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Separation of organic acids by capillary zone electrophoresis in buffers containing divalent metal cations

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Abstract

The addition of divalent metal cations to the background electrolyte influences the migration time of organic acids that are involved in complexation equilibria with the metals. This effect derives from a change in charge distribution and represent a means of manipulation of selectivity. In the present investigation, we evaluated the effects produced on the separation of a test mixture, composed of aromatic and aliphatic acids, by a series of divalent cations added to the background electrolyte. The use of a coated capillary allowed us to exclude the influence of wall effects on the separation.

Keywords: Background electrolyte composition; Organic acids; Metal cations

1. Introduction

Capillary zone electrophoresis (CZE) is becoming an important tool in the separation of polar charged molecules that offers an analytical method which is, in many cases, complementary to high-performance liquid chromatography (HPLC) and gas chromatography. The growing interest in this technique, that derives from its high efficiency and separation power, has led to a rapid development of various separation modes. Usually, the separation of the analytes by capillary electrophoresis (CE) is based on their differential electrophoretic mobility which is, in turn, strongly influenced by the background electrolyte (BGE) composition. For example pH, type of buffer, ionic strength type, concentration of organic cosolvents and buffer additives can have significant effects on migration behavior in CE. The increase in selectivity which is an important goal in all analytical techniques has been achieved through the addition of various additives to BGE. The application of CE to uncharged compounds required the development of electrokinetic chromatography [1,2]. This represents a brilliant solution to the problem of separation of uncharged compounds, as it combines an electrophoretic separation process with a partitioning process. Several other kinds of complexations have been also used in order to maximize separation selectivity in CZE. An example is the separation of racemates through the addition of cyclodextrins to the background electrolyte [3]. Chiral separations in CE have recently found a large application in the separation of a variety of pharmaceutical compounds.

A significant number of charged molecules with biological relevance such as amino acids and amino acid derivatives, including peptides and enzymes, and carbohydrates form complexes with metal ions. Complexation is a potential means of manipulation of the selectivity, as the species involved in complexation equilibria with metals undergo a change in charge distribution that may lead to a significant

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variation on electrophoretic mobility. It has been demonstrated that Cd^{2+} added to BGE can influence the effective mobility of anions (chloride, sulfate and nitrate) through complexation, changing the selectivity of the separation. Metallochromic ligands have been separated in BGEs containing metal ions exerting a potential influence on the separation efficiency and selectivity. In the field of biopolymer separations, the addition of Zn^{2+} and Cu^{2+} was used to optimize separations of oligonucleotides and peptides [4,5]. Several articles reported the use of this principle in separating cations [6,7]. However, the complementary principle that involves the use of complexation reactions to separate organic anions has been applied only to a limited extent. In a recent paper, Lalljie et al. [8] showed that the addition of Ca^{2+} to the buffer induces an acceptable separation of formate and tartrate via selective retardation of tartrate caused by its complexation with the cation. The addition of divalent cations to the buffer was reported to influence the migration time and the resolution in the separation of alkenesulfonate and arylsulfate [9]. This effect derives from a change in the electroosmotic flow (EOF) and is due to the cation-exchange between the cation additive and the silanols on the silica wall. More generally, however, the use of metal ions in uncoated capillaries negatively affects the separation as a result of a complexation between the free coordination sites of the sorbed metal and the ligand. Any complexation–decomplexation of the solute with the metal adsorbed on capillary wall would have a detrimental influence on the separation efficiency as reported by several authors [10,11].

In the present investigation, we evaluate the effect on the separation of a test mixture, composed of aromatic and aliphatic acids, by a series of divalent cations added to BGE. Several buffer systems containing different cations have been employed in the separations in order to evaluate the relationship between the structure of the anion and its ability to form complexes with different metals at various pH. All the separations were carried out in a new type of capillary column coated with linear poly[(acryloylaminoethoxy)ethyl]-β-D-glucopyranose (AEG)] [12]. The linear polymer formed by radical polymerization of this novel monomer suppresses EOF to a negligible value while the hydrophilic glucose substituent efficiently shields the silanol groups on the capillary surface. The silanols deactivation allowed us to exclude the influence of the wall effects on the separation leading to a highly reproducible electrophoretic behavior of the acid–metal complexes under study.

