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Review

Role of chemical equilibria in the capillary electrophoresis of inorganic substances

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Abstract

Electrophoretic behaviour of low-molecular-mass substances and their separation can be effectively manipulated employing selective interactions between the separated ions and components of working solutions. Proper selection of operational electrolytes can considerably enhance the selectivity of electrophoretic separations without expensive modifications of instrumentation. Complex-forming acid–base and ion association equilibria are most important for influencing the effective mobilities of inorganic cations and anions. These can be used for optimization of separations. On the other hand, capillary electrophoretic methods can be used for investigations of chemical equilibria and measurements of physicochemical characteristics, such as stability, dissociation/protonation, ion association, etc., constants. Formation of complexes of the separated ions with suitable ligands contained in the working solutions is especially useful in the case of the capillary electrophoretic determination of metals (transition and heavy metals, rare earth elements). As can be seen from a number of published operational systems, similar complexing agents are used in various electrophoretic modes, namely in capillary zone electrophoresis (CZE) and in capillary isotachopheresis (ITP). Hence the previously described systems for ITP separations can serve as an inspiration in the course of the development of new CZE methods. © 1999 Elsevier Science B.V. All rights reserved.

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Contents

1. Introduction	3
2. Electrophoretic mobility	6
3. Acid–base equilibria	7
4. Complex-forming equilibria	9
5. Other side equilibria	16
References	18

1. Introduction

Capillary electrophoresis (CE) belongs to electromigration techniques for separation (rarely for preparation – see isotachopheretic crystallization of inor-

ganic substances [1]) of compounds during their migration in solution under the influence of an electric field [2]. A unique feature of the electrophoretic (electromigration) techniques is their exceptional broad field of applications ranging from simple

inorganic ions to macromolecular species such as DNA and proteins. This review is focused primarily on analytical separations of inorganic cations and anions, however, some works dealing with separations of organic species are also mentioned, if they utilize similar principles and/or techniques as discussed for inorganic substances.

Although the basic principles of electrophoretic separation have been known for a relatively long time (see ‘milestones’ listed by Jandik and Boon in the preface of their book [3]), a real expansion of electromigration methods, especially in inorganic analysis, started in the 1990s, when the introduction and commercial availability of narrow-bore silica capillaries allowed electrophoresis in open capillaries – capillary zone electrophoresis (CZE) [4]. Some time before, another CE method had been applied in inorganic analysis – capillary isotachopheresis (ITP) [5,6]. The criteria for selection of an operational system are different for CZE and ITP. In CZE, the separation is carried out in one working solution – background electrolyte (BGE). The mobilities of the BGE components should match with the mobilities of the separated ions. In ITP, the sample is placed on the boundary between the leading electrolyte (LE) and terminating electrolyte (TE). LE contains ions with higher mobility and TE ions with lower mobility than the separated ions. Because of a rather complicated experimental arrangement, and maybe because of an uncommon shape of the isotachopherogram, ITP did not find widespread acceptance in analytical practice. Nevertheless, the principles of governing the migration behaviour of the analyte ions (and also the components of working solutions) are similar both in CZE as well as in ITP. Various chemical equilibria and other side effects can be used to adjust optimal conditions for successful separations. Operational systems designed for the ITP separations, especially those utilizing complex-forming equilibria, can be used as a useful guide for selection of BGE in CZE. Therefore selected papers on ITP of inorganic species will be involved in this review. Other CE methods, such as isoelectric focusing, micellar electrokinetic chromatography (MEKC) or electrophoresis in sieving media, are of less importance in separations of inorganic compounds (the use of MEKC for the separation of metal ions and metal-containing species recently reviewed Hadad et al. [7]).

In inorganic analysis, CE methods compete mainly with ion chromatography (IC). Comparison of both techniques is given in the recently published review [8] with respect to a stage of development, separation efficiency, separation selectivity, analytical performance parameters, method development procedures and applications. In many applications the IC methods may be replaced successfully by CE, advantageously utilizing its higher separation efficiency and speed of analysis. What is common for IC as well as for CE is that ‘chemistry’ plays a more significant role in the separation process in comparison with such techniques as gas chromatography or ‘conventional’ HPLC. As stated by Jandik and Bonn [3], CE requires a more active role of analyst in the method development and selection of working conditions. A significant improvement of the selectivity can be achieved not solely by the change of instrumentation (separation ‘hardware’), but often by the change of the working solutions – BGEs or mobile phases (separation ‘software’). A great advantage of CE in this context is the easily changed operational conditions, which facilitate development and optimization of methods. Strategies for selectivity control in CE of metal species were discussed by Timerbaev [9], who emphasized, in analogy to IC, the importance of secondary (side) equilibria, such as acid–base, complex-forming, ion association, etc., in the separation process. The term ‘secondary equilibria’ seems to be somewhat misleading in CE – the question is, what is the ‘primary equilibrium’ here. (In IC, it is obviously an ion-exchange between the analyte ions and driving ions of the eluent, taking place on active sites of stationary phase). Maybe the equilibrium between a driving force caused by an electrostatic attraction of the separated (charged) species towards the oppositely charged electrode and a frictional force affecting the movement of ions in solutions according to the Stokes’ law could be called the primary equilibrium in CE (although not in a chemical sense). The equilibria are shown schematically in Fig. 1.

It is evident that, in addition to the equilibria mentioned, a number of other auxiliary mechanisms (interactions with capillary walls, interactions with sieving media, ion-exchange in CE [3]) and further side equilibria are effective both in IC and CE, such as e.g. solvation, sometimes called tertiary effects [10].

