a fundamental expression for electrophoretic mobility can be written as:

$$\mu_{\text{eff}} = \frac{q_i}{6\pi\eta r_i} \quad (1)$$

where  $q_i$  is the charge of hydrated or complexed ion,  $r_i$  is its size (the hydrodynamic radius) and  $\eta$  represents the dynamic viscosity of the electrolyte medium. It follows from Eq. (1) that the mobility differences for metal species such as equally charged transition or lanthanide metal ions or their complexes with the same ligand number, possessing nearly identical charge and size, might be not sufficient for separation. A second consequence from Eq. (1) is that one can vary the selectivity of the electro-

phoretic separation that can be defined as given by the following equation:

$$p = \frac{\mu_{\text{eff}}(1) - \mu_{\text{eff}}(2)}{\mu_{\text{eff}}(2)} \quad (2)$$

(other expressions of selectivity have been compared by Dolnik [5]), by altering either the effective charge or size of a metal species, or both.

There is much that can be done to improve separation in CE. The majority of approaches employed for metal analytes are based on secondary chemical equilibria, such as complex formation, ion dissociation, ion pairing etc., established in the CE system as a result of compositional changes of the carrier electrolyte. By a judicious strategy for selecting a proper electrolyte additive(s), the electrophoretic mobilities can be greatly varied which provides the impressive separations for a wide range of metal species. In the same way, physicochemical equilibria like solvation or solubilization by a micelle can be applied as an auxiliary separation principle. Alternatively, employment of the approach relying on differences in migration of precapillary-formed metal complexes makes it possible to attain many innovative separations.

The regulation of the rate of electroosmotic flow is another way to enhance the separation in CE. Although the electroosmotic flow does not contribute to the selectivity changes (at least for the analytes of the same charge), it has been demonstrated to be useful in manipulation of the resolution, in particular, in micellar-mediated CE systems. However, as this volume is aimed to emphasise the factors that affect the selectivity, the discussion below is limited to the control of separation guided by altering the electrophoretic mobilities of metal analytes.

Evidently, the choice of experimental parameters that can be varied to influence the separation selectivity presents a challenge especially for those just beginning to work with CE method development. The separation of a particular mixture of metal

analytes without the necessity of carrying out a large number of experiments does require from the experimenter not only a certain training but also considerable chemical expertise. With that in mind, a detailed examination of separation strategies used in CE of metal species was undertaken with the aim of providing the reader with a knowledge necessary for the rational search of optimum separation conditions and to gain further insight into the mechanism of electrophoretic separation.

## 2. Adjusting the separation selectivity for metal cations

### 2.1. Partial complexation with organic acids

Efficient separations based solely on differences in ionic mobilities between cationic analytes can only be achieved for some alkali, alkaline-earth and a limited number of other group cations. Obviously, to improve the quality of the separation for metal ions belonging to other groups such as transition and lanthanide metal groups, it is necessary to modify their mobilities to a certain and different extent. At present, the most straightforward approach for such a modification, which was first proposed by Fanali et al. [6], is partial complexation with a suitable complexing reagent taking place within the capillary. Running a few steps further, it is worth noting that the wide acceptance of this complexation strategy appears to be logical because many researchers working in the area of CE have a strong chromatographic background. In HPLC, complexing reactions have been playing a paramount role since the initial stage of its evolution for metal ion analysis.

When a complex-forming equilibrium is established by introducing a weak complexing reagent into the electrolyte, several different complexes may exist simultaneously due to stepwise complexation schematically described by the equation:



where  $L^{-}$  is a monovalent, monodentate ligand. The number and amount of metal complexes formed depend mainly on the charge of the metal ion, the ligand-to-metal concentration ratio and pH. As a

result, the observed electrophoretic mobility of the metal ion is determined by the distribution among various possible complexed forms.

There are several ways to express  $\mu_{\text{eff}}$  under such CE conditions. In principle, it is possible to calculate  $\mu_{\text{eff}}$  from the weighted average of the mobilities of the free ion and the different complexed species [7–9]:

$$\mu_{\text{eff}} = \phi_M \mu_M + \phi_{ML} \mu_{ML} + \phi_{ML_2} \mu_{ML_2} + \dots + \phi_{ML_m} \mu_{ML_m} \quad (4)$$

( $\mu$  and  $\phi$  are the electrophoretic mobility and mole fraction of each species, respectively) by solving Eq. (4) for various electrolyte conditions, as was first done by Swaile and Sepaniak [10]. Note that  $\phi$  are the well-known functions of complex-formation constants and the concentration of free ligand,  $[L^{-}]$ . Another approach consists of describing the metal-complexing system as a single complex,  $ML_n^{(n-n)+}$  with the average number of ligands associated with a metal ion  $\bar{n}$  [9,11,12]:

$$\bar{n} = \frac{K_1[L^{-}] + 2K_2[L^{-}]^2 + \dots + mK_m[L^{-}]^m}{1 + K_1[L^{-}] + K_2[L^{-}]^2 + \dots + K_m[L^{-}]^m} \quad (5)$$

where  $K_i$  ( $i=1, 2, \dots, m$ ) represents the stepwise formation constants and  $m$  stands for the number of ligands involved. A validity of a modified version of Eq. (1), in which the term for the mole fraction of free metal ion is added to account for the reduction of free metal concentration due to complexation:

$$\mu_{\text{eff}} = \frac{q_i}{6\pi\eta r_i} \times \phi_M \quad (6)$$

was also proved [13] (we refer to this paper for a more detailed discussion).

#### 2.1.1. The nature of the complexing reagent

The first question to decide when a specific separation task is concerned, is which complexing reagent to choose. The variety of carboxylic and hydroxycarboxylic acids found to be suitable in aiding the separation of metal cations having mobilities similar to one another. Table 1 provides a comprehensive listing of organic acids related to the separation of various classes of metal ions by CE. Of various complexing reagents tested,  $\alpha$ -hydroxyisobutyric acid (HIBA) and lactic acid appear to be

Table 1  
Selected separations of metal cations based on partial complexation with organic acids

Cations <sup>a</sup>	Separation conditions	Separation time (min)	Ref.
K <sup>+</sup> , Na <sup>+</sup> , Mg <sup>2+</sup> , Li <sup>+</sup> , lanthanides (18)	4 mM HIBA, 30 mM creatinine (pH 4.8)	5	[6]
Alkali, alkaline earth, transition metals, lanthanides (19)	4 mM HIBA, 10 mM Waters UVCat-1 (pH 4.4)	2	[7]
Alkali, alkaline earth, transition metals, lanthanides (26)	4.2 mM HIBA, 0.2 mM Triton X-100, 6 mM <i>N,N</i> -dimethylbenzylamine (pH 5.0)	10	[14]
Alkali, alkaline earth, transition metals, lanthanides, Pb <sup>2+</sup> (27)	15 mM lactic acid, 5% methanol, 8 mM 4-methylbenzylamine (pH 4.25)	7	[11]
Alkali, alkaline earth, transition metals (12)	2.5 mM tartaric acid, 20% methanol, 6 mM <i>p</i> -toluidine (pH 4.8)	9	[11]
Alkali, alkaline earth, transition metals (14)	12 mM HIBA, 6 mM imidazole (pH 3.95)	4	[8]
Alkali, alkaline earth, transition metals, Pb <sup>2+</sup> , NH <sub>4</sub> <sup>+</sup> (16)	11 mM lactic acid–2.6 mM 18-crown-6, 8% methanol, 7.5 mM 4-methylbenzylamine (pH 4.3)	6	[15]
Alkali, alkaline earth, transition metals, Pb <sup>2+</sup> (17)	13 mM glycolic acid, 10 mM imidazole (pH 4.0)	14	[16]
Alkali, alkaline earth, NH <sub>4</sub> <sup>+</sup> , Mn <sup>2+</sup> , Cd <sup>2+</sup> (12)	6 mM glycine–2 mM 18-crown-6, 2% methanol, 5 mM 1,1'-diheptyl-4,4'-bipyridinium (pH 6.5)	5	[17]
Alkali, alkaline earth, transition metals, Pb <sup>2+</sup> , NH <sub>4</sub> <sup>+</sup> (17)	5 mM lactic acid–0.5 mM 18-crown-6, 10 mM imidazole (pH 4.5)	5	[18]
Alkali, alkaline earth, transition metals, Pb <sup>2+</sup> (15)	1 mM oxalic acid–100 mM acetic acid (pH 2.84)	15	[19]

<sup>a</sup> Given in parenthesis is the number of ions separated.

the most advantageous in terms of separation selectivity, followed by tartaric and oxalic acids. Figs. 1 and 2 show a pair of excellent separation examples for alkali, alkaline-earth, transition and rare-earth metal ions using HIBA and lactic acid, respectively. Remarkably, both separations require only 10 min or less. The performance at the level of 20 or more cations separated in a single run was obtained by other research groups [7,20]. For alkali and alkaline-earth metals the best separations were achieved with citric [7] or malonic [21] acid, while glycolic acid gave a higher selectivity for a mixture of alkali, alkaline-earth, transition metals, Al(III), Ag(I) and Pb(II) [16]. Lin et al. [21] have demonstrated that the effect of organic acids on the separation increases from monoprotic to di- and triprotic acids. Among

diprotic acids, the complexation effect of oxalic acid, which forms more stable chelate complexes with the polyvalent cations, tends to be more pronounced. Stronger complexing reagents, e.g. 8-hydroxyquinoline-5-sulphonic acid [10] or EDTA [22], also find some use to moderate differences in effective mobilities of alkaline-earth and magnesium cations. In this instance, the condition of incomplete complexation is due to the lack of complexing ability for such metals and to keeping modest free ligand concentrations in the electrolyte (see below). A noticeable improvement of the separation can be obtained by the combination of two ligands which possess different abilities for the complexation (like HIBA and acetate [9]).