2. Experimental

2.1. Instruments and reagents

All reagents used were of analytical grade. 3-(N-Morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino)ethanesulfonic acid (MES), tris(hydroxymethyl)aminoethane (Tris) and acetic acid were purchased from Sigma (St. Louis, MO, USA). Phthalic acid, benzoic acid, *p*-toluenesulfonic acid, caffeic acid, nicotinic acid and N-acryloylglycolic acid were from Aldrich (Steinheim, Germany). N-Acryloylglycine (Immobiline p*K* 3.6) and N-acryloyl-γ-aminobutyric acid were purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). Ammonium peroxydisulphate and N,N,N′,N′-tetramethylethylenediamine were obtained from Bio-Rad Labs. (Richmond, CA, USA). N-(Acryloylaminoethoxyethanol)-β-D-glucopyranoside was synthesized as described by Chiari et al. [12]. Salts used included reagent grade $Ni(NO_3)_2 \cdot 6H_2O$, $Cu(SO)_4 \cdot 5H_2O$, $MgSO_4 \cdot H_2O$, $CaSO_4 \cdot 2H_2O$, $Co(NO_3)_2 \cdot 6H_2O$ and $MnSO_4 \cdot H_2O$ purchased from Fluka (Buchs, Switzerland) and were used as received. All experiments were performed on a Waters Quanta 4000 capillary electrophoresis system, purchased from Millipore (Milford, MA, USA), equipped with Maxima 820 data evaluation software. For the experiments, 50 μm I.D.×370 μm O.D. capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) and coated as described elsewhere were used.

2.2. Coating procedure

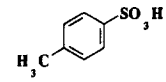
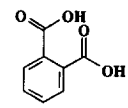
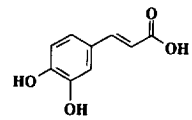
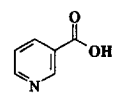
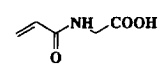
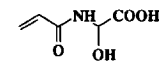
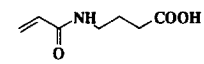
Capillaries were coated with linear poly(AEG) bonded through methacryloxypropylsilyl moieties prepared as described in [13]. The capillary was activated with γ-methacryloxypropyl bonded to the

hydride-modified support is formed by reacting triethoxysilane with a silica substrate in the presence of water and hydrochloric acid using dioxane as the solvent. The γ-methacryloxypropyl group residue was linked to the hydride-modified substrate by hydrosilation of allylmethacrylate in the presence of a Pt catalyst. Poly(AEG) coated capillaries were obtained by filling γ-methacryloxypropyl activated capillaries with a 15% water solution of N-(acryloylaminoethoxyethanol)-β-D-glucopyranoside containing the appropriate amount of catalyst (1 μl and 1 μl of 40% ammonium peroxydisulphate per ml of gelling solution) and degassed under vacuum (20 mmHg; 1 mmHg=133.322 Pa) for 40 min. Polymerization was allowed to proceed overnight at room temperature and then the capillary was emptied with an HPLC pump.

2.3. Separation condition

The mixture of acids was injected in a poly(AEG) coated capillary (37 cm total length, 30 cm to the detector) using three different buffer systems as running buffer: acetate–Tris, 50 m*M* pH 4.2, MES–Tris, 50 m*M* pH 5.5 and MOPS–Na 50 m*M* pH 7.0 containing, in some experiments, 1 m*M* Ni^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+} and Mn^{2+}. The samples were loaded by hydrostatic pressure and the separations were carried out at room temperature. The detector was set at 214 nm.

