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Analysis of metal complexes in the presence of mixed ion pairing additives in capillary electrophoresis

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Abstract

It was demonstrated that metal complexes were successfully separated by capillary electrophoresis with mixed ion pairing agents in the running buffer. The migration behavior of metal complexes in the presence of the ionic additives ammonium, tetramethylammonium, tetraethylammonium, and tetrabutylammonium is discussed. 4-(2-Pyridylazo)resorcinol (PAR) was selected as the chelating reagent for the pre-column derivatization with Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) ions. Under optimum conditions [10 m*M* NaH_2PO_4–Na_2HPO_4 as the running buffer containing $1\cdot10^{-4}$ *M* PAR, 5 m*M* tetramethylammonium and 5 m*M* tetrabutylammonium as the mixed ionic additives, pH 8.0, 70 cm (62.5 cm effective length)×50 μm capillary, separation voltage: 30 kV, injection time: 5 s, detection wavelength: 510 nm], the separation of six analytes was accomplished in 9 min. Excellent linearity with a dynamic range of two orders of magnitude from $1\cdot10^{-4}$ to $1\cdot10^{-6}$ *M* was obtained. The detection limits of Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) were $6.80\cdot10^{-7}$, $3.36\cdot10^{-7}$, $3.27\cdot10^{-7}$, $2.64\cdot10^{-7}$ and $6.63\cdot10^{-7}$*M*, respectively. This method was applied to the analysis of some metals in a pharmaceutical sample and an anode slime sample.

Keywords: Complexation; Metal complexes; Metal cations

1. Introduction

In earlier days, capillary electrophoresis (CE) was mainly utilized for analysing biological macromolecules such as proteins and nucleic acids, etc. However, the application of CE to the separation and the determination of smaller molecules e.g. inorganic cations, as their pre-column complexes with on-line direct UV–Vis detection has increasingly developed in recent years, due to the advantages of the combination of a highly sensitive detection with a high-performance separation technique [1].

The employment of sensitive chelating reagents overcomes the transparent character of metal ions in the UV–Vis region, and partly compensates for the limitation of the narrow optical length of the capillary for detectability. Many chelating reagents such as HQS [2], TBDA [3], EDTA [4], CDTA [5], PAR [6–9], 5-Br-PADAP [10] and 5-NO_2-PAPS [11] etc. are screened and exhibit satisfactory results for the sensitive detection. The detection limits ($\approx10^{-7}$ *M*) are at least one order of magnitude lower than those achieved with the indirect detection mode.

Although the formation of the complexes before injection enlarges the differences between metal ions, it seems to be more useful for improving the

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resolution between those analytes that have very similar or identical migration behaviour, if some other approaches [12] have been applied such as effect of solvation, micelle interaction, and ion association etc. Addition of organic solvent to the carrier electrolyte can improve the solubility of hydrophobic compound, adjust the behavior of electroosmotic flow (EOF), change the viscosity of the buffer, and accordingly improve the separation efficiency. Micelle interaction is widely used in CE, due to the contribution of Terabe et al. [13]. The enhancement of the selectivity is accomplished by the differences in the partition coefficient of the analytes between the aqueous and micellar phases. It has been shown that the migration of only those complexes possessing a hydrophobic character could be affected by the micelle interaction [6].

As an alternative methodology for ameliorating the resolution, ion association or ion pairing which has been widely used in high-performance liquid chromatography (HPLC), can also be successfully employed in CE. Iki et al. [14] proposed the first ion-association CE for the separation of equally and highly charged metal chelates. In that paper six ion paring agents as the single or independent additive in the buffer were employed to improve the separation selectivity. However, little further attention has been paid to the effect of multiple or mixed ion pairing agents on the separation of metal complexes.

In this study, we demonstrated the migration behavior of metal complexes in the presence of ion pairing additives in CE. Mixed ionic additives were employed for the separation and determination of some transition metal complexes. 4-(2-Pyridylazo)resorcinol (PAR) was selected as the pre-column chelating reagent for derivatization with these metals. Four ionic additives, ammonium, tetramethylammonium, tetraethylammonium and tetrabutylammonium were utilized for the investigation and optimization. It was concluded that specified ion pairing agents had a different effect on the electrophoretic migration of different metal complexes, owing to the variations of the affinity between the ion pairing additives and the complexes. Thus, the employment of the mixed ion pairing agents in the running buffer could bring about satisfactory analytical results. The method was applied successfully to assaying some metal ions in a pharmaceutical sample and in an anode slime.