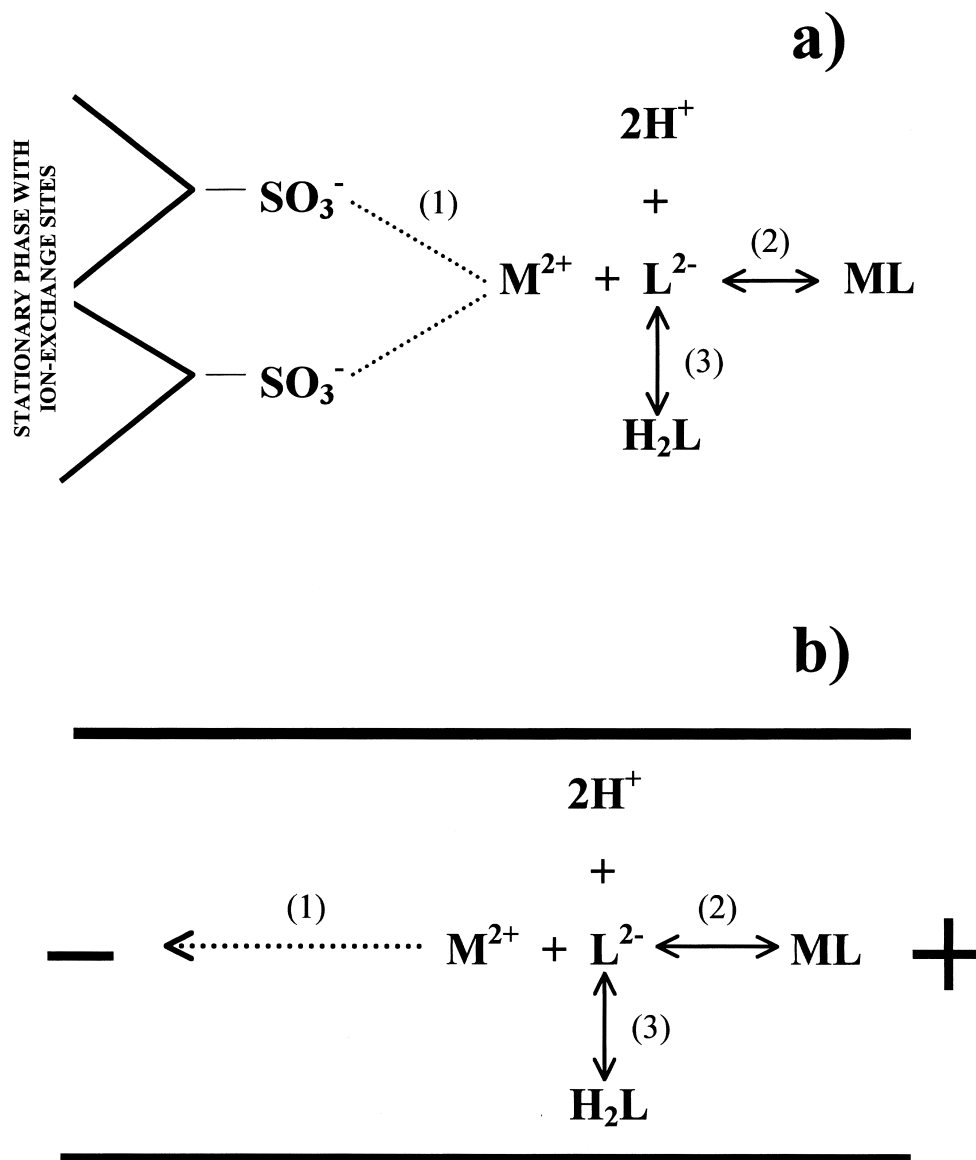


Fig. 1. Equilibria in separations of metal ions with the aid of IC (a) and CE (b) methods. (1) Retention of metal cations M^{2+} on the ion-exchange sites in IC or attraction of metal cations to the oppositely charged electrode in CE, (2) complex-forming equilibrium of metal ions with the complexing ligand L^{2-} , (3) protonation equilibrium of the ligand L^{2-} .

This review is focused on the role of side equilibria in controlling the effective mobilities of the separated compounds. However, it should be emphasized that an effect of some factors on migration and separation may be rather complicated. For example, the pH value of the working solution influences not only a dissociation of analyte and consequently its effective mobility, but also a chemistry of the

capillary wall and a magnitude of an electroosmotic flow (EOF). Similarly, the addition of quaternary ammonium salts can influence the effective mobility of the analyte and simultaneously dramatically change the magnitude or even the direction of EOF. The methods of how to govern the effective mobilities of analytes utilizing side equilibria for optimization of the separation will be discussed. Also

possibilities of investigations of these equilibria and measurements of physicochemical constants with the aid of CE methods will be presented here (with restriction to electrophoresis in open capillaries, although electrophoretic techniques in other arrangements, such as slab electrophoresis or paper electrophoresis, have been traditionally playing an important role in studying complex-formations [11–14]).

2. Electrophoretic mobility

In CE, the ionic substances are separated on the basis of differences in their mobilities, μ [15]:

$$\mu \approx \frac{q}{f} \quad (1)$$

where q is the electrical charge on the molecule and f is the frictional coefficient. According to the Debye–Hückel theory, the ions in solutions are surrounded by an ionic atmosphere, which affects their electrophoretic mobility in dependence on the concentration. An extrapolation of the concentration dependence of the electrophoretic mobility to the zero ionic strength yields a ionic mobility (or net mobility) – these ionic mobilities can be found in literature, or estimated from various empirical or semi-empirical equations, such as Jokl's equation [16]. Actual mobilities for the solutions with the given (actual) ionic strength are calculated from the ionic mobilities using e.g. the Onsager equation [16].

Compounds present in BGE not only originate the ionic atmosphere, but may also introduce specific interactions with the separated ions – take part in the above mentioned secondary equilibria. There are two basic situations (in this paragraph, let us assume for simplicity just two compounds A and B taking part in the equilibrium, where A is typically the analyte ion and B is the component of BGE) [17]:

(1) The equilibrium in the system is attained slowly in comparison with the duration of the experiment (inert systems).

(2) The time required for equilibrium is negligible compared with the duration of the electrophoretic experiment, i.e. with the separation time (labile systems).

In the case of the inert system where AB_n species is formed, $N+2$ boundaries are created during the

separation process and thus $N+2$ distinct peaks are observed. In the case of the labile system, a solution containing B, A, and AB_n species behaves as if it contained only two species B and A, moving with apparent mobilities. Discussions in this work will be focused on the labile systems.

The apparent mobility of the compound coexisting in the form of various species in BGE can be calculated from the weighted average of the mobilities of the respective species; this mobility is called an effective mobility, μ_{eff} [2]:

$$\mu_{\text{eff}} = \sum_{i=0}^N \mu_i x_i \quad (2)$$

where μ_i is the (actual) ionic mobility of the respective species and x_i is its mole fraction. For systems including more complex and/or acid–base equilibria, in which a series of complexes can be formed according to the general equation [17–20] (charges are omitted for the sake of simplicity):



the effective mobility for the component B can be expressed as follows (A is supposed to be mostly H^+ , C and D are complexing ligands):

$$\begin{aligned} \mu_{\text{eff}} &= \frac{\sum_{i=0}^N \mu_i [A_{p_i} B_{q_i} C_{r_i} D_{s_i}]}{\sum_{i=0}^N q_i \beta_i [A]^{p_i} [B]^{q_i} [C]^{r_i} [D]^{s_i}} \\ &= \frac{\sum_{i=0}^N \mu_i \beta_i [A]^{p_i} [B]^{q_i} [C]^{r_i} [D]^{s_i}}{B_{\text{tot}}} \quad (4) \end{aligned}$$

where μ_i represents the mobilities of the individual $A_{p_i} B_{q_i} C_{r_i} D_{s_i}$ species, β_i are the overall stability constants (β_0 is supposed to be equal to 1) and B_{tot} is the total (analytical) concentration of the component B. p , q , r , and s are stoichiometric indices.

In fact, there are several other ways to express the apparent mobility. Timerbaev [9] described the metal-complexing system (for metal M and complexing ligand L) as a single complex with the average number of ligands associated with the metal ion, \bar{n} :

$$\bar{n} = \frac{K_1[L^-] + 2K_2[L^-]^2 + \dots + NK_N[L^-]^N}{1 + K_1[L^-] + K_2[L^-]^2 + \dots + K_N[L^-]^N} \quad (5)$$

where K_i are the stepwise formation constants. Also a modified Stokes' equation was introduced by Timerbaev [21], 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:

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

where q_i is the charge of metal ion, r_i is its size (the hydrodynamic radius) and η represents the dynamic viscosity of the electrolyte medium.

A phenomenon called an electroosmotic flow (EOF) always accompanies electrophoretic experiments. EOF is caused by the influence of an electric field on a charged double layer formed on the interface between bulk solution and a capillary wall (more detailed explanation can be found elsewhere [2,3]). EOF is usually suppressed in ITP. Many important CZE analyses, on the other hand, can not be accomplished without EOF – e.g. simultaneous determination of cations and anions in a single run (unfortunately, this is usually not utilizable for the determination of low-molecular-mass species, such as inorganic cations and anions). Jandik and Bonn [3] distinguish between coelectroosmotic and counterelectroosmotic separation modes in CZE. An observed electrophoretic mobility, μ_{obs} , of the separated ions (directly measurable from an electropherogram) is given by the superposition of their effective mobility and the mobility of EOF:

$$\mu_{\text{obs}} = \mu_{\text{eff}} + \mu_{\text{EOF}} \quad (7)$$

The magnitude and even direction of EOF can be influenced and controlled by the chemical composition of the BGE as well as by the pretreatment of the capillary walls. This, certainly very important part of any CZE procedure, will not be discussed in detail in this review, because EOF itself does not contribute to the electrophoretic separation [22].