Table 1

Name	Formula	pK_a
1 Benzoic acid		4.1
2 *p*-Toluenesulfonic acid		
3 Phthalic acid		3.0 5.4
4 Caffeic acid		4.3 12.5 9.9
5 Nicotinic acid		2.1 4.8
6 N-Acryloylglycine		3.6
7 N-Acryloyl glycolic acid		3.1
8 N-Acryloyl-γ-aminobutryic acid		4.7

3. Results and discussion

The use of metal ions for the achievement of high selectivity is well known in liquid chromatography (LC). In ligand-exchange chromatography, for example, metal ions are immobilized on a stationary phase. An ion-exchange or a chelating resin is employed for metal binding. In this technique, a selectivity enhancement has been achieved in the separation of nitrogenous bases, amino acids [14] and carboxylic acids [15], but the system was characterized by poor efficiency and severe band asymmetry. These problems arise from the slow rates of desorption of ligands strongly bonded to metals [16] and from the slow rate of diffusion in the resin. An interesting approach that overcomes the efficiency problems involves the addition of metal ions to the mobile phase for selective complexation with particular entities. Nickel, for instance, has been used to complex with *o*-aminophenol [17] while complexation with transition metal ions was proposed to control retention in ion-exchange chromatography [18]. In other examples, the complexation was used to enhance the analyte hydrophilicity, thus reducing its retention in reversed-phase LC [19]. Alternatively, the metal ion may function as a species in which ion-pair or ligand association with solute can occur, thus enhancing hydrophobicity and retention in reversed-phase LC. In recent years, there has been great interest in the use of CE and the addition of various additives to the mobile phase has led to new developments which broadened the range of compounds separated by this technique. The aim of the present study was to evaluate the effects produced by the direct addition of divalent cations to the back-

ground electrolyte on the electrophoretic mobility and the migration behavior of organic acids. Table 1 lists the chemical structure of compounds selected as test substances. These compounds provide a wide variation in pK_a, hydrophobicity and functional groups responsible for various degrees of complexation. The influence of different metal ions on transit time and selectivity can be seen in Fig. 1 where the electrophoretic mobility in MES–Tris buffer at pH 5.5 is represented as a function of the cation contained in BGE. Mobility was calculated from Eq. (1):

$$\mu = lL/Vt \quad (1)$$

were l is the distance from the inlet to the window, L is the capillary length, V is the applied voltage and t the transit time. The metal concentration was 1 m*M*. Fig. 1 illustrates that the most significant changes in the mobility are caused by the addition of Cu^{2+}, producing a general decrease in analyte mobility. The effects caused by the addition of Ni^{2+} are less pronounced but still sufficient to produce significant change in the separation profile. Three of the test mixture components, benzoic acid, N-acryloylglycine and N-acryloylglycolic acid, change their mobility in the presence of the cation in such a way to be easily separated. Figs. 2–4 show some representative separations carried out at different pHs in the presence of Ni^{2+} or Cu^{2+} salts and in a buffer of the same composition but not containing metal additives. At pH 7.0, the addition of 1 m*M* Ni^{2+} (Fig. 2, trace B) improves the selectivity, separating nicotinic acid from benzoic and sulfonic acids. At pH 5.5, a selective shift in the position of nicotinic and phthalic acid is determined by the addition of Ni^{2+} (Fig. 3, trace B) while Cu^{2+} affects the migration behavior of a larger number of analytes in a most significant way (Fig. 3, trace C). At pH 4.2, the analyte migration is selectively influenced by the addition of Ni^{2+} (Fig. 4, trace B) and again the analytes involved in the complexation show a strong variation in their net electrophoretic mobility. Finally, in order to establish a relationship between analyte structure and complexation we have performed a systematic study on the selective effects produced by Ni^{2+}, Cu^{2+}, Mg^{2+} and Ca^{2+} at various pH values. The parameter taken into account has