2. Experimental

2.1. Apparatus and procedure

Analyses were carried out on the Spectra-Phoresis 1000 CE system (Thermal Separation Products, TSP, USA) equipped with a standard TSP untreated fused-silica capillary of 70 cm (62.5 cm effective length)× 50 μm I.D. Instrument control and data processing were carried using a Legend P5/100PCI computer (Legend, China) by using Spectra-phoresis software (TSP) with OS/2 Warp 3.0 as the operation system. Detection was performed by on-column spectrophotometric measurement at a specified wavelength or by scanning. The sample was injected at the anodic side by applying a vacuum for 5 s. Prior to use, the capillary was washed sucessively with 1 *M* NaOH and water for 15 min at 60°C, then preconditioned with the running buffer for 20 min at room temperature. Every five injections, the rinse cycle as described above was repeated to ensure reproducibility.

The pre-column derivatization was performed by mixing the metal ions and chelating reagent directly at room temperature before injection.

2.2. Chemicals

The standard solutions of metal ions were prepared from their nitrates, except for Cu(II) which was from $CuSO_4$, at a concentration of $2\cdot10^{-2}$ *M* in $1\cdot10^{-2}$ *M* HNO_3. The stock solution was $5\cdot10^{-3}$ *M* in $1\cdot10^{-2}$ *M* borate. Ionic additives including ammonium (THA), tetramethylammonium (TMA), tetraethylammonium (TEA), tetrabutylammonium (TBA) investigated in this work were purchased from Shanghai Chemical. The stock solutions were then prepared by dissolving them directly in pure water at a concentration of $5\cdot10^{-2}$ *M*.

The carrier electrolyte was prepared by mixing NaH_2PO_4–Na_2HPO_4 in appropriate ratios. PAR was added to the buffer to prevent chelates from dissociation during the electrophoresis at a concentration of $1\cdot10^{-4}$ *M*. The ion pairing agent was also added to the buffer if needed before the measurement of pH value. The electrolyte was prepared daily, degassed and filtered through a 0.45-μm membrane prior to

use. All chemicals were of analytical grade, and doubly distilled water was used for the preparation of all the solutions.

2.3. Sample preparation

Commercial Super-VATA in tablet form (0.6985 g) (Minsheng Pharmaceutical Hangzhou, China) was dissolved in of $1 \cdot 10^{-3}$ *M* HNO_3 solution, then filtered through a 0.45-μm membrane. The filtrate was transferred into a 100-ml calibrated flask and diluted to the mark with water. The other procedures were the same as those for the standard sample.

An anode slime from electrolytic refining of copper was crushed to a size of 100 mesh, and dried at 100°C for 2 h. An accurately weighed 5.000-g amount of slime powder was dissolved in 100 ml nitro-hydrochloric acid. After the slime had been completely digested, 1 ml 10% NaCl was added, then heated until it was evaporated to dryness. The residue was dissolved in pure water, transferred into a 100-ml calibrated flask, then diluted to the mark with water. The other procedures were the same as those for the standard sample.

2.4. Calculations

2.4.1. Electrophoretic mobility

The electrophoretic mobility (μ_{ep}) of the solute was calculated according to the equation:

$$\mu_{ep} = \mu_{eo} - \mu_{obs} = L_t L_d / V[1/t_{eo} - 1/t_{obs}]$$

where μ_{eo} is the observed mobility. L_t, L_d, V, t_{obs} and t_{eo} are the total length of the capillary, the effective length of the capillary, the applied voltage, the observed migration time and the migration time of EOF, respectively. The system peak (a negative peak) generated by water in the sample was used as the neutral marker for the determination of EOF.

2.4.2. Theoretical plate number

The theoretical plate number (N) was calculated from the following equation:

$$N = 5.54(T_m / WI)^2$$

where T_m is the migration time, and WI is the peak width at its half-height.

3. Results and discussion

3.1. Separation without ion-pairing additive

PAR can react with some transition metals, such as Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) etc., and form stable and water soluble complexes (ML_2) at room temperature provided the pH value was between 8 and 10. In our experiments, it was controlled within this range for optimizing the separation. With the increasing of the pH value, the migration time of analytes was decreased owing to the enhancement of the EOF. However, the resolution of complexes was decreased. Because our experiments were performed under the counter-electroosmotic conditions, the effect of EOF on the separation selectivity could be described as follows:

$$\begin{aligned}\Delta t &= t_2 - t_1 \\ &= [L_d L / V] \\ &\cdot \{(\mu_{ep}^1 - \mu_{ep}^2)/[(\mu_{eo} - \mu_{ep}^1)(\mu_{eo} - \mu_{ep}^2)\}\end{aligned}$$

where Δt is the difference of the migration time between two neighboring complexes, μ_{ep}^1, μ_{ep}^2 are the electrophoretic mobilities of the complexes, μ_{eo} is the electroosmotic mobility, L_d, L and V represent the effective length of capillary, the total length of capillary and the applied voltage, respectively. Obviously, increasing of the EOF would cause a decrease in Δt, thus, the decreasing the resolution. For this reason in our experiments we chose pH 8.0 as the optimized pH value.