As can be seen from Eqs. (4)–(6), in the presence

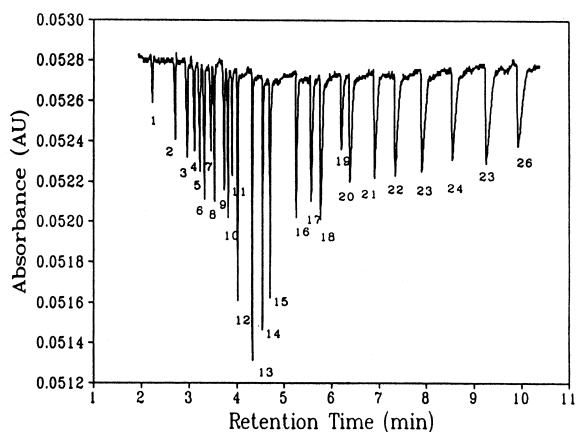


Fig. 1. Electropherogram for the separation of 26 metal ions. Capillary, fused-silica, 60 cm  $\times$  75  $\mu$ m I.D.; electrolyte, 4.2 mM HIBA, 6 mM dimethylbenzylamine (pH 5.0), 0.2 mM Triton X-100; applied voltage, +30 kV. Indirect UV detection at 214 nm. Peak identity, 1 = K<sup>+</sup>; 2 = Ba<sup>2+</sup>; 3 = Ca<sup>2+</sup>; 4 = Na<sup>+</sup>; 5 = Mg<sup>2+</sup>; 6 = Mn<sup>2+</sup>; 7 = Fe<sup>2+</sup>; 8 = Co<sup>2+</sup>; 9 = Ni<sup>2+</sup>; 10 = Zn<sup>2+</sup>; 11 = Li<sup>+</sup>; 12 = La<sup>3+</sup>; 13 = Ce<sup>3+</sup>; 14 = Pr<sup>3+</sup>; 15 = Nd<sup>3+</sup>; 16 = Sm<sup>3+</sup>; 17 = Eu<sup>3+</sup>; 18 = Gd<sup>3+</sup>; 19 = Cu<sup>2+</sup>; 20 = Tb<sup>3+</sup>; 21 = Dy<sup>3+</sup>; 22 = Ho<sup>3+</sup>; 23 = Er<sup>3+</sup>; 24 = Tm<sup>3+</sup>; 25 = Yb<sup>3+</sup>; 26 = Lu<sup>3+</sup> (reproduced with permission from Ref. [14]).

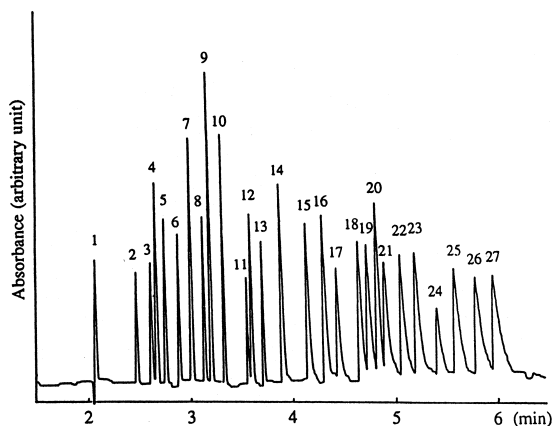


Fig. 2. Separation of 27 alkali, alkaline-earth, transition and lanthanide metal ions in a single run. Capillary, fused-silica, 60 cm  $\times$  75  $\mu$ m I.D.; electrolyte, 15 mM lactic acid, 8 mM 4-methylbenzylamine, 5% methanol (pH 4.25); applied voltage, +30 kV. Indirect UV detection at 214 nm. Peak identity, 1 = K<sup>+</sup>; 2 = Ba<sup>2+</sup>; 3 = Sr<sup>2+</sup>; 4 = Na<sup>+</sup>; 5 = Ca<sup>2+</sup>; 6 = Mg<sup>2+</sup>; 7 = Mn<sup>2+</sup>; 8 = Cd<sup>2+</sup>; 9 = Li<sup>+</sup>; 10 = Co<sup>2+</sup>; 11 = Pb<sup>2+</sup>; 12 = Ni<sup>2+</sup>; 13 = Zn<sup>2+</sup>; 14 = La<sup>3+</sup>; 15 = Ce<sup>3+</sup>; 16 = Pr<sup>3+</sup>; 17 = Nd<sup>3+</sup>; 18 = Sm<sup>3+</sup>; 19 = Gd<sup>3+</sup>; 20 = Cu<sup>2+</sup>; 21 = Tb<sup>3+</sup>; 22 = Dy<sup>3+</sup>; 23 = Ho<sup>3+</sup>; 24 = Er<sup>3+</sup>; 25 = Tm<sup>3+</sup>; 26 = Yb<sup>3+</sup>; 27 = Lu<sup>3+</sup> (reproduced with permission from Ref. [11]).

of complexing reagent in the carrier electrolyte, the apparent mobility of a metal ion co-existing in equilibrium with complexes ML ... ML<sub>m</sub> is reduced in accordance with the values of the formation constants  $K_i$ . The separation selectivity is in turn dominated by differences in complexing ability which are greater than differences in the ionic mobility. Thus, an evident criterion on selecting the optimal complexing reagent is that the range of stability constants for a given metal mixture should be as broad as possible.

Shi and Fritz [15] have also outlined the requirement of maintaining the equilibrium conditions during the separation. This means that the kinetics of complexation must be rapid enough to produce the complexes within the time frames of a typical CE run. This requirement seems to be more critical in CE than in HPLC owing to both smaller run times and a low flow mixing in narrow-bore capillaries [23]. The equilibrium kinetics between the free metal cation and the complexed species should also not be too slow. Otherwise, the various forms with a given metal functionality would migrate with different velocities resulting in electrophoretic zone broadening.

The additional benefit of using the complexing reagents is in their effect on peak symmetry [7,24,25]. The complexed cations possess lower apparent mobilities, which may match more closely to the mobility of a visualisation co-ion in the carrier electrolyte. Therefore, the peak shapes exhibit better symmetry for metal ions having a higher mobility than the mobility of the electrolyte.

### 2.1.2. The concentration of free ligand

The second important parameter of the CE system, which is necessary to take into consideration and optimise in order to attain the best separation, is the concentration of the complex-forming ligand. As follows from the above discussion, it affects the degree of complexation and resulting migration velocity. The majority of organic acids used as complexing reagents are of a weak acidic nature and hence undergo acid–base dissociation equilibria in the electrolyte buffer expressed, in the simplest case of a monoprotic acid, by the following equation:



$$K_a = \frac{[H^+][L^-]}{[HL]} \quad (8)$$

where  $K_a$  is the acid dissociation constant of HL. Consequently, it is not the total concentration of a given reagent added to the buffer ( $C_L$ ) but the concentration of free ligand which determines the ability of differentially reducing the mobilities of metal ions. Nonetheless, at a pH fixed above the  $pK_a$ , increasing  $C_L$  means necessarily increasing the amount of ligand resulting in an increase of complex formation.

Many separation studies include a detailed investigation of the  $C_L$  effect [7,9,14,18,22,25–29]). Fig. 3 presents a typical dependence of electrophoretic mobilities of metal ions on the complexing reagent concentration in the carrier electrolyte, which can be used for selecting the most appropriate  $C_L$ . While the lower concentration level depends primarily on the average degree of complexation quantitated by  $K_i$  (see Eq. (5)) and the concentration of metal ions in the sample, the upper level may be limited by non-compliance with the condition of incomplete complexation. As demonstrated by Vogt and Conradi [9], in the state of a maximum complexation with  $\bar{n}=m$ , the mobility differences are lost and separation becomes impossible. Also, excessively high ligand concentrations should be avoided to prevent high ionic strength effects, accompanied by significant Joule heating, and enlarged migration times of metal analytes. Likewise, the use of indirect UV detection sets the upper concentration limit for the electrolyte complexing ions [30]. As seen from Table 1, the typical operating  $C_L$  are in the range from 1 to 10 mM. In some cases, an increase in  $C_L$  changes the migration order of metal analytes [8,14,16,21,24] (see also Fig. 3), since the complexing reagent has a stronger effect on the mobility of cations of higher complexing ability, e.g., transition metal ions. This provides a wide option to adjust selectivity in accordance with the sample composition.

Finally, several theoretical approaches are to be mentioned suitable for the calculation of the optimum ligand concentration if the formation constants of the complexes are known. For example, the optimum  $C_L$  for HIBA and lactate predicted on the base of the theoretical calculation of  $\bar{n}$  (or  $\mu_{\text{eff}}$ ) versus  $\log [L^-]$  dependencies allowed satisfactory

separations of rare earth metal ions [9]. Wang and Li [22] have derived an equation indicating the existence of maximum differences in  $\mu_{\text{eff}}$  of cation M(1) and M(2) that form 1:1 complexes with ligand L at:

$$[L] = (K'_{M(1)L} K'_{M(2)L})^{-1/2} \quad (9)$$

where  $K'$  is the apparent stability constant. On the other hand, applying the same complexometric methods one can solve an opposite task – that is, how strongly metal ions should be complexed for optimal separation by CE. Under conditions of the complete separation of the lanthanides (4.0 mM HIBA at pH 4.3 [11])  $\bar{n}$  ranges from 0.61 to 1.95 across the lanthanide series.

### 2.1.3. The pH of the electrolyte

Eq. (8) shows that the free ligand concentration and, subsequently, the metal complexation can also be effectively affected by variation of the running buffer pH, which is another controllable electrolyte parameter. With increasing pH the dissociation of the complexing reagent increases, leading to the shift of the complex-forming equilibrium (Eq. (3)) towards slower migrating metal-complexed forms (Fig. 4). The optimal pH should be selected with due account for the  $pK_a$  value, ranging for typical complexing acids from 2.8 (malonic acid) to 4.2 (succinic acid); at more acidic pH values, much of the complexing ability of HL is lost. The practical upper pH limit is about 5, which in most cases guarantees the negligibility of sample metal hydrolysis and sufficient protonation of a UV active component of the electrolyte. The increase in the differences in effective mobilities resulting from increasing complexation favoured by increased pH may also be overwhelmed by an adverse effect on resolution of the large electroosmotic flow at high pH.