3. Acid–base equilibria

For the analyte ion undergoing protonation/dissociation according to the equation:



the general Eq. (2) or Eq. (4) for the effective mobility can be rewritten as follows [17]:

$$\mu_{\text{eff}} = \frac{\sum_{i=0}^n \mu_i [H_{n-i}A^{i-}]}{\sum_{i=0}^n [H_{n-i}A^{i-}]} \quad (9)$$

In the simplest case of monobasic acid, Eq. (9) can be further simplified and rewritten using dissociation constant K_a [2]:

$$\mu_{\text{eff}} = \frac{\mu_A - K_a}{[H^+] + K_a} \quad (10)$$

This equation exhibits a dependence of the effective mobility on pH of BGE and can be utilized for an optimization of the separation of two compounds (A_1 and A_2) differing in their dissociation constants (K_{a1} and K_{a2}), and, as the case may be, in their ionic mobilities, μ_{A_1} and μ_{A_2} . Solving an equation:

$$\frac{\partial(\mu_{\text{eff},A_1^-} - \mu_{\text{eff},A_2^-})}{\partial(\text{pH})} = 0 \quad (11)$$

we obtain after a rearrangement an optimal pH for separation [2]:

$$\text{pH}_{\text{opt}} = \frac{(pK_{a1} - pK_{a2})}{2} - \log \frac{\left(\sqrt{\frac{\mu_{A_2^-}}{\mu_{A_1^-}}} - \sqrt{\frac{K_{a2}}{K_{a1}}} \right)}{1 - \sqrt{\frac{\mu_{A_2^-} K_{a2}}{\mu_{A_1^-} K_{a1}}} \quad (12)$$

Dolník [22] used tabulated values of ionic mobilities and K_a values to optimize CE separations and to find the optimum pH of BGE, using various optimization functions, such as selectivity, differential mobility, and resolution. Selectivity, differential mobility or resolution was calculated for the hard-to-separate pairs of analytes and plotted vs. pH. The pH, at which the function reaches its maximum, corresponds to the optimum pH for the separation. Havel et al. [20] suggested for similar purposes an application of artificial neuron networks in combination with an experimental design approach. This represents so called 'soft modeling' when the exact

description of the system is missing or if it is too complex ('no prior knowledge' is needed), whereas 'hard modeling' requires full description of the system (the above mentioned equations and others) [23]. In the first step, the effective mobilities were calculated for a system of two weak acids using the above presented equations. In the following step, those data were used in the neural networks training. The inputs to the neurons were the effective mobilities and pH. The difference in effective mobilities was used as the output. The training of the neural networks was done for several different structures. Finally, the neural network with the optimal structure was able to predict the best separation conditions (an optimal pH) from a low number of experiments. The prediction power of the soft modeling (neural networks) and hard modeling (a set of equilibrium equations) was recently compared for some separation methods, such as ion-interaction chromatography [24].

Manipulation of the separation selectivity using the control of the electrolyte pH has been used mainly in separations of organic acids and bases. The influence of the pH of chromate–tetradecyltrimethylammonium bromide-based BGE on the separation of inorganic anions was investigated by Harakuwe and Haddad [25]. From a number of anions studied, hydrogencarbonate–carbonate showed the most pronounced changes in migration time in an alkaline region. The resolution between fluoride and phosphate was improved greatly using BGE with pH 7. Separation of disparate levels of fluoride and phosphate was shown to be possible and the fully resolved separation of these anions in toothpaste was demonstrated. Dependencies of the effective mobility on the pH value were measured for inorganic anions, such as I^- , $S_2O_3^{2-}$, NO_3^- , NO_2^- [26], MoO_4^{2-} , S^{2-} , NO_3^- , I^- , $S_2O_3^{2-}$ [27], and some selenocompounds [28]. For most inorganic anions, no changes in mobility and selectivity occur between pH 7.5 and 11.5. Above pH 12, the effective mobility of sulphide slightly increases, because it is near to its second pK_a [27]. The effective mobility for nitrite decreased dramatically at low pH below 4 due to the protonation of nitrite ions [26]. The separation of nitrate and nitrite ions is rather difficult with BGEs of pH above 4.5, because these ions are very similar in their ionic mobility. The separation efficiency was

enhanced using an acidic BGE containing monochloroacetate buffer and sodium sulphate with pH 3.2 [26].

A selection of working solutions in ITP is more complicated than in CZE. When an analysis of a sample is to be carried out by ITP, a certain pH interval in the sample zone is required to ensure the following:

(i) The substances in question show sufficient effective mobilities so they reach the detector in a reasonable time.

(ii) The isotachophoretic migration of the substances is not disturbed by H^+ and OH^- (the 'unstable region', where H^+ or OH^- ions contribute significantly to the electrical charge transfer, should be avoided).

The above presented equations for an analyte separability should be combined with the isotachophoretic conditions of zone stability. The criteria for the selection of electrolyte system were formulated in [29,30] and a concept of the zone existence diagram was introduced, where zone pH vs. effective mobility is plotted for μ_i and K_a as parameters. More recently, a similar concept was utilized to predict zone patterns in CZE [31].

CE is not only a powerful separation technique, but also a useful tool for the determination of physicochemical characteristics. Protonation/dissociation constants of weak acids, bases, and ampholytes can be determined from the effective mobility – pH dependencies using Eq. (9) (or similar ones) [32–38]. The simplified form of Eq. (10) can be rearranged to:

$$\frac{1}{\mu_{\text{eff}}} = \frac{[H^+]}{K_a \mu_{A^-}} + \frac{1}{\mu_{A^-}} \quad (13)$$

or

$$\text{pH} = pK_a - \log \frac{\mu_{\text{eff}}}{\mu_{A^-} - \mu_{\text{eff}}} \quad (14)$$

Eq. (13) shows that a plot of $1/\mu_{\text{eff}}$ against $[H^+]$ should be a straight line with a slope equal to $1/K_a \mu_{A^-}$ and an intercept equal to $1/\mu_{A^-}$; hence, the K_a values can be readily calculated from the ratio of the intercept to the slope. The values of K_a as well as ionic mobility μ_{A^-} can both be determined from this plot. According to Eq. (14), a plot of pH against

$\log [\mu_{\text{eff}}/(\mu_{\text{A}^-} - \mu_{\text{eff}})]$ results in a straight line with a slope equal to -1 and an intercept equal to $\text{p}K_{\text{a}}$. This plot, however, requires that μ_{A^-} is known, or determined from independent measurements. More general equations for di-, tri-, and tetraprotic acids were presented in [35]. Both graphical and numerical approaches have been used to find the K_{a} values from the μ_{eff} vs. pH dependencies. The general computer program CELET [17] as well as artificial neural networks [20] were used for calculations of the K_{a} values from CE measurements.

A specific approach was used for determinations of dissociation constants of aldohexose isomers [39]. In this case it is assumed that the ionic mobilities of the isomers having the same molecular mass are identical and on that account Eq. (13) represents a linear dependence between the reciprocal of μ_{eff} (or migration time) and the reciprocal of K_{a} . When the parameters of this straight line plot are determined from separate experiments (in [39] glucose was used as a 'standard'), the K_{a} values for all isomers can be

measured from the single electrophoretic run at constant pH (for confirmation see Fig. 2).

Also ITP methods, despite some limitations caused by more complex experimental arrangements, were employed for studying the acid–base equilibria. Hirokawa et al. [40–45] derived computational expressions for isotachophoretic equilibria and used a computer simulation to determine ionic mobilities and $\text{p}K_{\text{a}}$ values for many organic acids as well for some inorganic ones (phosphorus oxoacids [42]) in various media.