Phosphate buffer was chosen as the carrier electrolyte because of its effect on acidity and ionic strength at the chosen pH value of 8.0. The influence of phosphate concentration in the buffer was investigated. It was found that increasing the concentration of the buffer electrolyte could slightly improve the selectivity of the separation if the concentration was below 30 m*M*. This was attributable to the effect of the ionic strength of the buffer on the EOF. Increasing the buffer ionic strength would result in the

compression of the double layer of the inner capillary wall, and consequently reduce of the velocity of the EOF. Further increasing the ionic strength would bring about the band broadening resulting from Joule heating. Because the ion pairing agent would be added to the buffer in our further experiments, we chose 10 m*M* phosphate for the running buffer to keep the total concentration of the buffer below the level at which the Joule heating began to take effect.

Fig. 1 illustrates the typical separation of complexes of Fe(II), Co(II), Ni(II), Cu(II), Zn(II) and free PAR ligand in the absence of the ion pairing agent in the running buffer. The peak of free PAR partly overlapped the peaks of Cu(II) and Fe(II). Moreover, the complexes of Zn(II) and Ni(II) co-eluted under the selected conditions. Therefore, a complete separation could not be achieved relying solely on the distinctions (e.g. differences in mass, charges and volume of complex) of the complexes formed before injection.

3.2. Effect of ion pairing additives

THe effect of ion pairing agent is based on the

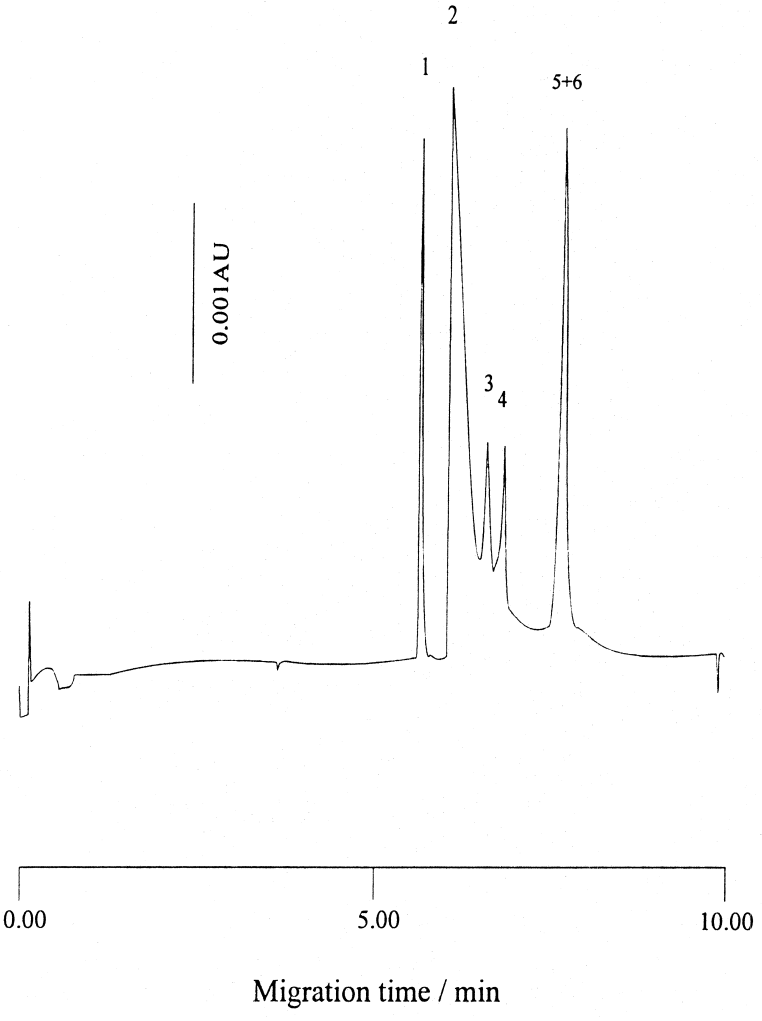

Fig. 1. Electropherogram in the absence of ion pairing agent in buffer. Conditions: 10 m*M* NaH_2PO_4–Na_2HPO_4 buffer containing $1\cdot10^{-4}$ *M* PAR, pH 8.0; 25°C; λ=510 nm; V=−30 kV; introduction time: 5 s; capillary: 70 cm (effective length 62.5 cm)×50 μm. Peaks: 1=Co(II), 2=Cu(II), 3=PAR, 4=Fe(II), 5=Zn(II), 6=Ni(II).