It must be pointed out that when varying the pH of the electrolyte, the nature of acid used for pH adjustments is of special concern. For instance, sulphate anion can act as a complexing agent and compete with an organic ligand for the cations, such as alkaline earths, affecting their migration [21,28,31,32]. Therefore, adjustment of the pH with sulphuric acid instead of, e.g., hydrochloric acid may give very different migration orders and should be avoided. The idea of association equilibria between

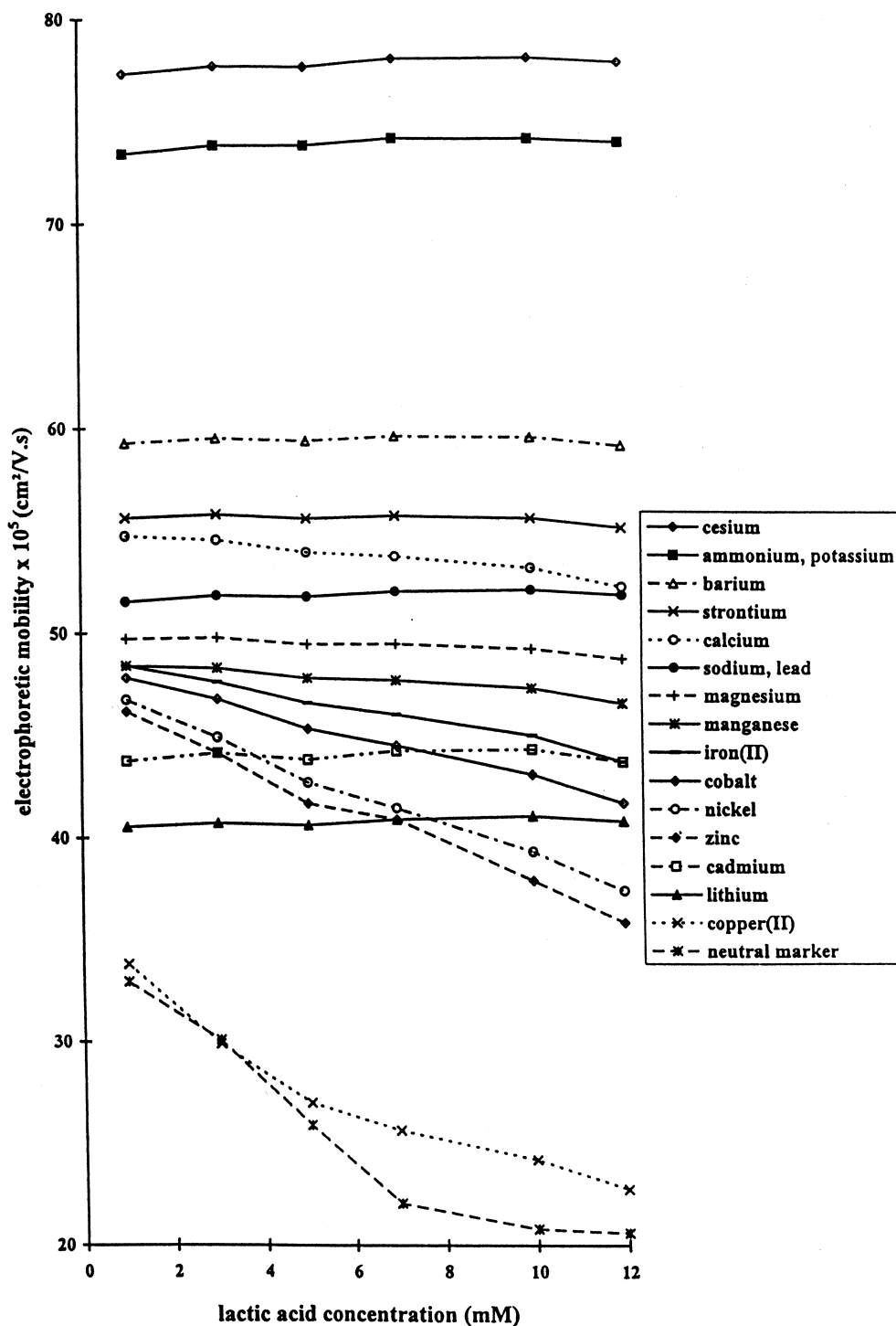


Fig. 3. Influence of lactic acid concentration on the electrophoretic mobility of 17 inorganic cations. Capillary, fused-silica, 57 cm  $\times$  75  $\mu\text{m}$  I.D.; electrolyte, lactic acid, 10 mM imidazole (pH 4.5); voltage, +20 kV. Indirect UV detection at 214 nm (reproduced with permission from Ref. [18]).

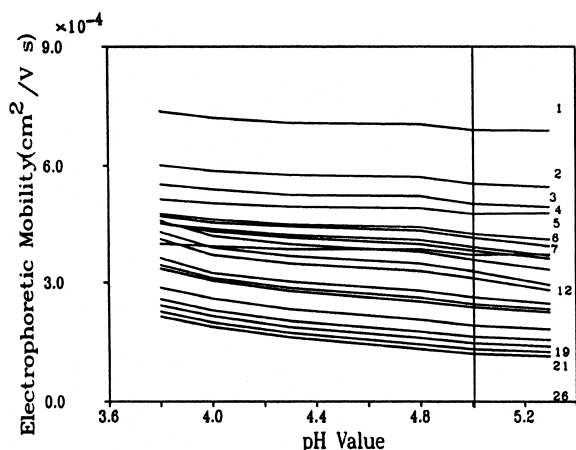


Fig. 4. Electrophoretic mobilities as a function of pH. Electrolyte, 4 mM HIBA, 6 mM dimethylbenzylamine, pH adjusted with acetic acid; other conditions as for Fig. 1 (reproduced with permission from Ref. [14]).

divalent cations and sulphate is supported by the experimentally observed increase in  $\mu_{\text{eff}}$  on increasing  $\text{SO}_4^{2-}$  concentration [33].

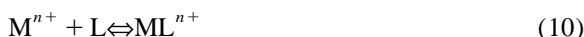
Similarly to the  $C_L$  effect considered above, the migration order depends greatly on the pH, which influences mostly the mobilities of the divalent cations [21]. Such pH-dependence is more marked in the case of diprotic acids than monoprotic acids, and for triprotic acids the effect is even greater. For the separation of divalent cations in the presence triprotic citric acid, the pH not only has an effect on the degree of complexation but also on the charge carried by the metal complexes.

Thus, when the nature of the complexing reagent is specified, the optimisation of the separation selectivity in metal–ligand CE systems can be handled by varying the ligand concentration and pH. As indicated above, these electrolyte variables interact, exerting simultaneously their effects on the separation. This complicates the assessment of the impact of each parameter and the choice of optimum electrolyte composition while only one parameter is varied at a time. Therefore, the use of computer-assisted experimental designs is extremely beneficial in CE method development. In this regard, several methods for predictive optimisation of the electrolyte composition published in recent CE literature

[8,27,28,34,35] can be recommended for the interested readers.

## 2.2. Ion-exclusion complexation with macrocyclic ligands

The enhanced selectivity for the separation of alkali and alkaline-earth cations can also be gained due to a somewhat different partial complexation mechanism with a suitable macrocyclic polyether incorporated into the carrier electrolyte. If a metal cation fits closely inside the cavity of the crown ether, this can induce inclusion (or host–guest) complex formation described by Eq. (10):



so that the stability of the resulting metal–crown complex depends on the match in the ionic radius of the cation and that of the crown ether. The number and position of the electron-donor oxygen atoms in the crown ether ring are also playing a role. According to this complexation principle, macrocyclic ligands magnify differences in mobility (relative to the other separands) of only certain cations like  $\text{K}^+$ ,  $\text{Ba}^{2+}$  and  $\text{Sr}^{2+}$  which interact specifically with 18-crown-6 (Fig. 5). As the charge of a metal ion remains unchanged during the crown complexation, the retardation effect on the migration velocity, clearly seen from the electropherograms in Fig. 5, is owing to the charge density alterations for much bulkier crown-complexed cations. Apart from the resolution of ammonium/potassium and calcium/strontium pairs, other changes in selectivity, most dramatically the large change in migration time of barium, are noted.

Since the crown ether ligands are electro-neutral, free ligand concentration and thereby complexation is virtually unaffected by the pH (i.e.,  $[\text{L}] = C_L$ ), and the only electrolyte variable to improve the selectivity is the total concentration of crown ether. In general, higher electrolyte concentrations of crown ether are favourable as they widen the range of the effective mobilities of the cations. This is demonstrated in Fig. 6, where changes in migration times are shown as a function of 18-crown-6 concentration. To achieve the best total resolution of ammonium, alkali and alkaline-earth cations, the  $C_L$  of 18-crown-6 should be in the range between 2 and 5 mM. For



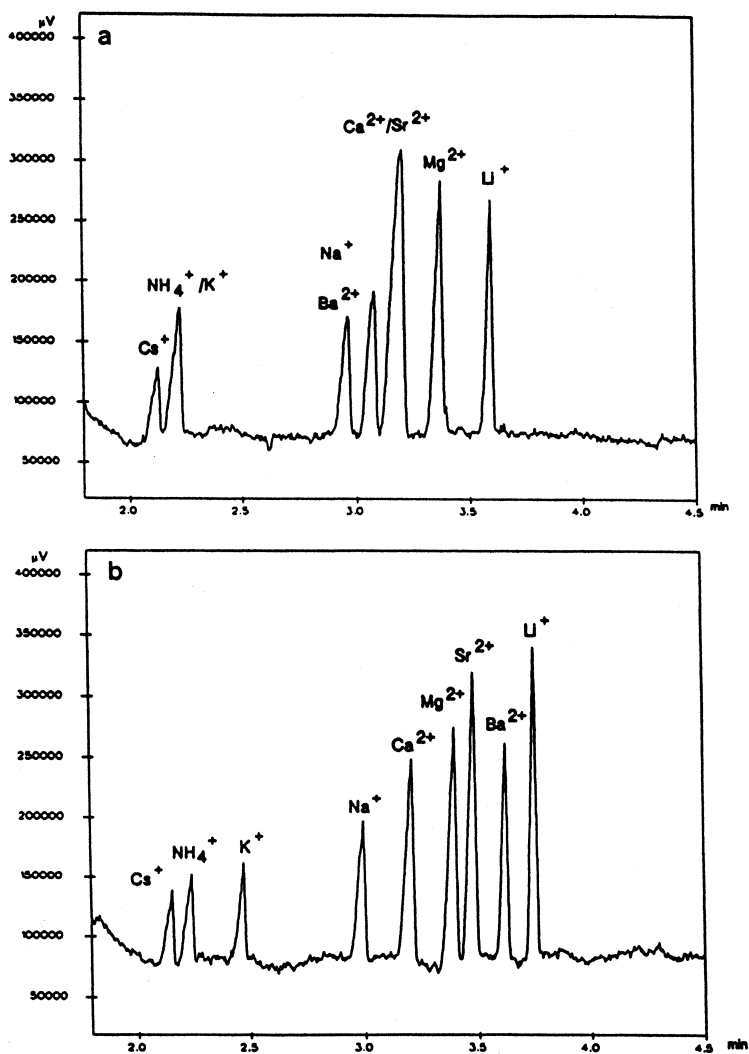


Fig. 5. Electropherograms of an alkali and alkaline earth standard. Capillary, fused-silica, 80 cm  $\times$  75  $\mu$ m I.D.; electrolyte, (a) 4 mM 4-methylaminophenol sulphate; (b) 2 mM 18-crown-6, 4 mM 4-methylaminophenol sulphate; voltage, +30 kV. Indirect UV detection at 220 nm (reproduced with permission from Ref. [36]).

other crown ethers such as 15-crown-5 and 12-crown-4, noticeable selectivity changes become evident at much higher levels than with 18-crown-6 [38].