4. Complex-forming equilibria

For metal cations, many of which have identical charge and nearly identical hydrated ionic radius, the differences in ionic mobilities are not sufficient to provide a good separation. Complex-forming equilibria between the separated metals and components of working solutions are frequently used for improving

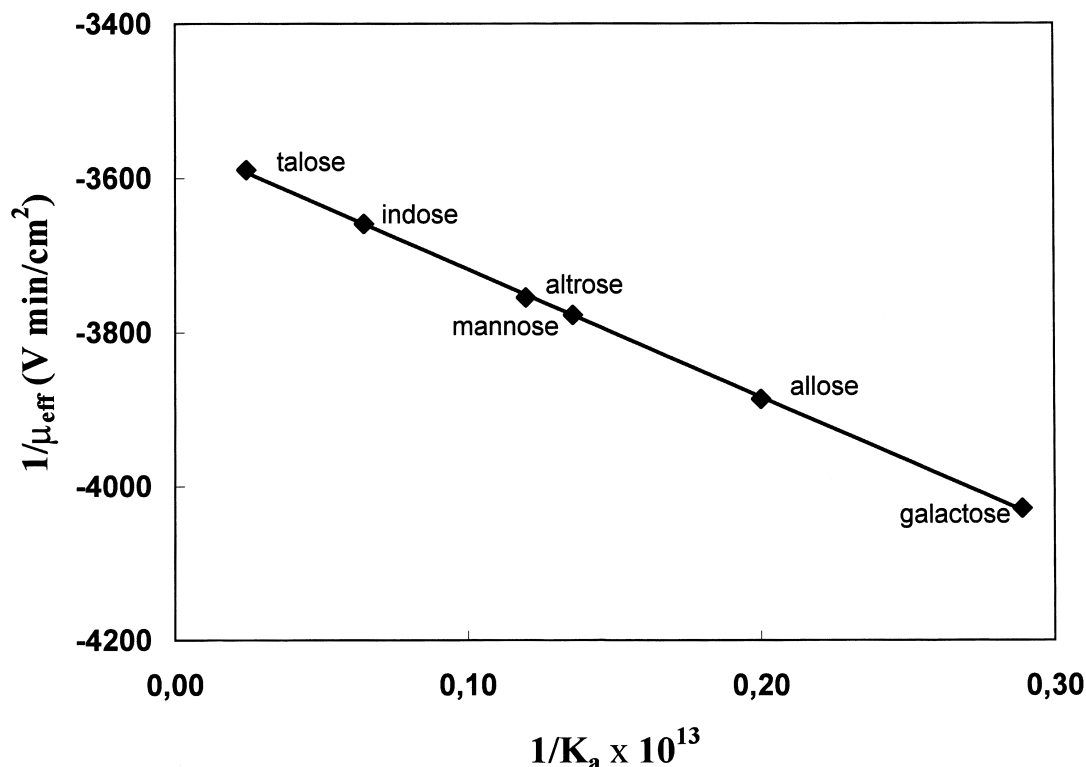


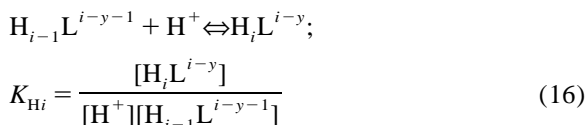
Fig. 2. Dependence between reciprocal effective mobility and dissociation constants. Data from [39].

the separations [46]. For effective mobility of the metal cation, M, in the presence of complexing ligand, L, under the formation of a series of mononuclear ML_i complexes, the general Eq. (2) or Eq. (4) can be rewritten as follows (charges are omitted):

$$\mu_{\text{eff}} = \frac{\sum_{i=0}^N \mu_{ML_i} \beta_i [L]^i}{M_{\text{tot}}} = \frac{\mu_M + \mu_{ML} \beta_1 [L] + \mu_{ML_2} \beta_2 [L]^2 + L + \mu_{ML_N} \beta_N [N]^N}{1 + \beta_1 [L] + \beta_2 [L]^2 + L + \beta_n [L]^N} \quad (15)$$

This relation (or slightly modified one), based on the concept of Tiselius [47], can be found in many books and papers [2,5,6,16–20,48–50].

Complexing agents employed in this kind of separations are usually weak organic acids undergoing dissociation/protonation according to the following equations:



where K_{Hi} are the protonation constants. As the amount of ligand bound to the complexes is small in comparison with the total amount of ligand in the working electrolyte, the total ligand concentration, c_L , can be expressed:

$$c_L = \sum_{i=0}^y [H_iL^{i-y}] = [L^{y-}] \sum_{i=0}^y K_{Hi} [H^+] \quad (17)$$

On combining Eq. (17) with Eq. (15) we can describe the dependence of the effective mobility on the main parameters of the working solutions (in metal separations) – the concentration and kind of complexing ligand and pH. The derived equations can be used for a prediction and optimization of separations. The basic physical constants, such as β_i and K_{Hi} , are usually known. The ionic mobilities μ_{ML_i} of the complex ions, on the other hand, can be rarely found in literature and are not experimentally accessible in a simple way. For their estimation, at least in the first approximation, known relationships between mobilities of ions and their charges and formula weights can be used (e.g. Jokl's equation

[16]). As an example, the dependence of the effective mobility of the Cu^{2+} ion on the pH value and total ligand (lactate) concentration was calculated (Fig. 3). In Fig. 4, the dependence of the simplest separation characteristics – relative difference of mobilities, selectivity [$\text{selectivity} = (\mu_A - \mu_B) / \mu_B$] – on the pH and total ligand concentration is predicted for the Cu^{2+} / Ce^{3+} pair. As can be seen, and as can be expected, an addition of lactate improves the separation of the model mixture of the Cu^{2+} and Ce^{3+} ions. Decreasing the pH value, the dissociation of lactic acid and consequently its complexing ability is suppressed, which causes worse separation. However, in optimization of the separation the effect of pH on EOF must also be taken into account.

In ITP, the complex-forming equilibria are usually utilized in such a way that the complexing agent is a component of the leading electrolyte and, during the migration, it passes through the sample zones as counter ion. Most often, the complex-forming equilibria were used in ITP of cations. The above presented equations holds true also in ITP and could be used for an optimization, but attention should be paid to the correct choice of the leading electrolyte concentration with regard to possible interferences due to uncontrolled migration of H^+ ions through the isotachophoretic zones. It follows from the theory of the correct ITP migration that the effective mobility of the separated metal ion in the respective zone must be greater than the effective mobility of the H^+ ions. The critical concentration of the complexing agent can be calculated using relationships for the effective mobility together with conditions of constant current density and electroneutrality [53–55]. Detailed theoretical equations for the complex-forming equilibria in ITP derived Hirokawa et al. [40,56,57]. They used a computer simulation at the steady state to choose the optimum separation for various models as well as real systems, e.g. calcium tartrate system [40] or zinc- α -hydroxyisobutyrate system [57].

Most frequently used complexing agents in CE separations of metals are simple organic acids, such as formic, acetic, lactic, α -hydroxyisobutyric (HIBA), glycolic, oxalic, malonic, tartaric, succinic and citric. Note that they are essentially the same agents as commonly employed in IC [58]. However, the addition of a stronger (di-, or polyvalent) com-