interaction between the charged compounds and their counterions as described in the equation:

$$A^{m(+/-)} + B^{n(-/+)} \rightarrow AB^{(m-n)\pm}$$

where $A^{m(+/-)}$, $B^{n(-/+)}$ and $AB^{(m-n)\pm}$ represent the charged compound, its counterion and the formed ion pair, respectively. In CE, the ion pairing agent was added to the running buffer. When the sample was introduced into the capillary and the electrophoresis begun, the ion pairing agent took effect. It tended to slow down the electrophoretic migration of the analytes in their own directions. Because each compound has its special characteristics, the affinities of different compounds with the same ionic additive are quite different. Therefore, the selectivity of separation was improved.

In our investigation, we studied the effects of four ion pairing agents on the electrophoretic mobility of the metal complexes.

3.2.1. Effect of ionic additives on EOF

Fig. 2 shows the effect of ionic additives on the EOF. Increasing the ionic strength of the buffer could be partly responsible for the decreasing the EOF. It led to the compression of the double layer of the inner capillary wall. Also, the adsorption of ion paring agent onto the capillary wall gave rise to a lowering of the EOF. In addition, more attention should be paid to the different lengths of hydrophobic alkyl chain of the ionic additives, which could result in different effects on the behavior of the EOF. With the increasing of the length of hydrophobic alkyl chain of ionic additive, EOF decreased as shown in Fig. 2. This could be attributed to a difference in adsorption onto the capillary wall. From THA to TBA, the hydrophobic characteristics of ion pairing agents increased, thus, the adsorption onto the inner capillary wall was increased, which led to a decrease in the zeta potential of the double layer and further reduction of EOF to different degrees as follows: THA<TMA<TEA<TBA.

3.2.2. Effect of ionic additives on separation

The complete separation of the five metal complexes with free ligand could not be accomplished in the absence of ion pairing agent. As 5 m*M* THA was added to the buffer, the resolution between Fe(II) and PAR was improved slightly as shown in Fig. 3A, but that between Cu(II) and PAR became worse.

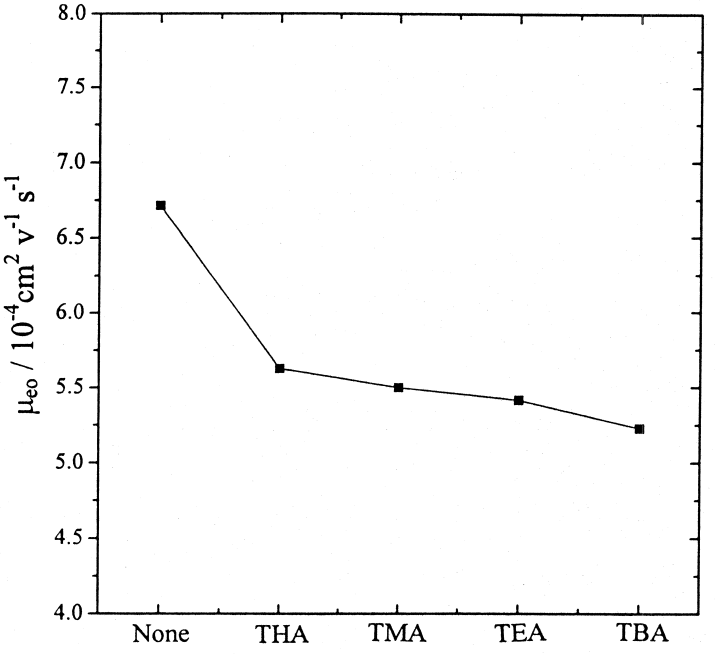

Fig. 2. Effect of ion pairing agents on EOF.