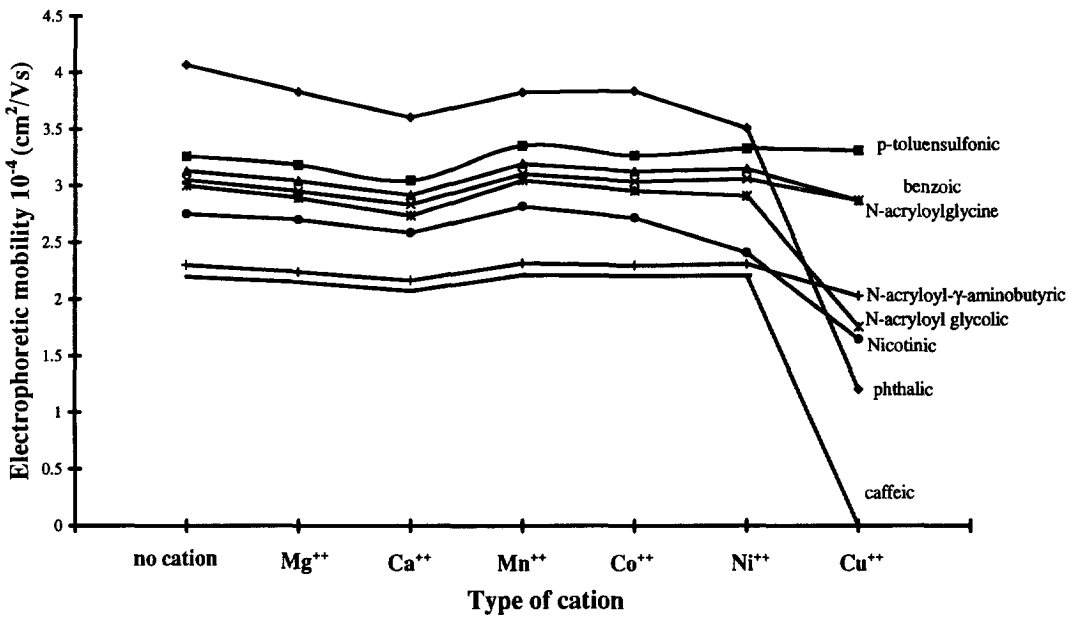


Fig. 1. Electrophoretic mobility of organic acids in 50 m*M* MES–Tris buffer, pH 5.5, containing different cations (1 m*M*). Electrophoretic conditions: capillary coated, 37 cm long (30 to the window); applied potential 15 kV reversed-polarity; electrokinetic injection, 1 s; sample concentration 0.5 m*M*; detector UV, 214 nm.