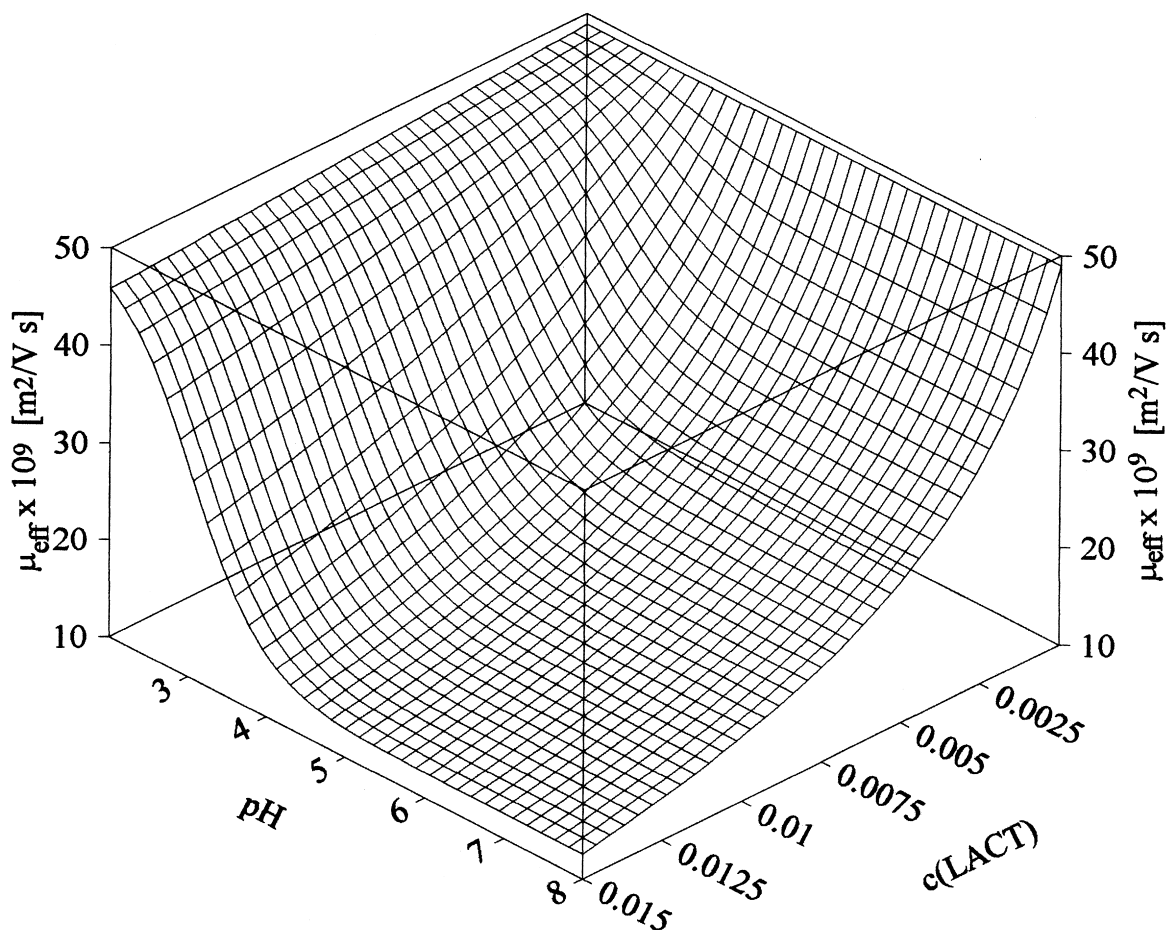


Fig. 3. Dependence of the effective mobility of the Cu^{2+} ion on the pH value and total lactate concentration. Stability and dissociation constants from [51], ionic mobilities of charged complexes estimated from the Jokl's equation [16,52].

plexing ligand will manifest more markedly in CE than in IC because of the formation (in many cases) of anionic complexes. These complexes do not take part in the ion-exchange retention process in IC, but may contribute significantly to the migration process in CE. Therefore, relatively weak complexing agents (typically lactate, which was also used by Hjertén [59] in his pioneer's work) are utilized for fine adjustment of operational conditions in CE, whereas tartrate or citrate are most typical components of mobile phases in IC.

Monovalent cations of alkaline metals and ammonia do not form complexes with the above mentioned 'classical' complexing agents. For manipulation of their effective mobilities, electroneutral

ligands, such as linear [60] or, more often, cyclic polyethers are used, from which 18-crown-6 ether is most convenient for an improvement of the mutual separation of the K^+ and NH_4^+ ions. Dependencies between effective mobilities and the concentration of the complexing agent clearly demonstrate that 15-crown-5 ether also effectively decreases the mobilities of alkaline cations, especially Na^+ and K^+ cations. A modified imidazole BGE containing 15-crown-5 ether, oxalic acid or dipicolinic acid as complexing agents allowed the determination of ammonium, alkaline and alkaline-earth trace cations in samples with a calcium, sodium or magnesium matrix [61].

Alkaline earth metal cations [62] and also uranium

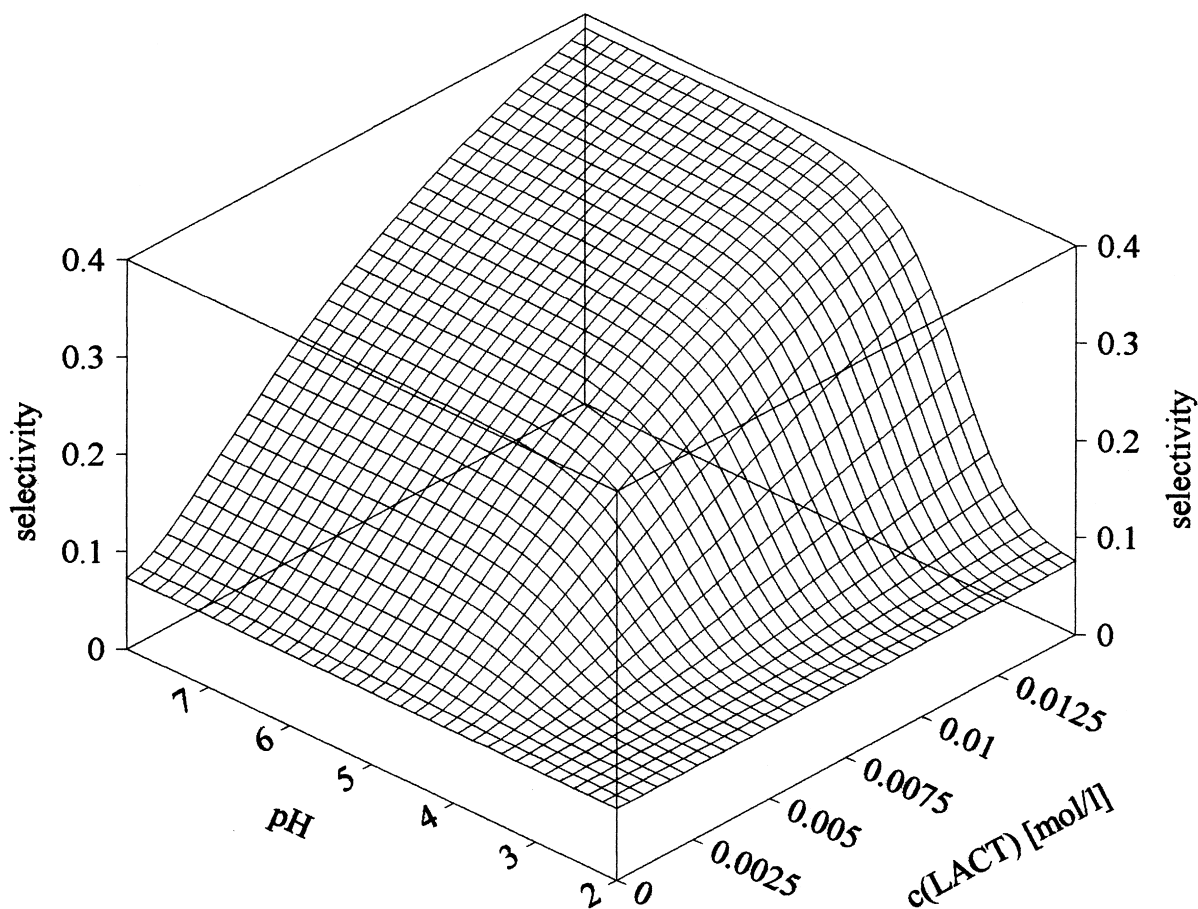


Fig. 4. Dependence of selectivity (relative difference of effective mobilities) on the pH value and total lactate concentration.

(VI) and lanthanides [63] were successfully separated in the presence of more voluminous ligands, such as arsenazo III or sulfonazo III. Noble metals and platinum-group metals can be separated in the form of their anionic chloride [64] or cyanide [65] complexes in anionic separation mode. This approach was used in ITP, where a suitable agent was added to the terminating electrolyte. Hirokawa et al. [66] applied bidirectional ITP to separate eighteen metal cations using ethylenediamine tetraacetic acid (EDTA) as complexing agent. Alkaline and alkaline earth metals were recovered as cations, whereas heavy metals and lanthanides were recovered as chelate anions.

Lanthanides have very similar radii to each other and very similar ionic mobilities [67], it is therefore very difficult to separate them simultaneously. The

addition of HIBA, the very popular agent in the chemistry of rare earths, allows separation of all lanthanides by ITP (first published by Nakatsuka et al. [68]) as well as by zone electrophoresis [69] and CZE [70]. Especially in analytical chemistry of rare earths, it is possible to show that modern CZE systems employing HIBA as complexing agent could be relatively easily derived from older ITP systems, in which the same agent was used years before.

Some operational systems for the CE separations of metal ions are listed in Table 1.