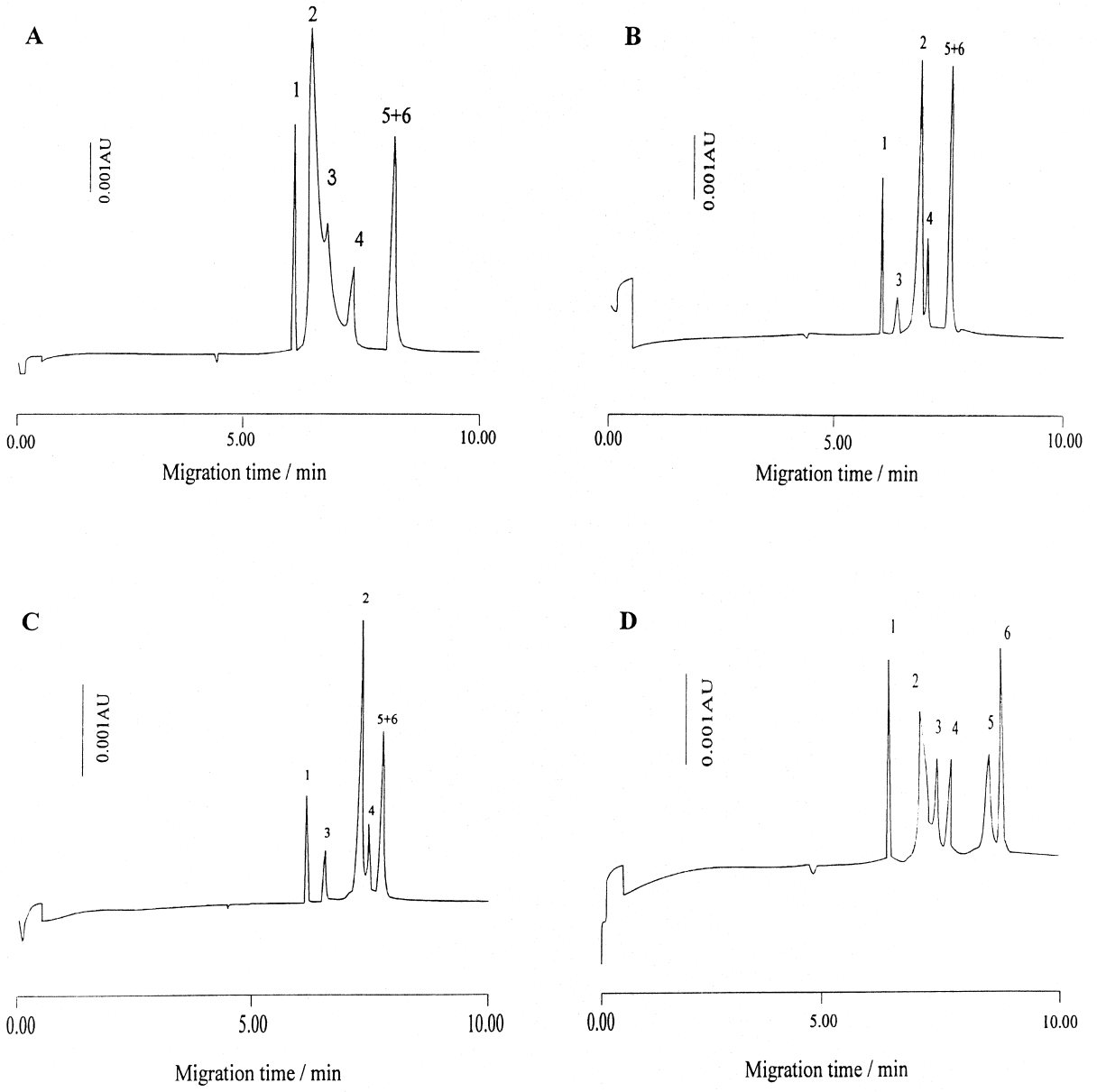

Fig. 3. Electropherograms in the presence of ion pairing agents in the buffer. (A) 5 m*M* THA; (B) 5 m*M* TMA; (C) 5 m*M* TEA; (D) 5 m*M* TBA; other conditions as in Fig. 1.

When 5 m*M* TMA replaced 5 m*M* THA, Cu(II), PAR and Fe(II) were completely resolved. Furthermore, the elution order was also changed. The zone of ligand surpassed that of Cu(II) (see Fig. 3B). If the ion pairing agent was changed to TEA (Fig. 3C), no further improvement was found compared to TMA. In Fig. 3A–C, Ni(II) and Zn(II) still co-eluted without any improvement. However, this problem could be solved by using TBA as shown in Fig. 3D, but Cu(II) and PAR still had some overlapping. The

whole elution order in the electropherogram was: Co(II)<Cu(II)<PAR<Fe(II)<Zn(II)<Ni(II).

From Fig. 3, it could be immediately concluded that the affinities between different complexes and ionic additives were quite different. A further understanding of the effect of four ion pairing agents on the migration behavior could be gained (Fig. 4) by using the change of the electrophoretic mobility ($\Delta\mu_{ep}$) of the complex as the parameter, which was calculated according to the following equation:

$$\Delta\mu_{ep} = \mu_{ep} - \mu'_{ep}$$

where μ_{ep}, μ'_{ep} represent the electrophoretic mobilities without or with ion pairing agent in the running buffer, respectively. The μ_{ep} values of the analytes with THA were very similar, and therefore separation was not improved as desired. In the presence of TMA or TEA, the difference of the μ_{ep} between any two analytes was enlarged. The cationic counterions TMA and TEA could be beneficial to resolve the complexes of Cu(II), Fe(II) and PAR. No difference of $\Delta\mu_{ep}$ in the co-eluted complexes of Zn(II) and Ni(II), however, was found. Although the difference of $\Delta\mu_{ep}$ resulting from the addition of TBA to the buffer was limited, it made a difference of $\Delta\mu_{ep}$ between the complexes of Zn(II) and Ni(II) which was adequate to discriminate the two analytes.