From the point of view of many practical applications, the utility of crown ether additives is especially encouraging for separating potassium from ammonium which has become an accepted practice since a remarkable work by Bächmann et al. [31].

Potassium and ammonium ions not only co-migrate because of identical mobilities under acidic pH conditions but also cannot be separated using the above-considered negatively-charged ligands showing weak interaction with these two cations. By varying the concentration of 18-crown-6 the migration time of  $K^+$  can be controlled, so that its peak can be shifted relatively to the peaks of ammonium and other highly mobile ions [26,31,36,37,39]. In

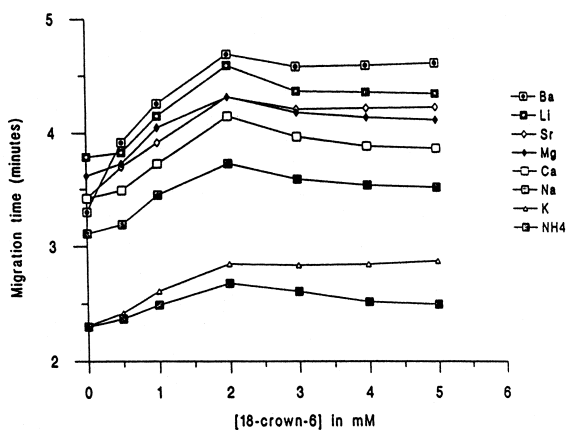


Fig. 6. Effect of 18-crown-6 on migration times of ammonium, alkali and alkaline-earth metal cations. Capillary, fused-silica, 50 cm  $\times$  50  $\mu$ m I.D.; electrolyte, 18-crown-6, 4 mM copper(II) sulphate, 4 mM formic acid; voltage, +20 kV. Indirect UV detection at 215 nm (reproduced with permission from Ref. [36]).

such a way the CE system can be optimised for the analysis of various types of samples.

Macrocyclic polyethers, mostly 18-crown-6, are commonly used in combination with a weak complexing acid in order to separate  $K^+/NH_4^+$  [15,17,18,25,28,30,40] or  $Na^+/Pb^{2+}$  [18] ions in the presence of other group cations (see also Table 1). An impressive example of separation selectivity gained by addition of 18-crown-6 to the lactate system is the complete resolution of Mg, Ca, Sr and Ba against a 1000-fold excess of sodium [15]. Another type of macrocyclic compounds that can promote better CE separation of several cations is cryptands, e.g., cryptand-22 [41], possesses a remarkable cation complexing ability.

### 2.3. Solvation

Another approach that can be adopted, when the mobilities of the free cations are similar, is to add an organic modifier to the carrier electrolyte. The use of organic solvents offers the advantage of selectivity manipulation due primarily to an effect on the solvation of cations. This effect is accompanied by the changes in charge density, since in comparison to aqueous electrolytes, polar organic solvents impact the actual size of ions via forming ion–solvent dipole

bonds. The mobilities of solvated cations are also influenced by changes in the viscosity of water–organic mixtures (see Eq. (1)). Although they have little to do with adjusting the separation selectivity, the organic modifier causes the migration times to be longer and resolution to be better.

There are a few examples where the solvation effect acts differently for different metal groups resulting in changes of the migration order even without the need to employ the complexing electrolytes. For instance, introducing ethylene glycol in the electrolyte causes a general decrease in the mobilities of divalent cations relative to monovalent cations attributed to the changes in the cation hydration [33]. With the amount of organic modifier at the level of 60% it is possible to separate more than 10 cations including some transition metal ions. As can be seen in Fig. 7, divalent cations showing comigration in aqueous electrolyte solution are nicely separated in a 20% acetonic solution, because their mobilities tend to be more decreased than the mobilities of monovalent cations.

In metal-complexing CE systems, the separation of the cationic components having adjacent migration times can be improved with higher concentrations of an organic solvent, mostly methanol [11,15,17,27–29] (see also Fig. 2). The two factors are actually responsible for this behaviour. First, methanol alters favourably the degree of complexation affecting the complexation reaction equilibria. As already mentioned, an increase in the viscosity of the electrophoretic medium simultaneously takes place. A combined effect of increasing the percentage of methanol in the electrolyte on  $\mu_{eff}$  is shown in Fig. 8. As the fraction of methanol increases, an increase in migration time leading to an increase in peak tailing occurs because the increased viscosity results in a decline of both the electrophoretic and electroosmotic flows. Therefore, overuse of organic modifier should be avoided in order to avoid prolonged migration times and broadening of the peak shape. Also, highly organic electrolytes can impair analytical performance owing to evaporation during CE measurements and to lowering the electrical current down to electrical breakdown. On the other hand, a certain advantage of organic solvents, especially with regard to ionic complexing additives, is that the ionic strength of the capillary electrolyte is

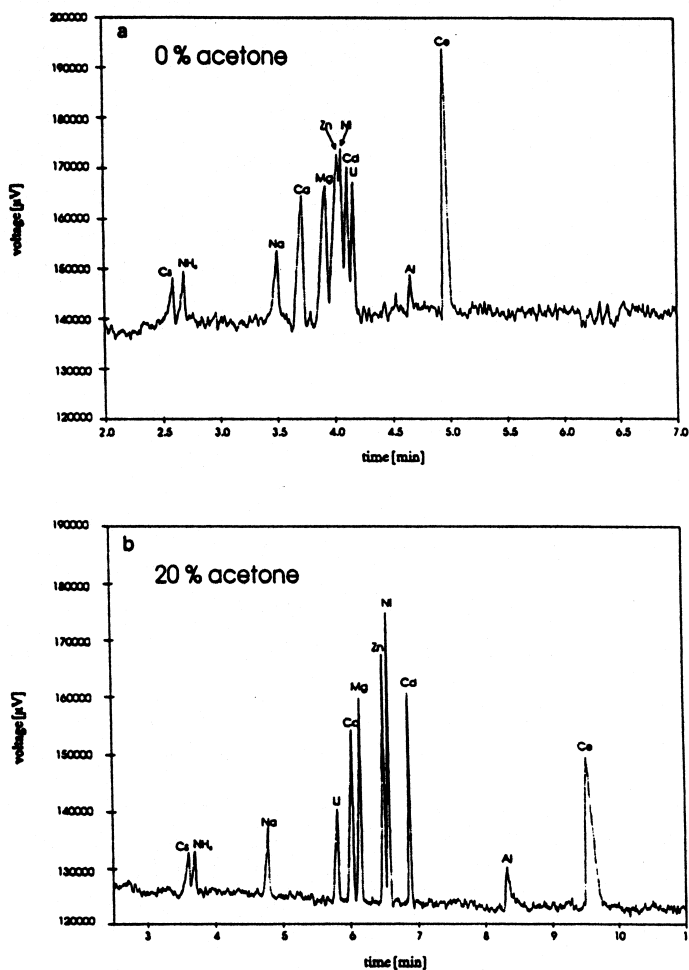


Fig. 7. Effect of acetone on the electrophoretic separation of metal cations. Capillary, fused-silica, 74 cm  $\times$  50  $\mu$ m I.D.; electrolyte, (a) 4 mM 4-methylaminophenol sulphate; (b) 4 mM 4-methylaminophenol sulphate, 20% acetone; voltage, +30 kV. Indirect UV detection at 220 nm (reproduced with permission from Ref. [36]).

not increased which prevents possible thermal effects [36].

Organic modifiers of polymeric nature such as polyethylene glycols also hold promise for selectivity control in CE metal ion separations. In spite of the similar ion-dipole type of interaction with the cations taking place through oxygen atoms, the differentiating power of polyethylene glycols differs slightly from that of organic solvents. The enlarged differences in effective mobility are assumed to be due to the conformational flexibility of polyethylene glycols and the formation of somewhat stronger

complexes with the cations. On the other hand, this complex formation is not specific compared to that existing in the crown-ether electrophoretic systems (see Section 2.2).

Stathakis and Cassidy [42] have found that changes in electrophoretic mobilities of various cations, resulting from the addition of polyethylene glycol, depended on the nature of the metal ion. Smaller, more hydrated monovalent metal ions or polyvalent metal ions are usually more strongly complexed. The trend for decreasing  $\mu_{\text{eff}}$  is a function of the relative molecular mass and con-

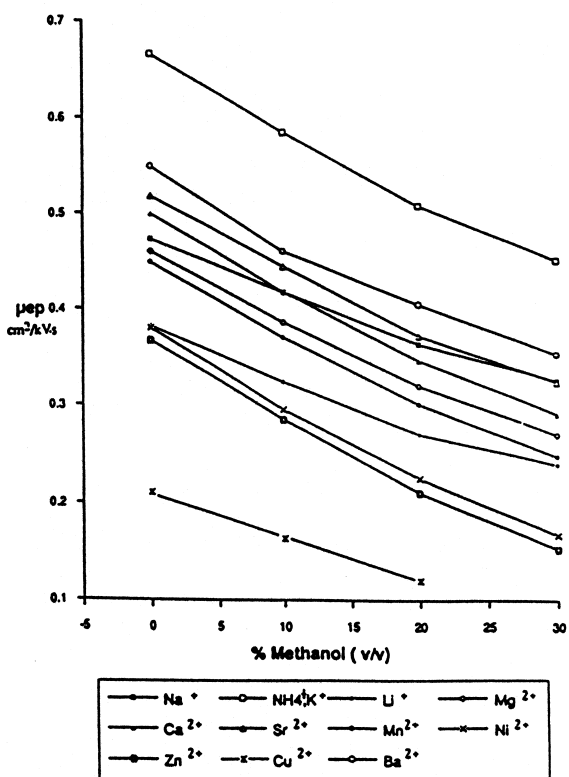


Fig. 8. Dependence of the ionic mobility on the methanol concentration in the carrier electrolyte. Capillary, fused-silica, 60 cm  $\times$  75  $\mu$ m I.D.; electrolyte, 6.5 mM HIBA, 5 mM imidazole (pH 4.5); applied voltage, +20 kV. Indirect UV detection at 214 nm (reproduced with permission from Ref. [28]).

centration of polyethylene glycol. Ito and Hirokawa [43] have attained the nearly baseline separation of a mixture of ten alkali, alkaline-earth and ammonium cations applying water–polyethylene glycol (>35%, v/v) electrolytes. Noticeably, with the polyethylene glycol systems, different migration patterns were observed in comparison with conventional metal–ligand systems [42,43].