An effective mobility of anions can be governed using complex-forming equilibria in a similar way to that of cations adding a suitable agent capable of forming complexes with the separated anions. The presence of Cd^{2+} , for example, may strongly influence a migration behaviour of halides in zone

Table 1
CE separations of metal ions

Analyte	Separation mode	Operational solution(s)	Detection	Application, matrix	Ref.
Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺	CZE	5 mM Imidazole, pH 4.5	Indirect UV	Mineral waters, apple vinegar	[71]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Li ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺	CZE	0.5 mM Cerium(III) sulphate, 2 mM 18-Crown-6,	Indirect fluorimetry	Rain water, cola beverage	[72]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Li ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺	CZE	4 mM Copper(II) sulphate, 4 mM 18-Crown-6, 4 mM formic acid	Indirect UV		[73]
Na ⁺ , Li ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺	ITP	LE: 10 mM NH ₄ ⁺ -ACES, 3 mM HIDA, 0.1% HEC, pH 6.8 TE: HIS, pH 6.3	Conductivity	Human blood, mineral water, drinking water	[74]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Li ⁺ , Cs ⁺ , Rb ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺	CZE	30 mM Creatidine, 12 mM HCl, 1.5 mM tartrate, pH 5.0, in 50% PEG	Indirect UV		[50]
Na ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺	ITP	LE: 10 mM NH ₄ OH, 1 mM CyDTA, succinic acid, pH 5.7 TE: 10 mM β-Alanine, acetic acid, pH 4.4	Potentiometry		[75]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺ , Zn ²⁺	CZE	10 mM Histidine, 2 mM 18-crown-6, 8 mM lactic acid, pH 4.0	Indirect UV	Air particulate matters	[76]
K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺	CZE	2.5 mM Copper(II) nitrate, 1 mM fumaric acid, 5 mM ethylenediamine, pH 8.5	Indirect UV		[77]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺	CZE	5.05 mM Methylbenzylamine, 3.78 mM, 18-crown-6, 13.06 mM HIBA	Indirect UV	Atmospheric aerosol	[78]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Li ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺	CZE	10 mM Imidazole, 2.5 mM 18-crown-6, pH 4.5	Indirect UV		[79]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Li ⁺ , Cs ⁺ , Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺	ITP	LE: 5 mM <i>p</i> -toluenesulfonic acid, 20–40 mM 18-crown-6, 0.01% Triton TE: 5 mM TBA–Br	Potentiometry		[80]
K ⁺	ITP	LE: 10 mM HCl, 3 mM 18-crown-6 TE: 10 mM TBA–Cl	Conductivity	Lysine	[81]
NH ₄ ⁺ , K ⁺	ITP	LE: 5 mM HCl, 30% glycerol, 3 mM, 3 mM 18-crown-6 TE: 10 mM lithium sulphate	Potentiometry	Sea water	[82]
K ⁺	ITP	LE: 5 mM CsOH, 2 mM 18-crown-6, 0.01% HPMC, 70% methanol TE: 5 mM TBA–Br, 0.01% HPMC, 70% methanol	Potentiometry	Sea water	[83]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺	CZE	6 mM Aminopyridine, 2.7 mM H ₂ CrO ₄ , 0.03 mM CTMA–Br, pH 8	Indirect UV	Tap water, rain water, Process water	[84]
NH ₄ ⁺ , K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺	ITP	LE: 1.25 mM EDA, 3.75 mM acetic acid, 50 mM 18-crown-6, 0.1% HEC, pH 5.0 TE: 3 mM acetic acid	Conductivity	Rain water	[61]
K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺ , Mn ²⁺	CZE	5 mM Imidazole, 6.5 mM HIBA, 0.53 mM 18-crown-6, 20% methanol, pH 4.5	Indirect UV	Green tea infusion	[85]
K ⁺ , Na ⁺ , Li ⁺ , Ca ²⁺ , Mg ²⁺	CZE	4 mM Copper(II) sulphate, 4 mM 18-crown-6, 4 mM formic acid, pH 3.1	Indirect UV	Mineral waters	[86]
Sr ²⁺	ITP	LE: 10 mM HCl, 0.1% HEC, Tris, pH 9 TE: 10 mM Na hexanoate	Potentiometry	Sea water	[87]
Ca ²⁺ , Mg ²⁺ , Sr ²⁺ , Ba ²⁺ , Mn ²⁺ , Fe ²⁺ , Co ²⁺ , Ni ²⁺ , Zn ²⁺ , Pb ²⁺ , lanthanides	ITP	LE: 20 mM NH ₄ ⁺ , 10 mM HIBA, pH 4.8 TE: 10 mM carnitine hydrochloride	PIXE		[88]
Sr ²⁺ , Ba ²⁺	CZE	0.3 mM Sulfonazo III, 20 mM MES, 10 mM Tris, pH 6.2	Direct photometry	Mineral waters	[62]

Table 1. Continued

Analyte	Separation mode	Operational solution(s)	Detection	Application, matrix	Ref.
Fe ²⁺ , Cu ²⁺ , Ni ²⁺ , Cd ²⁺ , Co ²⁺ , Zn ²⁺ , Pb ²⁺ , Mn ²⁺	ITP	LE: 20 mM Na ⁺ (K ⁺) TE: 5 mM acetic acid	Conductivity	River water, serum	[89]
Fe ²⁺ , Cu ²⁺ , Ni ²⁺ , Zn ²⁺ , Pb ²⁺ Mn ²⁺ , Cd ²⁺	CZE ITP	5 mM Creatidine, 15 mM HIBA, pH 3.45 LE: 20 mM Na acetate, 0.4 mM xylenol orange, 0.1% HEC, pH 5.0 TE: 1 mM HNO ₃	Indirect UV Conductivity, direct photometry	Tap water	[90] [91]
Cu ²⁺ , Co ²⁺ , Ag ⁺ , Zn ²⁺ , Cd ²⁺ , Hg ²⁺	ITP (anionic)	LE: 5 mM HCl, Tris, PVA, pH 7.5 TE: 10 mM KCN, 5 mM Ba(OH) ₂	Potentiometry		[92]
Cd ²⁺ , Hg ²⁺ , Zn ²⁺ , Pb ²⁺ , Mn ²⁺ , Fe ³⁺ , In ³⁺ , Ga ³⁺	ITP (anionic)	LE: 5 mM HClO ₄ in dimethylformamide TE: 10 mM HCl in dimethylformamide	Potentiometry		[93]
Fe ³⁺ , Ga ³⁺	ITP (anionic)	LE: 5 mM HCl, 3 mM β-alanine in 45% acetone TE: 5 mM EDTA	Potentiometry		[94]
Pb ²⁺ , Mn ²⁺ , Ni ²⁺ , Zn ²⁺ , Cu ²⁺	ITP (anionic)	LE: 5 mM HCl, 2 mM β-alanine in 45% acetone TE: 5 mM NaEDTA	Potentiometry		[95]
La ³⁺ –Lu ³⁺	ITP	LE: 27 mM KOH, 15 mM HIBA, 0.0025% PVA, pH 4.92 TE: β-alanine, pH 4.00	Potentiometry		[68]
La ³⁺ , Ce ³⁺ , Pr ³⁺ , Nd ³⁺	ITP	LE: 30 mM KOH, 15 mM HIBA 0.05% PVA, pH 5.5 TE: 10 mM β-alanine, pH 5	Conductivity	Polishing powders	[96]
La ³⁺ , Ce ⁴⁺ , Pr ³⁺ , Nd ³⁺	CZE	10 mM Phosphate buffer, 8 mM HIBA, 0.064 mM Co ²⁺	On-column chemiluminescence		[97]
La ³⁺ –Lu ³⁺ , Y ³⁺	ITP	LE: 20 mM NH ₄ ⁺ , 7.5 mM HIBA, 2.0 mM malonic acid, pH 4.80 TE: 20 mM carnitine hydrochloride	conductivity	Didym	[98]
	CZE	30 mM Creatidine, 4 mM HIBA, 0.4 mM malonic acid, pH 4.8	Indirect UV	Didym	
Gd ³⁺ , Eu ³⁺ , Sm ³⁺ , Pr ³⁺ , Ce ³⁺ , La ³⁺	ITP (anionic)	LE: 10 mM HCl TE: 5 mM EDTA	Potentiometry		[99]
U(VI), lanthanides	CZE	15 mM Tartaric acid (acetic, citric), 0.025 arsenazo III, 20 mM Tris, pH 4.1	Direct photometry		[63]
Ni ²⁺ , Pt ²⁺ , Ag ⁺ , Au ⁺	ITP (anionic)	LE: 6 mM HCl, Tris, zephiramine, 0.01% PVA in 20% acetonitrile TE: 10 mM KCN, 50 mM Ba(OH) ₂	Potentiometry		[100]
Au ³⁺ , (Pd ²⁺ , Pt ⁴⁺)	CZE (anionic)	100 mM HCl, 400 mM NaCl through liquid membranes	Direct UV	Study of AuCl ₄ ⁻ transport	[64]
Pd ²⁺ , Pt ⁴⁺ , Ir ⁴⁺ , Au ³⁺ , Rh ³⁺	CZE (anionic)	50 mM Na acetate, 30 mM HClO ₄ , 50 mM NaBr, pH 3	Direct UV		[101]