The influence of the addition of ion pairing agents on the separation efficiency was also discussed. The results are shown in Fig. 5. The separation efficiency is described by the theoretical plate number. The values of the theoretical plate number were the averages calculated from seven mixed sample solutions utilized to evaluate the calibration ($1\cdot10^{-4}$ *M*, $4\cdot10^{-4}$ *M*, $8\cdot10^{-4}$ *M*, $4\cdot10^{-5}$ *M*, $8\cdot10^{-6}$ *M*, $4\cdot10^{-6}$ *M* and $1\cdot10^{-6}$ *M*). With the addition of the ion pairing agent the separation efficiency increased. It could be interpreted by the variation in the concentration of the buffer. The increase of the ionic strength of the electrolyte could reduce the influence of the electrodispersion. Moreover, the increase of the length of hydrophobic alkyl chain of these cationic counterions could result in the decrease of the theoretical plate number. This phenomenon was in agreement with the adsorption onto the inner

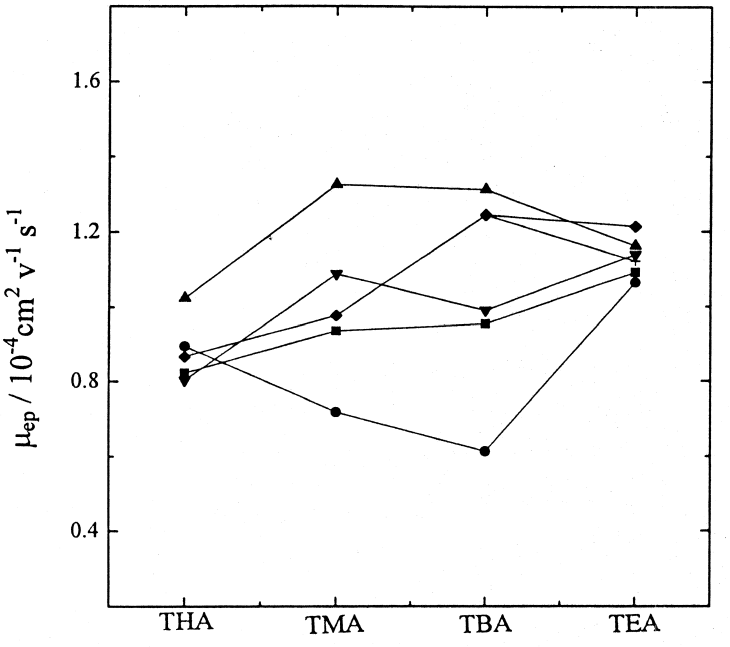

Fig. 4. Influence of ionic additives on electrophoretic migration of metal complexes. Curve identification: ♦: Zn(II), ▲: PAR, ●: Cu(II), +: Ni(II), ■: Co(II), ▼: Fe(II). conditions as in Fig. 1.

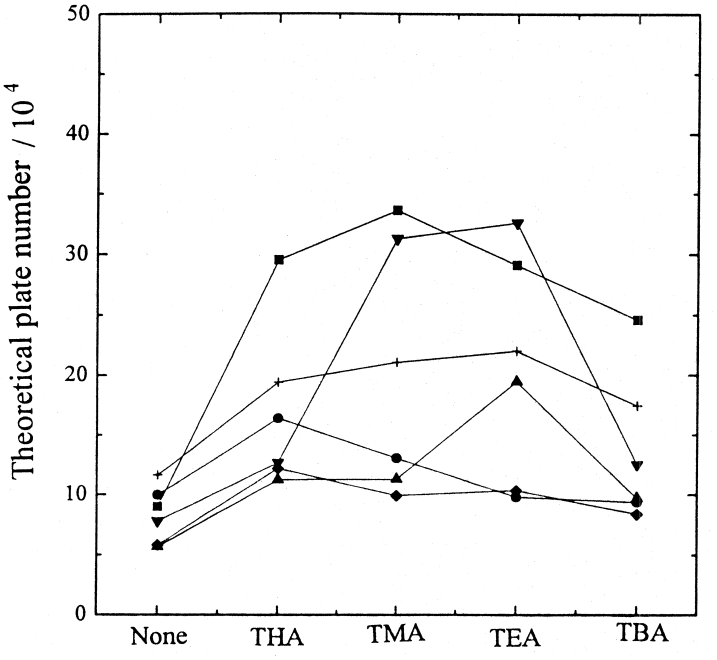

Fig. 5. Influence of ionic additives on separation efficiency. Curve identification: ♦: Zn(II), ▲: PAR, ●: Cu(II), + Ni(II), ■: Co(II), ▼: Fe(II). Conditions as in Fig. 1.

capillary wall. It was attributed to the interaction between the complexes and the ionic additives adsorped on the capillary wall.