#### 2.4. Ion pairing

Of other separation principles, which are of minor significance in facilitating the selectivity for non-complexed metal ions, is ion pairing. Positively-charged ions can interact with a negative ion-pairing reagent according to the following equilibrium:



( $X^{-}$  is the monovalent counter ion of the ion-pairing reagent). The degree of ion pairing may differ from cation to cation which allows their effective mobilities to be varied to a different extent upon varying the concentration of ion-pairing reagent. For example, the most pronounced effect of sodium dodecyl sulphate on delaying the migration of a group of alkaline-earth and transition metal ions tested by Buchberger et al. [33] was observed for strontium and barium. This finding suggests that the equilibrium (11) is involved in the electrophoretic separation of these two ions.

Non-ionic surfactants, for instance, Triton X-100, strongly reducing the electroosmotic flow velocity, can be used to adjust resolution of partially complexed metal ions (Fig. 1 and ref. [14]) but without any effect on their effective mobilities.

#### 2.5. Complete complexation

Separation of metal ions in the precapillary-complexed form presents a viable supplementary approach of improving selectivity. In this way, complete transformation into moderate to strong complexes before injection of the sample mixture into the capillary imparts different mobilities to metal ions with a separation selectivity that differs from that attainable with the aforementioned CE methodologies. Depending on their charge and size, metal ion complexes formed can move towards different electrodes, so that several migration modes can be realised here and more flexibility with regard to choice of separation conditions can be afforded [3].

Previously reported studies reveal the variety of complexing reagents suitable for the CE separation of metal cations. Most commonly used reagents are listed in Table 2 together with metal analytes separated and typical carrier electrolyte compositions. In general, the complexing reagent designed for separation of metal ions by CE is required to be nonselective, i.e., capable of forming, under the same conditions, complexes with a decent number of metal ions. In some instances, however, when selective determination of a particular metal (or a group of metals) is requested, a selective reagent is of better

Table 2  
Examples of chelating reagents for the separation of metal ions by CE

Chelating reagent	Metal ions separated	Separation conditions	Ref.
<i>trans</i> -Cyclohexane-1,2-diaminetetraacetic acid (CDTA)	Fe(III), Sr(II), Ca(II), Mg(II), Cd(II), Co(II), Ni(II), Cu(II)	20 mM sodium borate, 3 mM CDTA	[44]
	Ce(III), Pr(III), Y(III), Nd(III), Sm(III), Eu(III), Gd(III), Tb(III), Dy(III), Ho(III), Er(III), Tm(III), Yb(III), Lu(III), Sc(III)	20 mM borate buffer, 1 mM CDTA (pH 11.1)	[45]
	Tl(III), Ce(III), Sb(III), Bi(III), Fe(III), Fe(II), Co(III), V(V), Ag(I), Zr(IV), Cr(III), Sn(IV), Mo(VI), Ba(II), Sr(II), Ca(II), Mg(II), Hg(II), Co(II), Ni(II), Cu(II), Zn(II), U(VI)	20 mM sodium borate, 5% ethylene glycol, 1 mM CDTA	[46]
8-Hydroxyquinoline-5-sulphonic acid (HQS)	Mn(II), Cu(II), Al(III), Cd(II), Fe(III), Zn(II), Co(II), Ni(II)	10 mM sodium borate, 0.1 mM HQS (pH 9.0)	[47]
4-(2-Pyridylazo)-resorcinol (PAR)	Co(II), V(V), Cu(II), Fe(III), Ni(II)	10 mM NaH <sub>2</sub> PO <sub>4</sub> , 10 mM sodium borate (pH 8.4)	[48]
	Cr(III), Co(II), Cu(II), Pb(II), Ni(II), Fe(II), Zn(II), Fe(III), Cd(II)	10 mM ammonium phosphate buffer, 75 mM SDS, 0.1 mM PAR (pH 8.0)	[49]
2,2'-Dihydroxyazo-benzene-5,5'-disulphonic acid	Cu(II), Fe(III), Co(III), Cr(III), Al(III), V(V)	20 mM NaH <sub>2</sub> PO <sub>4</sub> (pH 7.0), 25 mM tetrabutylammonium bromide	[50]
2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol (5-Br-PAPS)	Cd(II), Zn(II), Pb(II), V(IV), Hg(II), Cu(II), Co(II), Ni(II), Fe(II)	24 mM acetate buffer (pH 4.9), 0.12 mM 5-Br-PAPS	[51]
Arsenazo III	Ce(III), La(III), U(VI), Cu(II), Pb(II), Co(II), Fe(III)	10 mM ammonium phosphate buffer, 0.1 mM Arsenazo III (pH 8.0)	[49]
2,6-Diacetylpyridine bis(N-methylenepyridinio)hydrazone	Mo(VI), Sc(III), Fe(III), Y(III), Zn(II), Cd(II), Zr(IV), Co(II), U(VI), Cu(II), Sn(IV), Ta(V), Hg(II)	10 mM sodium borate, 75 mM tetradecyltrimethylammonium bromide, 10 mM sodium <i>n</i> -octanesulphonate	[52]

choice. Similarly to HPLC, the stability of metal complexes in the CE system is also highly desired as it ensures the existence of only one metal-containing species within each zone and guarantees the constancy of its composition and hence the migration behaviour. If this is not so, the complexation conditions must be chosen carefully in order to prevent the less stable complexes from partially dissociating during the CE run. Sometimes it is sufficient to

simply add an excess of the complexing reagent to the sample and then adjust the pH to an appropriate value. However, as a more universal way, a certain concentration of the complexing reagent is incorporated into the carrier electrolyte together with maintaining a proper reagent-to-metal ion ratio in the injected sample [23]. It should be stressed that under specific conditions, an on-capillary complexation method, in which the complexing reagent is included

directly in the electrolyte to create the complexes, may be suited [53–57].

Perhaps the only one requirement relevant for complexing reagents in CE, unless the micellar-mediated CE systems are concerned, is the formation of charged complexes. To satisfy this requirement the ligand must contain at least one ionizable group, such as sulphonate, carboxylic or a quaternary ammonium group, which does not participate in complexation, i.e., located in a somewhat remote position away from the chelating group. It is also advisable that this group is able to express the difference in a complexed metal nature (e.g., an electron-acceptor ability) by means of the magnitude of the effective charge.

From the point of view of the above requirements, multidentate organic reagents, i.e., chelating reagents, are more preferable. That is why precapillary derivatization using chelating reagents is increasingly being used to carry out metal ion separations since the work of Motomizu and co-authors [44] on separation of a mixture of alkaline-earth and transition metals complexed with *trans*-cyclohexane-1,2-diaminetetraacetic acid (CDTA) in free-solution CE. It can be seen from Table 2 that the variety of metal ions separated by CE following the conversion into stable chelate complexes can be increased substantially. Amongst these, are such cations that possess a slow rate of complexation [Cr(III), Co(III), Al(III), etc.] or that are incompatible with carrier electrolytes commonly used in CE, being in uncomplexed form (for example, readily hydrolysed cations). Neutral organic reagents, e.g., 1,10-phenanthroline [58,59] and 2,2'-dipyridyl [60], and inorganic ligands, like cyanide [61,62] and chloride [63–66], are generally less applicable as they suppose more rigid control on complexation conditions.

One particularly advantageous aspect derived from the underlying complexation chemistry, whatever type of metal complexes is utilised, is the absence of special restrictions on the types of ions that can be used in the electrolyte. Accordingly, it is possible to manipulate the separation selectivity in a broad range by varying the electrolyte composition using various auxiliary interactions between separands and electrolyte components such as those involved in acid–base equilibria, dissociation, mixed-ligand complex formation, ion pairing and micellization. The most

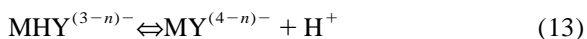
significant of these options will be commented on in the following subsections to demonstrate their effect on resolution.

### 2.5.1. Variation of pH

The electrophoretic mobility can reflect the changes in the electrolyte pH provided that the analytes undergo certain charge density alterations. For charged metal complexes, once formed, this may concern the influence of pH on acid–base and ligand dissociation equilibria or, at the extreme pH values, hydrolytic processes.

Some ligand functional groups responsible for the complex's charge can change the degree of dissociation over the pH range typical for CE buffer electrolytes (pH 3–11). This makes variations of electrolyte pH a useful tool in selectivity enhancement. For example, the changes in the mobility of metal ion complexes of 4-(2-pyridylazo)resorcinol, well known as PAR, as a function of the pH of the phosphate/borate electrolytes have been reported by Iki et al. [48]. These changes, different in the magnitude, are well interpreted in terms of the  $pK_a$  of the corresponding protonated metal–PAR species. The corresponding dependencies (not shown) indicate two groups of complexes which show a different pH-dependence pattern. The pattern, a slight decrease in the migration time with increasing pH, culminating eventually to a situation where there is essentially no change in the effective mobility (rectified for simultaneously increased electroosmotic flow velocity), is typical for the vanadium and cobalt complexes having the  $pK_a$  values of a non-bound hydroxy group of the resorcinol moiety far beyond the pH region studied (pH 7–9.5). In contrast, the mobility of iron ( $pK_{a2}$  7.66), copper ( $pK_{a1}$  8.2) and to a lesser extent, nickel ( $pK_{a2}$  7.1) chelates is clearly reduced with the decrease in the pH, directly affecting their effective charge.

The large differences in migration behaviour of a series of alkaline-earth and transition metal complexes with N,N'-di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid ( $H_4Y$ ), as shown in Fig. 9, seem to be owing to the existence of similar acid dissociation equilibrium:



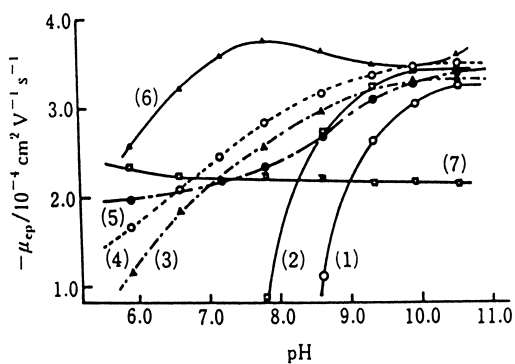
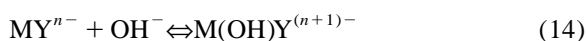


Fig. 9. Effect of pH on the mobilities of metal complexes with *N,N'*-di(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED). Capillary, fused-silica, 72 cm × 50 μm I.D.; electrolyte, 20 mM sodium borate, 2 mM HBED (pH 9.3); applied voltage, -25 kV. Direct UV detection at 294 nm (reproduced with permission from Ref. [68]).