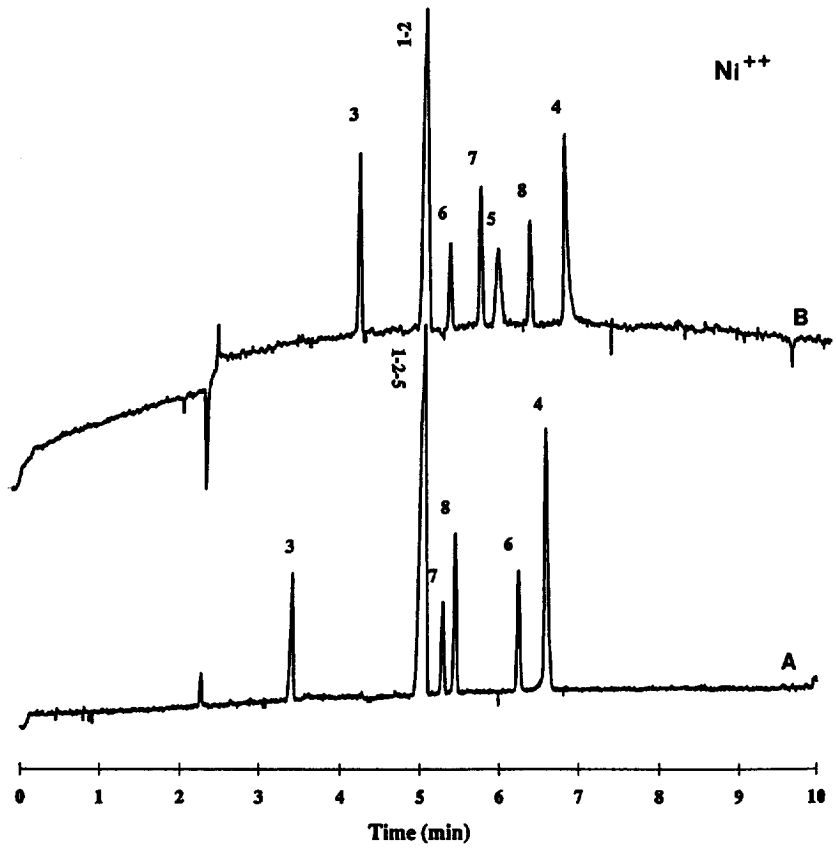


Fig. 2. CZE separation of organic acids carried out in 50 m*M* MOPS–Na buffer, pH 7.0. Trace A: buffer without cation additive. Trace B: buffer containing 1 m*M* $Ni(NO_3)_2$. For peak identification, see Table 1. Applied potential 12 kV, other conditions as in Fig. 1.

been the resolution between two adjacent analytes. Since the pH influences the charge distribution of the acids according to their p*K*, different pairs of analytes have been considered at each pH. As shown in Fig. 5, the addition of Ni^{2+} to acetate buffer at pH 4.2 was found to be beneficial for the separation of the majority of analytes whereas the effect of Cu^{2+} is very pronounced in both positive and negative directions, indicating that a high number of analytes undergo a complexation reaction with this metal ion. Fig. 6 illustrates the resolution changes induced by different cations at pH 5.5. The resolution between benzoic and sulfonic acid (sample 1–2) increases by adding Ni^{2+} while the resolution of the other considered compounds, N-acryloylglycine and N-acryloylglycolic acid (sample 6–7), or benzoic acid and N-acryloylglycine (sample 1–6) is not influenced by the additive. The effects of Cu^{2+} complexation on the resolution of the same pairs of analytes is more pronounced, leading to an enhancement in the separation of samples 1–2 and 6–7 and a decrease for the other pair of compounds.

The strongest retarding effects caused by Cu^{2+} are not unexpected, in view of the strongest binding properties exhibited by this ion in the series of metal ions investigated [20]. It is also interesting to note that with this ion the lowest mobilities are associated with the two acids (caffeic and phthalic) which can form neutral chelates. In the first case, the affinity of the catechol residue for Cu^{2+} is so strong that virtually all the analyte present must be converted into the neutral complex, since its mobility is reduced to zero (Fig. 1) In all other cases, since the metal ions are present in very large excess with respect to the analytes, it is likely that more complex and pH- and buffer-dependent complexation equilib-