3.3. Employment of mixed ion pairing agents

Since the specified ion pairing agents had different effects on the migration behavior of different metal complexes, this indicated that an improvement of the resolution could be achieved by incorporating mixed ion pairing agents according to the demands of the separation. In our studies, no complete separation of Cu(II), PAR, Fe(II) was accomplished without an ion pairing agent, and the complexes of Zn(II), Ni(II) co-eluted. Although the addition of TMA could resolve the complexes of Cu(II), Fe(II) and free ligand PAR, the complexes of Zn(II) and Ni(II) still co-eluted. Fortunately, they could be completely separated by using TBA as the ionic additive at the expense of decreasing resolution between the complexes of Cu(II), Fe(II) and PAR. So, TMA and TBA were simultaneously employed. Fig. 6 showed the dependency of electrophoretic mobility on the concentration ratio of TMA in the buffer. The whole concentration of ionic additives was controlled at a concentration of 10 m*M*. A concentration ratio of C_{TBA}: C_{TMA} (50:50) revealed good resolution. Higher or lower ratio of C_{TBA}: C_{TMA} in the running buffer would result in the decrease of selectivity between Cu(II) and PAR, or Zn(II) and Ni(II). In this experiment, 5 m*M* TMA and 5 m*M* TBA were added simultaneously to the running buffer, and a substantial improvement of selectivity was achieved.

Based on the results obtained, it is possible to propose the existence of a dynamic equilibrium involving the participation of the anionic metal chelate ML^- and the cationic ion-pairing additives A_i^+, which can be described as follows:

$$ML^- + A_i^+ \overset{K_{IP}^i}{\rightleftharpoons} ML^-A_i^+ \quad (1)$$

where

$$K_{IP}^i = \frac{[ML^-A_i^+]}{[ML^-][A_i^+]}$$

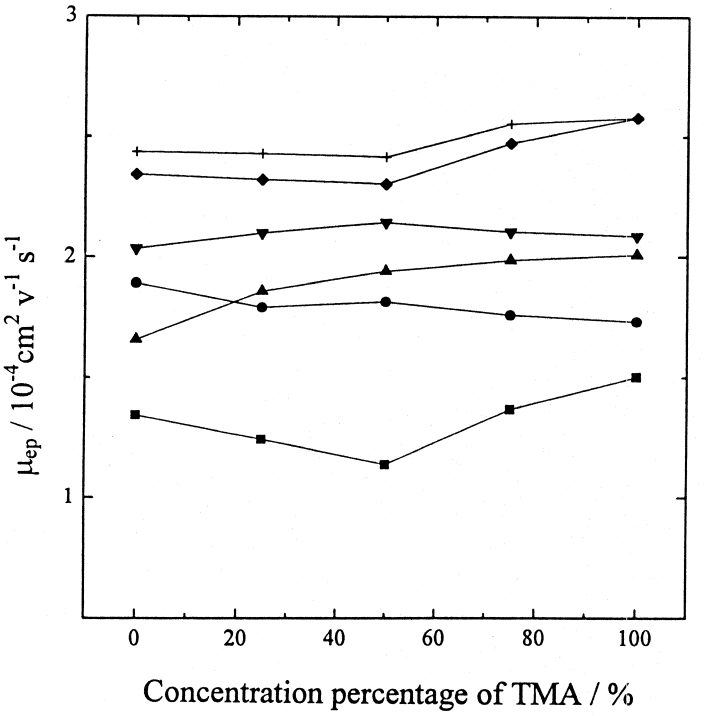

Fig. 6. Dependency of electrophoretic mobility on the concentration percentage of TMA. Curve identification: ♦: Zn(II), ●: PAR, ▲ Cu(II), +: Ni(II), ■: Co(II), ▼: Fe(II). Conditions as in Fig. 1 except for ionic additives in buffer.