Note a characteristic sigmoid shape of mobility vs. pH dependence for the copper chelate. On the other hand, relatively weak complexes of Ca and Mg are believed to incline to ligand dissociation, the lower the pH, the higher the degree of dissociation.

This resembles the character of the relationships between  $\mu_{\text{eff}}$  values and pH observed for metal complexes of 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulphopropylamino)phenol [51] or its 5-nitro-2-pyridylazo analog [69]. The authors have ascribed it to the fact that less stable chelates gradually became dissociated at acidic pH. Apparently, complete dissociation of the ligand sulphogroups over the wide pH range makes these chelates fully deprotonated species and, in this sense, their effective mobilities pH insensitive. The electrophoretic behaviour of lanthanide-CDTA complexes investigated in detail by Timerbaev and Semenova [45,70] was subjected to the changes in separands' composition as well. While at less basic pH, the mobilities of the complexes were very close or even identical, as for species with very similar  $pK_a$  (or effective charge) values, they increased significantly and became different with highly alkaline borate electrolytes (pH > 9.5). This phenomenon was thought to be related to the formation of hydroxo forms of lanthanide complexes:



(M and Y represent a lanthanide ion and the fully deprotonated form of CDTA or related polyamino-carboxylic acid, respectively). Presumably, the difference in increased negative charge of the lanthanide species, resulting from a shift in the equilibrium (Eq. (14)) in favour of the mixed-ligand form at higher pH, induced mobility differences between the complexes.

The work of Conradi et al. [71] presents the most recent example of mixed-complex formation as a factor influencing the separation of metal chelates. The introduction of a competing complexing ligand, salicylate, in the separation buffer appears to change the composition of parent Fe(II)-EDTA and Fe(III)-EDTA complexes and be the reason, among other factors, for the unexpected migration order of these iron species.

The electrolyte pH is also the parameter in varying the electrophoretic mobility of metal complexes of non-chelate nature such as chloro complexes of precious group metals [63–67], which tend to hydrolyse extensively even in acidic solution showing the complexity of existence states with different number of chloride and hydroxide ligands.

### 2.5.2. The electrolyte concentration of the complexing reagent

As mentioned earlier, because of the tendency of metal complexes to decompose while migrating through the capillary, the complexing ligand often has to be present in the electrolyte system. In this event, it may be practical to exploit discrepancies in the migration behaviour of metal complexes of different stability at varied ligand concentration in the carrier electrolyte. Those complexes which are not so stable show a continued increase in effective mobility with increasing  $C_L$  and finally reach the constant level of  $\mu_{\text{eff}}$ . This levelling-off corresponds to the state when all the complexes exist predominantly in the fully complexed form. The mobility of stable complexes remains virtually unaffected by  $C_L$ . There are several published reports [44,51,60,69,71] in which the resolution for metal chelates has been regulated as being based on the stepwise changes of their composition achieved by varying the electrolyte concentration of complexing reagent (cf. Section 2.1.2).

On-capillary decomposition of metal chelates

which happens to occur when using carrier electrolytes without ligand can be, in some instances, beneficial for the separation. The possibility of using such electrolyte conditions for selective separation of metal ions that form stable, non-labile complexes in the presence of labile, weakly complexing metal ions, has been demonstrated by at least two research groups [44,48,72].

### 2.5.3. Ion pairing

Association with the positive ion-pairing reagents is one of the challenges to regulating the separation selectivity for anionic complexes. In an important paper by the group of Yotsuyanagi [50], incorporation of tetraalkylammonium or tetraphenylphosphonium salts into the electrolyte was found to substantially improve the separation of metal chelates of 2,2'-hydroxyazobenzene-5,5'-disulphonic acid by virtue of an ion-association effect. According to the authors' experiments, a selective reduction of the effective charge and mobility is particularly marked for highly and equally ( $-5$ ) charged complexes of Al(III), Co(III), Cr(III) and Fe(III) capable of forming ion pairs of a higher degree of association. Fig. 10 shows that the bulkier the counter ion, the larger the hydrophobic interaction with metal

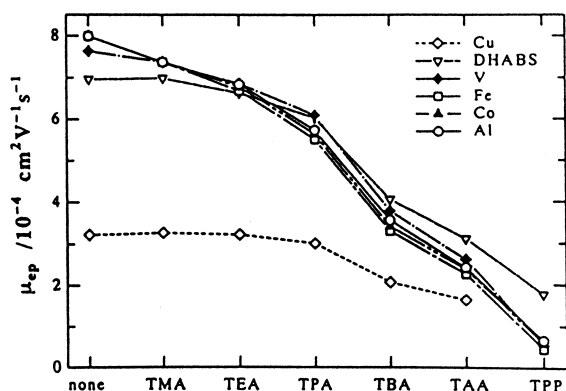


Fig. 10. Effect of counter cation on the migration behaviour of metal chelates of 2,2'-hydroxyazobenzene-5,5'-disulphonic acid. Capillary, fused-silica, 72 cm  $\times$  50  $\mu$ m I.D.; electrolyte, 25 mM counter cation, 20 mM  $\text{NaH}_2\text{PO}_4$  (pH 7.0). Direct photometric detection at 490 nm. Counter cations: TMA=tetramethylammonium; TEA=tetraethylammonium; TPA=tetra-propylammonium; TBA=tetrabutylammonium; TAA=tetraamylammonium; TPP=tetraphenylphosphonium (reproduced with permission from Ref. [50]).

chelates; however, the optimum resolution was observed using tetrabutylammonium cation. The effect on the separation of the concentration of ion-pairing reagent is also strong evidence. In a more recent paper [67], the same findings regarding the influence of the nature and electrolyte concentration of quaternary ammonium salts were reported for metal complexes of 2-nitroso-1-naphthol-4-sulphonic acid. Varied concentrations of a doubly charged and longer alkyl-chain cation, 1,6-bis(trimethylammonium)hexane (better known as hexamethonium) produce certain changes in migration order of metal cyanides due predominately to the ion-pairing effect [62].

Ion-pairing formation in micellar-mediated CE systems will be considered in the following subsection.

### 2.5.4. Micellar interactions

Regardless of the charge sign of metal complexes, their resolution can be substantially improved by applying micellar electrolytes, i.e. under the conditions of micellar electrokinetic chromatography. In fact, it is exactly micellar electrophoretic systems that extend the applicability of CE to electrically neutral metal complexes. The separation of such complexes, e.g., metal acetylacetonates [73,74], is governed by the partition between the bulk aqueous and micellar phase formed at supercritical micelle concentrations of an anionic surfactant, like sodium dodecylsulphate (SDS), and surrounded by the aqueous solution. This implies that the differences between migration parameters (capacity factors) of the complexes increase with increasing total SDS concentration which is actually the only controllable variable of the carrier electrolyte.

It is also well established [49,75,76] that anionic surfactants, most commonly SDS, have an effect on the electrophoretic mobility of metal complexes of the same charge type, provided that these complexes possess a hydrophobic nature so as to be solubilized by the micelle. In this case, the separation principle is based on two mechanisms, electrokinetic migration in the aqueous bulk phase and micellar solubilization, and this gives the experimenter a wider option in enhancement of the separation selectivity. In the pioneering work on a CE separation of metal ions involving pre-formed complexes [75], the feasibility of resolving transition metal-PAR com-



plexes with very similar electrophoretic mobilities using micellar CE was first demonstrated. Increased SDS micellar concentrations significantly influence the order of migration of the chelates. This clearly indicates that the partition processes are vital for the separation. The fact that the resolution of anionic species is accomplished very effectively with anionic SDS micelles but unsatisfactory with no added SDS can be explained in the most plausible way assuming that the distribution into the hydrophobic core of the micelle overwhelms the electrostatic repulsion between similarly charged metal–PAR complexes and micelle. In addition, the concomitant retardation of observed mobilities under the action of decreased electroosmotic flow extends the migration range and thus plays a role in the peak resolution. The separation behaviour of the chelates also varies with the electrolyte pH as a result of changes in acid dissociation equilibria (see, e.g., Eq. (13)); the latter obviously affect the hydrophobicity of separands.

Later, an improved peak capacity for separations of metal–PAR complexes has been achieved due mainly to ion-pair formation between anionic solutes and the cationic component of the micellar electrolyte buffer [49]. The electropherograms shown in Fig. 11 illustrate the influence of compositional buffer changes on the resolution of closely migrated PAR complexes. When sodium as a counter cation was replaced by ammonium, the effective mobilities were decreased through ion association that leads to the almost complete separation of paired complexes from the test mixture. A minor effect of the micellar partitioning on mobility differences was observed for relatively polar complexes of 8-hydroxyquinoline-5-sulphonic acid [47] in micellar SDS solutions containing tetraalkylammonium salts as ion-pairing reagents. Separation of manganese and cobalt  $\alpha,\beta,\gamma,\delta$ -tetrakis(4-carboxyphenyl)porphyrinates presents another example of exploiting the subsidiary interactions to favour the partition of solutes into the SDS micelle [77]. The addition of imidazole to the micellar buffer gives rise to an enhanced retention of the cobalt complex via seemingly adduct formation with the co-ordination of imidazole molecules onto the axial sites of this chelate. Nevertheless, the separation in the micellar medium of less hydrophobic complexes, such as highly ionized chelates of Arsenazo III [49], is essentially controlled by the