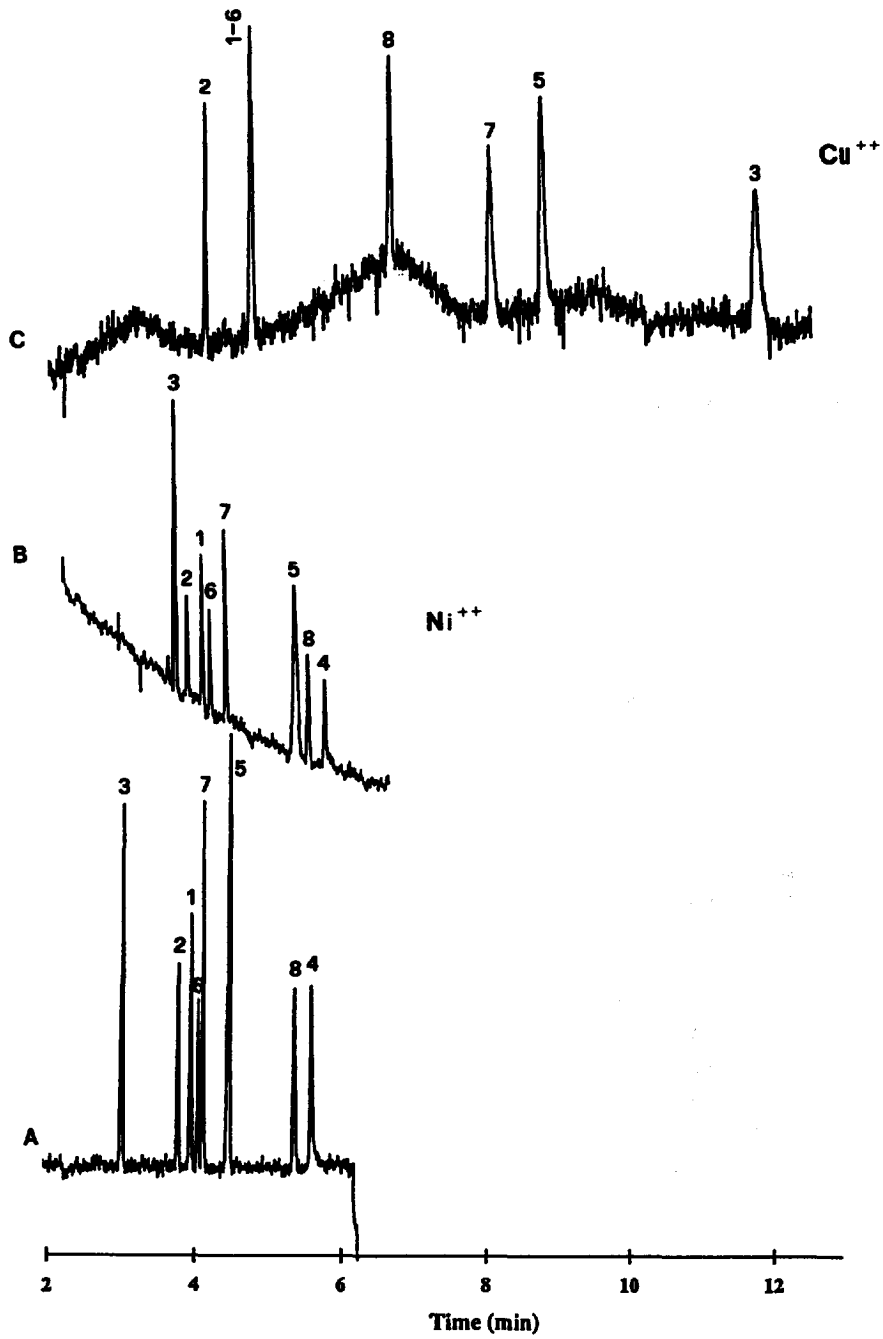


Fig. 3. CZE separation of organic acids carried out in 50 m*M* MES–Tris buffer, pH 5.5. Trace A: buffer without cation additive. Trace B: buffer containing 1 m*M* $Ni(NO_3)_2$. Trace C: buffer containing 1m*M* $CuSO_4$. For peak identification, see Table 1. Other conditions as in Fig. 1.