LE=leading electrolyte, TE=terminating electrolyte, ACES=*N*-(2-acetamido)-2-aminoethane sulphonic acid, HIDA=*N*-hydroxyethylimido-diacetic acid, HEC=hydroxyethylcellulose, HIS=histidine, PEG=poly (ethylene glycol), CyDTA=1,2-cyclohexanediamine-*N,N,N',N'*-tetraacetic acid, TBA=tetrabutylammonium, HPMC=hydroxypropyl methylcellulose, CTMA=cetyltrimethylammonium, EDA=ethylenediamine, PIXE=particle-induced X-ray emission, Tris=tris(hydroxymethyl)aminomethane, MES=2-morpholinoethanesulphonic acid, PVA=poly(vinyl alcohol).

electrophoresis to the degree that they migrate towards the cathode [102]. The first application of complexation in ITP was described by Boček et al. [103] who used Cd²⁺ for improving the separation of

fast migrating anions, such as sulphates and halides. Typical agents employed for complexation with inorganic anions are divalent metal cations (Ca²⁺, Mg²⁺, Cd²⁺) or organic cation bistrispropane. Some

Table 2
CE separations of inorganic anions employing complex-forming equilibria

Analyte	Separation mode	Operational solution(s)	Detection	Application, matrix	Ref.
Cl^- , Br^- , I^- , SO_4^{2-}	ITP	LE: 2–10 mM cadmium(II) nitrate TE: 10 mM citric acid	Electric gradient	Mineral waters,	[103]
Cl^- , NO_3^- , SO_4^{2-}	ITP	LE: 5 mM $\text{Ca}(\text{OH})_2$ TE: 1 mM formic acid	Conductivity	Well waters, surface waters	[104]
NO_3^- , SO_4^{2-} , NO_2^- , H_2PO_4^-	ITP	LE: 10 mM Cl^- , 2.5 mM Mg^{2+} , β -alanine, 0.2% HEC, pH 3.50 TE: 5 mM citric acid	Conductivity	Study of absorption of inorganic ions by roots	[105]
NO_3^- , SO_4^{2-} , NO_2^- , H_2PO_4^-	ITP	LE: 10 mM Cl^- , 2.5 mM Mg^{2+} (Ca^{2+} , BTP^{2+}), β -alanine, pH 2.9–3.6 TE: 5 mM citrate	Conductivity	Plant material	[106]
Phosphate, arsenate, germanate, silicate, molybdate	ITP	LE: 10 mM Cl^- , 3 mM Mg^{2+} , Tris, 0.01% PVA, pH 8.5 in 5% methanol	Potentiometry		[107]

BTP^{2+} = bistrispropane.

examples of the operational systems for the separations of anions are presented in Table 2.

Complexation of the separated anions with suitable agents has frequently been used in ITP. In CZE, on the other hand, the separation efficiency is usually sufficient to separate all common anions without such an auxiliary effect as complexation.

If the metal cations are present in the sample as matrix elements, they can interfere with the determination of some anions due to the complex formation, as was observed for Fe^{3+} ions in ITP [108] as well as in CZE [109]. This disturbing effect was eliminated by the addition of masking agent (EDTA [108], thiocyanate [109]). Metal complexation was used also for the CE separation and improved detection of organic substances (tetracyclines) [110].

Electrophoretic measurements have been traditionally used for investigations of complex-forming equilibria and measurements of stability constants [111]. In principle, the stability constants (binding constants, formation constants) can be calculated from the relationship between ligand concentration and a measured electrophoretic mobility. In its general form [Eq. (15)] this relationship is rather complicated and a great amount of experimental data is needed for reliable calculations. If the stability of individual complexes (ML_{i-1} , ML_i) is sufficiently different, the separate part of the μ_{eff} vs. ligand

concentration dependence corresponding to the $\text{ML}_{i-1} \rightarrow \text{ML}_i$ reaction is described as follows [48]:

$$\mu_{\text{eff}} = \frac{\mu_{\text{ML}_{i-1}} \beta_{i-1} [\text{L}]^{i-1} + \mu_{\text{ML}_i} \beta_i [\text{L}]^i}{\beta_{i-1} [\text{L}]^{i-1} + \beta_i [\text{L}]^i} \quad (18)$$

Ionic mobilities μ_{ML_i} can be calculated from the Jokl's equation [52] and the value of the stability constant is then found graphically on the $\log [\text{L}]$ axis as the point where [52]

$$\mu_{\text{eff}} = \frac{\mu_{\text{ML}_{i-1}} + \mu_{\text{ML}_i}}{2} \quad (19)$$

The separate parts of the μ_{eff} vs. ligand concentration dependence may be expressed also in a logarithmic form [112]:

$$\begin{aligned} f(\mu_{\text{eff}}) &= \log \frac{\mu_{\text{ML}_{i-1}} - \mu_{\text{eff}}}{\mu_{\text{eff}} - \mu_{\text{ML}_i}} \\ &= \log \frac{\beta_i}{\beta_{i-1}} + \log [\text{L}] \end{aligned} \quad (20)$$

from which the stability constants can be determined graphically. In the simplest case (unfortunately not typical for real systems) when only one kind of complex with zero charge is formed (e.g. interaction of divalent cation, M^{2+} , with divalent ligand, L^{2-} , at low ligand concentrations), Eqs. (15) and (18) can be simplified to [86]:

$$\frac{1}{\mu_{\text{eff}}} = \frac{1}{\mu_{\text{M}}} (1 + \beta_1[\text{L}]) \quad (21)$$

From the dependencies of reciprocal effective mobilities on the ligand concentration, the stability constants can be easily determined. Both graphical as well as numerical approaches were compared for an evaluation of the $1/\mu_{\text{eff}}$ vs. (ligand concentration) dependencies in the study of metal-sulphate complexes [86].

Yang et al. [49] also used Eq. (21) to estimate stability constants of the M^{2+} -HIBA complexes assuming that ionic mobilities of the charged complexes (ML^+) are negligible in comparison with the mobility of free metal cation. Although an agreement between the determined stability constants and literature values was satisfactory, this approach seems to be not quite correct and generally applicable, because it was shown that a contribution of the charged complexes to the effective mobility must not be neglected [98]. In Fig. 5a, the dependencies of reciprocal effective mobility on the ligand concentration are calculated for some model systems and compared with experimental dependencies for Cu^{2+} -lactate and Ce^{3+} -lactate systems (Fig. 5b).

Because in real metal–ligand systems several complexes are formed with unknown ionic mobilities, a simple graphical approach is seldom utilizable and more sophisticated numerical methods employing computers are recommended. A general least-squares CELET program was developed for an evaluation of the CZE data [17], which is able to treat the data of effective mobilities as a function of pH, ligand or metal concentration up to quaternary systems.

Relationships between various expressions that can be used to calculate equilibrium constants from CE data together with advantages and limitations of various approaches are discussed in [112].

CZE was also applied to measure stability constants of metal cations with neutral ligands (18-crown-6) [79]. The stability constants were determined from the abscissa of the inflection point on the μ_{eff} vs. (ligand concentration) curve. Stover [81] determined stability constants for 18-crown-6 with alkaline metals from ITP data using a computer simulation.