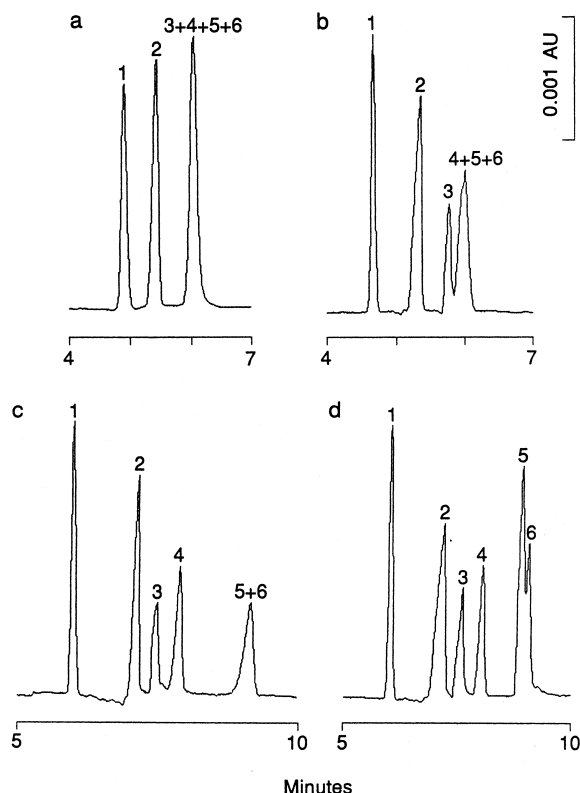


Fig. 11. Electropherograms of metal–PAR complexes with different micellar carrier electrolytes: (a)  $\text{Na}_2\text{B}_4\text{O}_7$ ; (b)  $\text{Na}_2\text{HPO}_4$ – $\text{NaH}_2\text{PO}_4$ ; (c)  $(\text{NH}_4)_2\text{HPO}_4$ – $\text{NaH}_2\text{PO}_4$ ; (d)  $(\text{NH}_4)_2\text{HPO}_4$ – $\text{NH}_4\text{H}_2\text{PO}_4$ . All buffers were 10 mM and contained 50 mM SDS and  $1.10^{-4}$  M PAR. Capillary, fused-silica, 42 cm  $\times$  75  $\mu\text{m}$  I.D.; voltage, –15 kV. Direct UV detection at 254 nm. Peaks: 1 = Co(III); 2 = PAR; 3 = Cu(II); 4 = Ni(II); 5 = Fe(III); 6 = Zn(II) (reproduced with permission from Ref. [49]).

electrophoresis in the bulk carrier electrolyte without any observable interaction with the micellar phase. Electrostatic repulsion is most likely the decisive factor in reducing the role of the partitioning processes.

In the same way, cationic alkyltrimethylammonium surfactants added to the electrophoretic medium permit an improved resolution of positively charged metal chelates of 2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) [52]. The gain in resolution observed with increasing the content of surfactant was considered by the authors as a result of co-operative effects of changes in the phase ratio (i.e., ratio of the volume of micellar phase to that of

the bulk aqueous phase) and electroosmotic mobility (due to increasing viscosity and ionic strength). The latter does not enhance the selectivity but provides the enlarged migration range. At concentrations above the critical micellar concentration, cationic surfactants may also show a retardation effect on the migration of anionic metal–chloride complexes [63] owing to the electrostatic attraction with the micelle.

### 2.5.5. Effect of organic solvents

Although organic modifiers are unlikely to produce any significant selectivity changes for metal complexes, employing carrier electrolytes containing various organic solvents can be of great support to fine-tune the resolution [44,46,62,78]. This is especially true for ethylene glycol with the help of which the currently best separation record with as much as 23 complexed metal ions simultaneously resolved has been obtained (Fig. 12). There is only one separation example [62] known to the author, where several changes in migration order of metal cyanides, distinctively small and highly charged complexes, have been attributed to alterations in hydration in the presence of methanol, the solvent with a rather strong solvating capability with respect to anionic species.

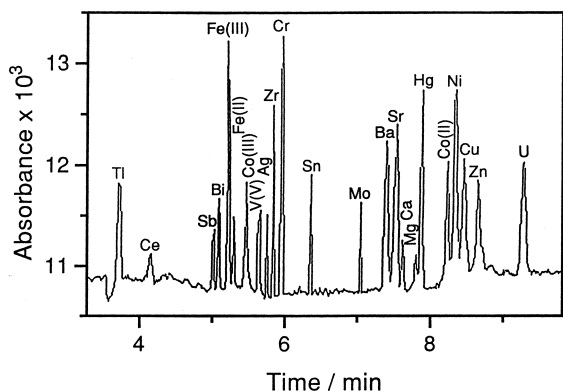


Fig. 12. Separation of metal–CDTA using ethylene glycol as an electrolyte additive. Capillary, fused-silica, 50 cm × 75 μm I.D.; electrolyte, 20 mM sodium borate, 5% ethylene glycol, 1 mM CDTA; voltage, –12.5 kV. Direct UV detection at 214 nm (reproduced with permission from Ref. [46]).

### 3. Manipulation of separation selectivity for metal–ligand complexes

The separation of metal-complexed forms is growing in significance as an important application area of CE. The most useful accounts published to date concern the metal complexes of biological and industrial relevance. Evidently, all the opportunities for selectivity control commented in the previous section are suitable here. Some particular examples are considered as follows.

Advantage can be taken of micellar partitioning phenomenon for the separation of diastereomers of 1,2-di(aminomethyl)cyclobutaneplatinum(II) lactate, a promising anti-cancer therapy agent [79], otherwise having nearly the same effective mobilities. With increasing amount of added SDS, an increase in resolution was observed and reasonably explained by the different distribution coefficients for the two diastereomers between the bulk aqueous buffer and the SDS micelle. The feasibility of the same anionic surfactant micellar solutions for the simultaneous CE separation of bioporphyrins of different central metal ions and porphyrin structure, i.e., copper and zinc complexes of haematoporphyrin IX and protoporphyrin IX [80] as well as naturally occurring magnesium porphyrines, chlorophylls [81], was also demonstrated. The electrostatic interaction with the cationic micelles of cetyltrimethylammonium bromide proved to be a highly sensitive separation mechanism for the copper–polyaminocarboxylic acid chelates as evidenced by the baseline resolution of the model mixture (Fig. 13). As the most highly charged species,  $[\text{Cu(II)EDT}[\text{A}]^{2-}]$ , migrates the slowest, micelle affinity appears to impose the dominating effect on the separation behaviour rather than the higher electrophoretic mobility of this metal chelate in solution. Interestingly, the migration order of iron(III) complexes with diethylenetriaminepentaacetic acid and EDTA (widely used as metal-masking additives in various industries) at nonmicellar concentrations of tetradecyltrimethylammonium bromide [78] is opposite to that observed for the corresponding copper chelates. The anionic micellar system incorporating SDS also involves interaction between the micelle and the copper-complexed polyaminocarboxylic acids; however, this system is less

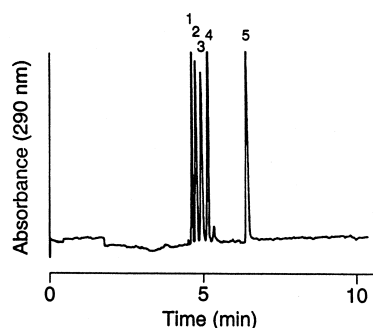


Fig. 13. MEKC separation of copper complexes of polyamino-carboxylic acids using cetyltrimethylammonium bromide (CTAB) as surfactant. Capillary, fused-silica, 80 cm  $\times$  50  $\mu$ m I.D.; electrolyte, 70 mM acetic acid, 10 mM copper(II) acetate, 100 mM CTAB; voltage, -20 kV. Direct UV detection at 290 nm. Peak identity: 1 = [Cu(II)-ethylenediaminetriacetic acid]<sup>-1</sup>; 2 = [Cu(II)-diethylenetriaminepentaacetic acid]<sup>-1</sup>; 3 = [Cu(II)-hydroxy-ethylenediaminetriacetic acid]<sup>-1</sup>; 4 = [Cu(II)-nitrilotriacetic acid]<sup>-1</sup>; 5 = [Cu(II)-ethylenediaminetetraacetic acid]<sup>-2</sup> (reproduced with permission from Ref. [76]).

generally suitable for the separation of such analytes because of much longer migration times. Metallo-cyanides existing in environmental and industrial samples present an important class of metal-inorganic complexes. Generally, they can be nicely separated without special adjustments of the electrolyte composition. Nonetheless, using ion-pairing reagents (Section 2.5.3) and/or organic solvents (Section 2.5.5) in the capillary electrolyte has well broadened the range of metal cyanides separated by CE.

#### 4. Optimisation of electrophoretic selectivity for metal oxoanions

To date, relatively few papers have been published on the CE separation of metal oxoanions such as  $\text{CrO}_4^{2-}$ ,  $\text{MoO}_4^{2-}$ ,  $\text{WO}_4^{2-}$  and  $\text{VO}_3^-$ . Analysis of literature data available reveals that the pH provided significant selectivity control over these analytes. As anions of polybasic acids, many oxoanions are pH-sensitive species showing a strong dependence of  $\mu_{\text{eff}}$  on the carrier electrolyte acidity [82–84]. Importantly, the most pronounced deviations in analyte mobility induced by alterations in ionic dissociation

are observed in the pH region around the  $\text{p}K_a$  of the corresponding conjugated acids. The second path shows how the electrophoretic behaviour of metal oxoanions displaying pH-dependence is behind their inclination to a series of complex condensation-polymerisation reactions (e.g.,  $\text{CrO}_4^{2-}$ – $\text{Cr}_2\text{O}_7^{2-}$ ,  $\text{MoO}_4^{2-}$ – $\text{Mo}_7\text{O}_{24}^{6-}$  or  $\text{VO}_3^-$ – $\text{H}_2\text{V}_{14}\text{O}_{13}^{4-}$ ) [85,86]. Obviously, the formation of isopolyanions strongly affects the charge-to-size ratio of parent oxoanions.

The addition of a cationic surfactant to reverse the direction of the electroosmotic flow naturally occurring in fused-silica capillaries is the common practice for anionic analyses by CE. When the cationic surfactant is used above the critical micellar concentration, an anion is electrostatically attracted to the surface of the cationic micelle, reducing the migration velocity differently for each anion. This interaction was observed by Martinez and Aguilar [87] for chromate in their experiments on varying the concentration of tetradecyltrimethylammonium bromide in different buffers. Using nonmicellar concentrations of an electroosmotic flow-modifier often has the same effect on mobility differences owing to ion-pairing interactions between anionic analytes and quaternary ammonium cations. However, cetyltrimethylammonium bromide did not yield appreciable changes in selectivity for chromate, molybdate and vanadate [86] presumably because of the too low concentration levels utilised.

#### 5. Selectivity control for organometallic compounds

Recently, CE is being used to an increasing extent for the separation of organometallics. Both free-resolution and micellar electrophoretic systems are suitable to simultaneously separate different species of one metal, often possessing nearly the same mobilities, and means of enhancing their resolving power are abundant.