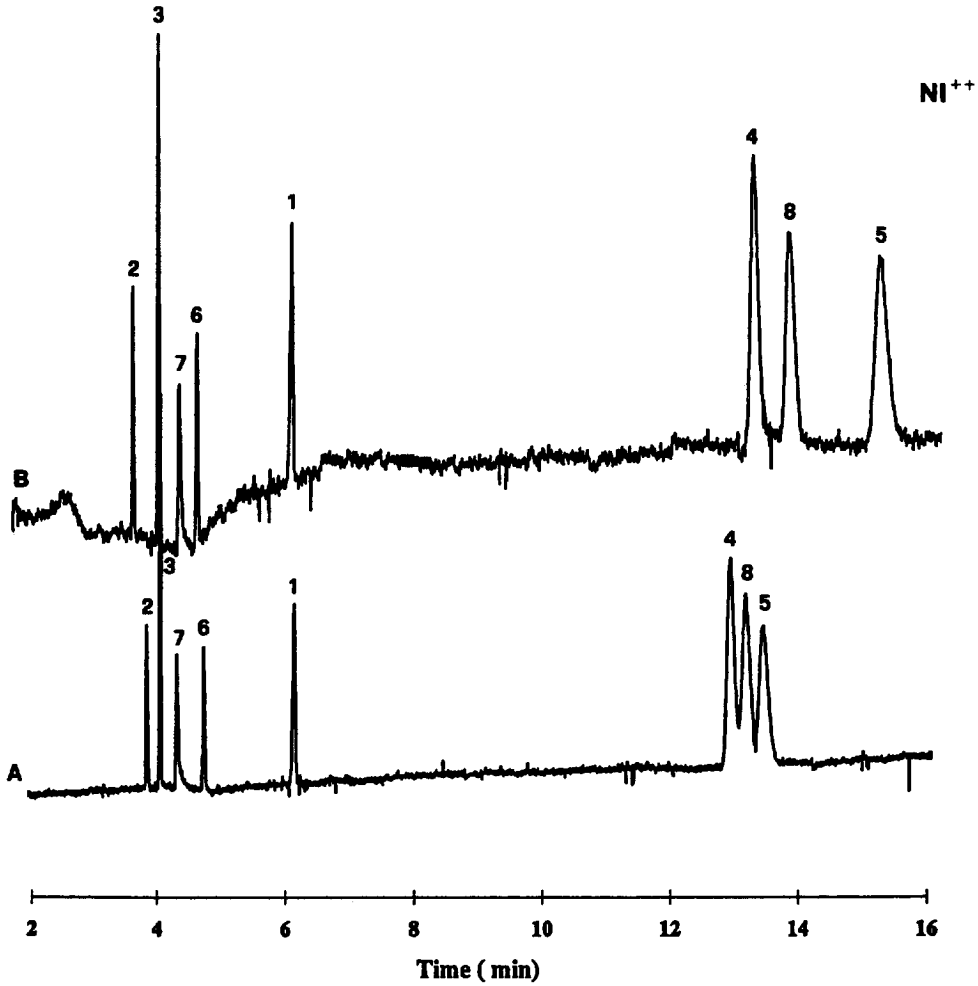


Fig. 4. CZE separation of organic acids carried out in 25 m*M* acetate–Na buffer, pH 4.2. Trace A: buffer without cation additive. Trace B: buffer containing 1 m*M* $Ni(NO_3)_2$. For peak identification, see Table 1. Other conditions as in Fig. 1.

ria will be established for each analyte. Moreover, while the reduction in mobility of the acids is due to the loss of negative charge occurring on metal binding, the observed mobility is strongly dependent also on the rate of exchange between free and bound forms of the acids. Since these are generally considered as rather weak ligands for metal ions, the relatively modest effects we observe here indicate that it is probably only a small fraction of the analyte which is involved in metal binding, particularly at acidic pH. However, this is sufficient to change appreciably and often selectively the electrophoretic mobility of the acids so that their separation is improved. In this respect, the flexibility of metal ions and their binding properties towards a large number of polar molecules can enormously increase the scope and potentiality of CZE separations.

4. Conclusions

The direct addition of metal ions to the mobile phase produces changes in the ionization of a series of organic compounds that determine a significant variation in their electrophoretic mobility. This provides an efficient and rapid means of manipulating