Complex-forming equilibria were studied iso-

tachophoretically by Hirokawa et al. [113–117]. The migration model in ITP is more complex than in CZE because of the varying composition of sample zones. The equations for effective mobilities hold true similarly as in CZE, but concentrations of compounds as well as actual pH in the zones have to be derived from the composition of the leading electrolyte. The computer simulation was used to obtain compositions of complexes formed in the sample zones, stability constants and mobilities of complex ions for the systems M^{2+} -acetate [113], M^{2+} -tartrate and M^{2+} -citrate [113,114], M^{2+} -glycolate, lactate, β -hydroxybutyrate, α -hydroxybutyrate, α -hydroxyisobutyrate [115], lanthanide-acetate, lanthanide- α -hydroxyisobutyrate [116], lanthanide-2-ethylbutyrate and lanthanide-malonate [117]. Recently, Hirokawa and Mao [118] suggested a new ITP method to measure stability constants of lanthanide–EDTA complexes, based on the relationship between the stability of complexes and the bleeding effect. The stability constants were determined from the linear dependence $\log K$ vs. \log (zone reduction).

5. Other side equilibria

Electrophoretic mobility can be influenced employing some other side equilibria, such as ion pairing or ion association, host–guest interaction, etc., that are, however, more effective in separations of organic substances and less frequently are used to manipulate the mobility of inorganic ions. Recently, Shelton et al. [119] reviewed applications of ion pairing equilibria in CZE and presented the fundamental theory of ion-interactions as applied to CZE separations. Conventional HPLC ion pairing agents were used for the CZE separations of inorganic complexes. Buchberger and Haddad [120] used hexamethonium bromide as a means to simultaneously control EOF and act as ion pairing agent for metallo-cyanide complexes. Tetraethylammonium perchlorate and tetrabutylammonium hexafluorophosphate were used to alter selectivity for eleven inorganic cations in non-aqueous media [121].

More often ion pairing equilibria were utilized in ITP separations of inorganic anions. Quaternary

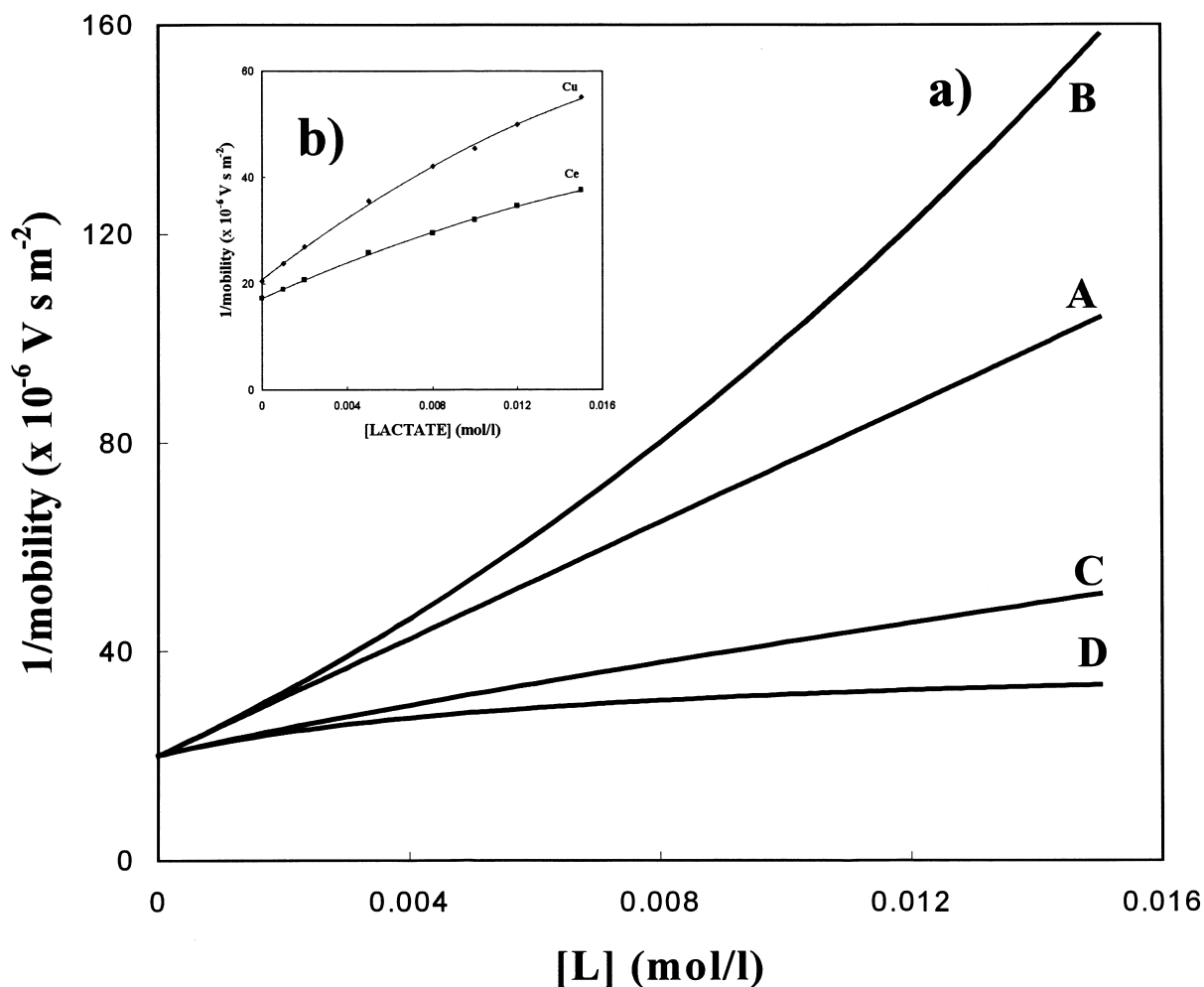


Fig. 5. (a) Dependencies of reciprocal effective mobilities of metal cations on the concentration of complexing agent for various model systems. (A) only one complex is formed with zero mobility (e.g. neutral complex), (B) more complexes are formed, all of them have negligible mobilities, (C) more complexes are formed, their mobilities estimated using the Jokl's equation (system Cu^{2+} -lactate), (D) one complex is formed (ML^+ type) with its mobility estimated from the Jokl's equation. (b) Experimental dependencies of reciprocal effective mobilities of the Cu^{2+} and Ce^{3+} cations on the lactate concentration. BGE contained 20 mM Na^+ , 0–15 mM lactate, pH 6 ± 0.05 adjusted with perchloric acid (data from [17]).

ammonium salts (tetradecyldimethylbenzylammonium) were used to facilitate the ITP separation of bromide and iodide in an aqueous electrolyte system [122]. An interaction with α -cyclodextrin was suggested for improving the separation of iodide from chloride [123]. Yoshida et al. [124] demonstrated a decrease of the effective mobilities of molybdate, tungstate, and vanadate in the presence of quaternary ammonium ions, such as tetradecyl-

dimethylbenzylammonium and cetyltrimethylammonium, whereas a short-chain tetraethylammonium ion had no influence on the effective mobilities. A migration behaviour of some phosphorus oxoanions was studied in the presence of tetrabutylammonium and cetyltrimethylammonium salts in aqueous and mixed water–methanol electrolyte systems [125].

Highly charged metal chelates of Al(III), Co(III), Cr(III) and Fe(III) ions with 2,2'-dihydroxy-

azobezene-5,5'-disulphonate were separated by CZE utilizing ion association of the anionic metal chelates with tetrabutylammonium cation [126].

The use of the ion association reaction in CZE seems to be promising not only for separations of metal chelates [126,127] but also inorganic and organic anions [128,129]. A multipoint ion association with divalent quaternary ammonium ions (e.g. 1,5-bis (triethylammonium) pentane) was used for the CZE separation of aromatic anions [130,131]. The change (decrease) in mobility of anions due to the ion association was also used for calculations of ion association constants [130]. The ion association constants were calculated from the dependencies of effective mobility on the concentration of the quaternary ammonium ion using a non-linear least-squares method.

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