##### 5.1. Free-solution CE

Separation between cationic organomercurials as

well as inorganic mercury(II) is rather poor due to the species' close electrophoretic mobilities [88]. Advantageously, the resolution can be facilitated by the pre-capillary formation of cysteine complexes [89]. In this case, the pH is an important operational parameter of the electrophoretic buffer since it determines the net charge of the complexes. In alkaline medium, i.e., at pH over the isoelectrical point of cysteine, complexes formed by this complexing reagent are negatively charged. Therefore, the use of a simple borate buffer enabled the separation of the three organomercurials and mercury(II) as shown in Fig. 14. The Hg(II)–cysteine complex bearing two negative charges appeared as the last migrating peak in the electropherogram.

Most of the species of the organotin family show similar migration velocity under conventional acidic electrolyte conditions [90,91], where they exist as cations. The influence of various electrolyte additives (tartaric acid,  $\beta$ -cyclodextrin, camphorsulphonic acid), at variable concentrations, was examined in an attempt to attain the complete resolution of four triorganotin, viz., trimethyltin, triethyltin, tributyltin and triphenyltin [90]. The slowly migrating species of tributyl- and triphenyltin were the most difficult pair to resolve. Satisfactory results were obtained with electrolytes containing tartaric acid as an effective complexing reagent for the modulation of the electrophoretic mobilities of triorganotin cations.

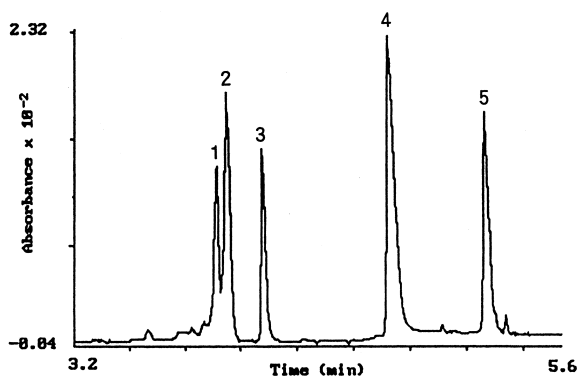


Fig. 14. Speciation of mercury forms complexed with cysteine. Capillary, fused-silica, 50 cm  $\times$  75  $\mu$ m I.D.; electrolyte, 100 mM borate buffer (pH 8.35), 10% methanol; applied voltage, +15 kV. Direct UV detection at 200 nm. Peaks: 1=ethylmercury; 2= excess cysteine; 3=methylmercury; 4=phenylmercury and 5= inorganic mercury (reproduced with permission from Ref. [89]).

Another complexing additive employed in this study,  $\beta$ -cyclodextrin, capable to form inclusion complexes with different types of solutes (see, e.g., Section 5.2), allows only a partial separation of tributyl- and triphenyltin. In a more recent work [92], somewhat better speciation performance – five triorganotin in one run – was achieved by CE with a zwitterionic buffer of N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, though the zwitterion concentration permitted only subtle changes in selectivity. The migration order of dimethyltin, dibutyltin and tributyltin can be reversed by increasing the electrolyte pH from 2.75 to 4.5 [91]. The most possible explanation is that this is a result of the difference of hydrolytic properties of the organotin moieties; diorganotins are known to undergo hydrolysis at a pH greater than 3.0. The choice of the buffer anions is also critical in the CE separation of organotins [91]. Results of the experimental series with different acids used to adjust the pH suggest that neutral complexes appear to form with diorganotins in the presence of oxalate and citrate.

## 5.2. MEKC

Micellar electrokinetic chromatography has a proven potential for multi-elemental analysis of organometallic compounds of different metals. By adding the anionic surfactant, that is, SDS to the electrophoretic buffer, Li et al. [93,94] readily achieved the MEKC separation of a group of organoleads and organotins. As expected, the migration order is dominated by the distribution ratios of the species between the aqueous electrophoretic and micellar phase, i.e., by their hydrophobicities estimated from the size of the alkyl substituent and metal atom. Fig. 15 presents the electropherograms of selected organolead and organotin compounds at different buffer pH. As can be seen, the pH largely affects the resolution by changing the degree of ionization and stability (Fig. 15C) of the solutes. The opposite order of migration for trimethyllead and triethyllead under CE conditions [95] is noteworthy. The authors attributed the slower migration of the former species to a higher degree of ion pairing conceivably existing between organoleads and the borate ions of the electrolyte buffer.

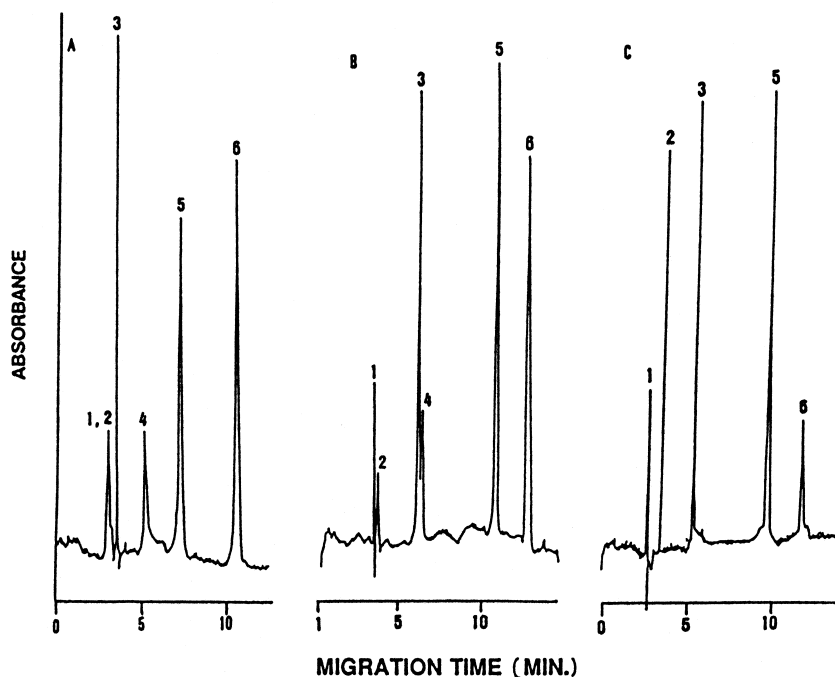


Fig. 15. MEKC separation of organolead and organotin compounds at various electrolyte buffer pH values: A=10.2; B=9.3; C=7.65. Capillary, fused-silica, 55 cm $\times$ 50  $\mu$ m I.D.; electrolyte, 50 mM SDS in different ratios of 25 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>–75 mM NaH<sub>2</sub>PO<sub>4</sub>; voltage, +20 kV. Direct UV detection at 200 nm. Peaks: 1=methanol; 2=trimethyltin; 3=trimethyllead; 4=dibutyltin; 5=triethyllead; 6=tributyltin (reproduced with permission from Ref. [93]).

The main advantage of MEKC compared to free-solution CE is that both charged and neutral organometallics can be separated simultaneously. This was demonstrated [95] for a mixture of cationic organolead and cationic and neutral organoselenium compounds using  $\beta$ -cyclodextrin-modified micellar SDS solutions. The competitive interactions of the separands with electrolyte components, SDS and  $\beta$ -cyclodextrin, taking place via hydrophobic and inclusion (host–guest) complexation mechanism, respectively, appear to govern the migration pattern observed in this work. This conclusion was supported, in particular, by a crossover of peaks for triethyllead and diphenylselenium that occurs at higher  $\beta$ -cyclodextrin concentrations; the latter species tends to be incorporated more readily into the cavity of  $\beta$ -cyclodextrin and thereby migrates earlier. Satisfactory separation of neutral organometallic compounds of iron, viz., ferrocene and ferrocene derivatives, can also be achieved as based on the interaction inside the micelles of SDS [79].

## 6. Conclusions

The key conclusion drawn from this overview is that a diversity of different metal species are already amenable to separation by CE and a wide range of possibilities for selecting advanced separation conditions are acknowledged. A variety of electrolyte additives have been studied to change the electrophoretic mobility of metal analytes and resolve otherwise comigrating species. Table 3 summarises the ways of modifying selectivity discussed here which can easily be adapted for CE method development. Further, manipulation of these parameters allows the separation selectivity for metal species to be optimised for particular application problems. More knowledge of CE separations makes the choice of electrolyte variables and, to a certain extent, the variable space more accurate, and the author believes that this work offers a lot of knowledge on this. However, while we do know the main types of interactions influencing separation of metal species

Table 3

Main factors affecting the separation of metal species by CE

Metal species separated	Electrolyte parameter	Separation principle
Metal ions	The nature of weak complexing reagent (e.g., hydroxycarboxylic acid) Concentration of complexing reagent pH The type of counter ions of the buffer Concentration of organic solvent (methanol) The nature of macrocyclic ligand (18-crown-6) Concentration The type and concentration of organic solvent or polymeric organic modifier (polyethylene glycol) The nature and concentration of ion-pairing reagent	Differences in ionic mobilities induced by the stepwise complexation equilibria  Differences in ionic mobilities magnified specifically by inclusion complexation Differences in mobilities of solvated cations  Differences in ionic mobilities affected by ion pairing
	Metal complexes	pH Concentration of complexing reagent The nature and concentration of ion-pairing reagent The nature and concentration of organic solvent  The nature of surfactant Concentration  The concentration of SDS
Metal oxoanions	pH The nature and concentration of cationic surfactant	Differences in ionic mobilities facilitated by changes in ionic dissociation, the formation of isopolyanions or the electrostatic attraction with the micelle
Organometallic compounds	The nature and concentration of weak complexing reagent pH The type of counter ions of the buffer The concentration of $\beta$ -cyclodextrin The concentration of SDS  The concentration of SDS The concentration of $\beta$ -cyclodextrin	Differences in electrophoretic mobility of cationic organometallics induced by partial or inclusion complexation, hydrolytic changes or ion pairing Differences in electrophoretic mobility of cationic organometallics enhanced by differential micellar partitioning Differential distribution of neutral organometallics between the bulk aqueous electrolyte and micellar phase including inclusion complexation mechanism

in CE, there are still some things to be investigated. Therefore, further development optimising chemical selectivity of CE systems is anticipated in the years ahead.

## Acknowledgements

The author wishes to thank Dr. Olga Semenova, Interuniversity Research Institute for Agro-Biotechnology, Tulln, Austria for proof reading and for technical assistance in preparation of the manuscript.

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