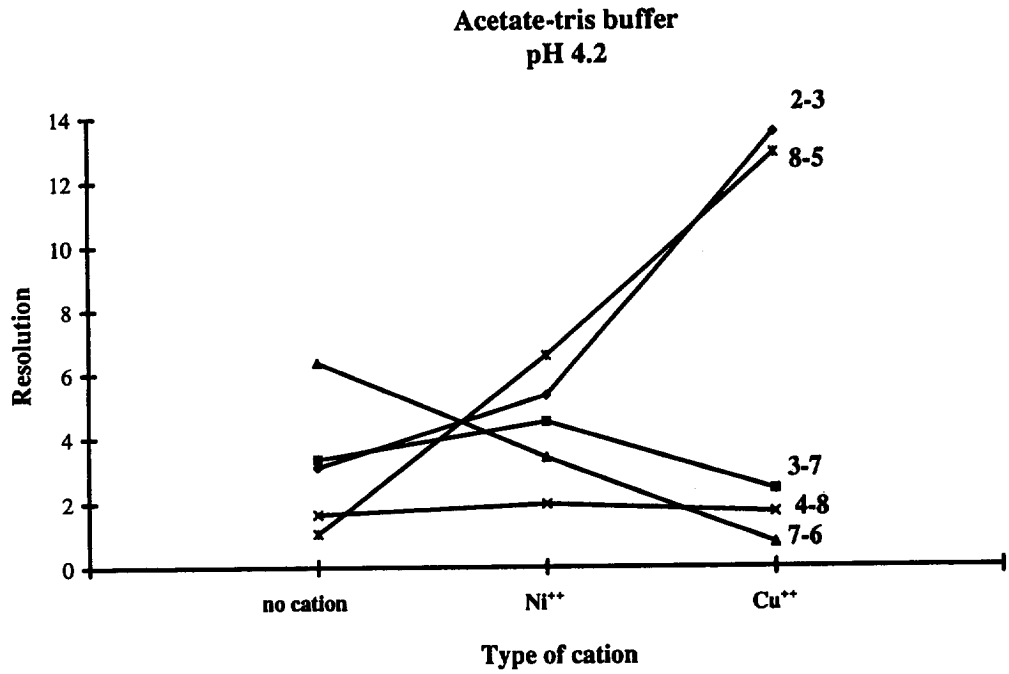


Fig. 5. Resolution of different pairs of organic acids separated in 25 m*M* acetate–Tris buffer, pH 4.2, containing different cations. For sample identification, see Table 1. Electrophoretic conditions as in Fig. 1.

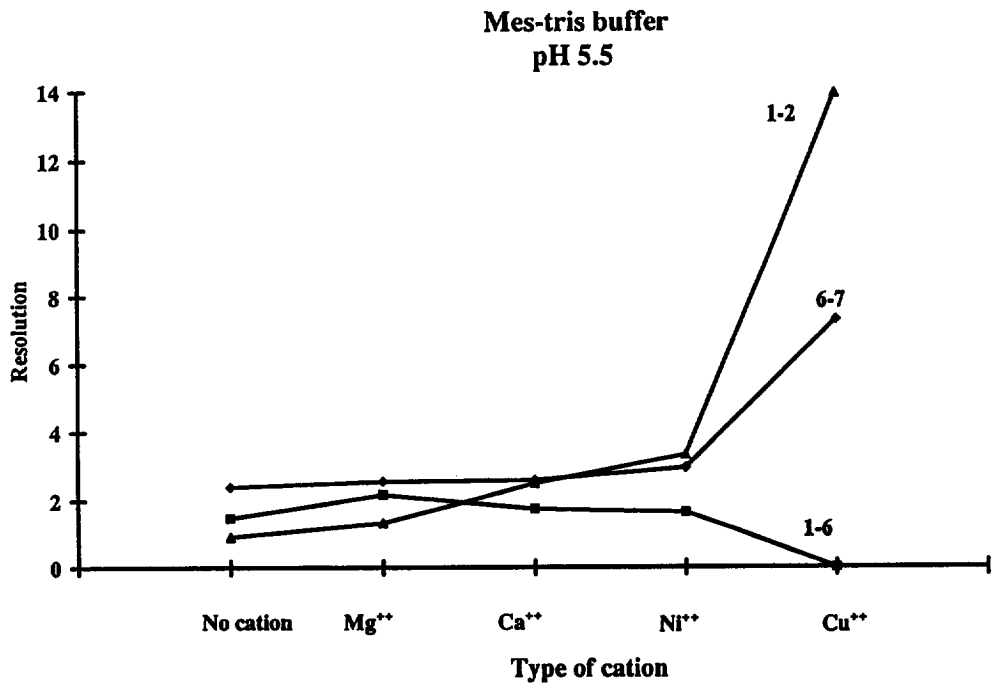


Fig. 6. Resolution of different pairs of organic acids separated in 25 m*M* MES–Tris buffer, pH 5.5, containing different cations. For sample identification, see Table 1. Electrophoretic conditions as in Fig. 1.

selectivity. The usefulness of this approach has been demonstrated in the separation of a series of organic acids which have been efficiently separated via a mechanism independent from pH variation.

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