giving

$$[ML^-A_i^+] = K^i_{IP}[ML^-][A_i^+] \quad (2)$$

While mixed ion pairing additives were added to the buffer, the total concentration $[ML]_{total}$ of ML^- in the sample zone can be given by:

$$[ML]_{total} = [ML^-](1 + \sum K^i_{IP}[A_i^+]) \quad (3)$$

because

$$a_i = \frac{[ML^-A_i^+]}{[ML]_{total}} \quad (4)$$

Where a_i represents the molar fraction of certain ion pairing species $[ML^-A_i^+]$. Substituting Eqs. (2 and 3) into Eq. (4) we get the following relationships:

$$a_i = \frac{K^i_{IP}[A_i^+]}{1 + \sum K^i_{IP}[A_i^+]} \quad (5)$$

The electrophoretic mobility of chelate (μ^*_{ep}) can be assumed to be a combination of the mobility of the free chelate and various ion pairings, which can be described by:

$$\mu^*_{ep} = \sum a_i \mu^i_{IP} + \left(1 - \sum a_i\right)\mu_{ep} \quad (6)$$

so we get

$$\mu^*_{ep} = \sum \left(\frac{K^i_{IP}[A_i^+]}{1 + \sum K^i_{IP}[A_i^+]} \cdot \mu^i_{IP} \right) + \left(1 - \sum \frac{K^i_{IP}[A_i^+]}{1 + \sum K^i_{IP}[A_i^+]} \right) \cdot \mu_{ep} \quad (7)$$

From Eq. (7) it could be concluded that the migration behavior of the metal chelate could be conveniently manipulated by selecting different ion pairing agent or different mixture of ion pairing additives and varying their concentrations.

3.4. Selection of other conditions for optimization

Some other conditions such as sample size and

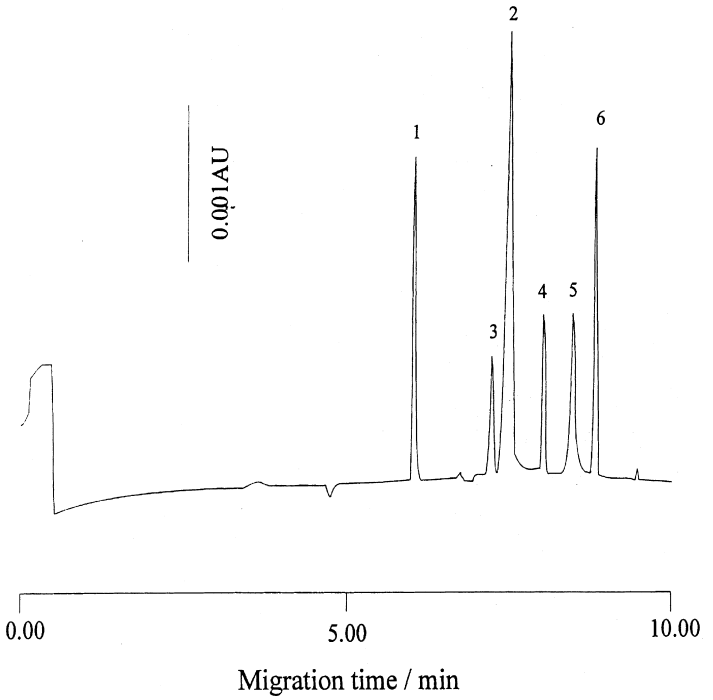

Fig. 7. Electropherogram under the optimum conditions: 5 m*M* TMA and 5 m*M* TBA in buffer, other conditions as in Fig. 1. Peak identification: 1=Co(II), 2=Cu(II), 3=PAR, 4=Fe(II), 5=Zn(II), 6=Ni(II).

applied voltage were investigated, which had minor effect on the separation.

3.4.1. Sample size

Increasing the injection time could increase the length of sample plug and the detection sensitivity without any loss of the resolution if the injection time was shorter than 10 s. If the injection time was <1 s the reproducibility disappeared, due to the non-reproducibility. We chose 5 s as the optimized injection time ($\approx$9 nl injected).

3.4.2. Applied voltage

With the decreasing of the applied voltage, the separation time was distinctively increased, and the detection limit decreased because of more band broadening caused by the diffusion. So, we chose 30 kV as optimum.

Table 1[a]

Metal ions	Calibration curve $y = ax + b$	γ	Linear range $\cdot 10^{-5}$ *M*	Detection limits $\cdot 10^{-7}$ *M*
Co(II)	$y=2779x-648.2$	0.9991	0.1–10	3.36
Cu(II)	$y=6506x+834.6$	0.9993	0.1–10	2.64
Fe(II)	$y=286.8x+2871$	0.9998	0.1–10	6.80
Zn(II)	$y=956.3x+714.6$	0.9991	0.1–10	6.63
Ni(II)	$y=1074x+3027$	0.9994	0.1–10	3.27

[a] *y*, peak area; *x*, concentration of analyte.

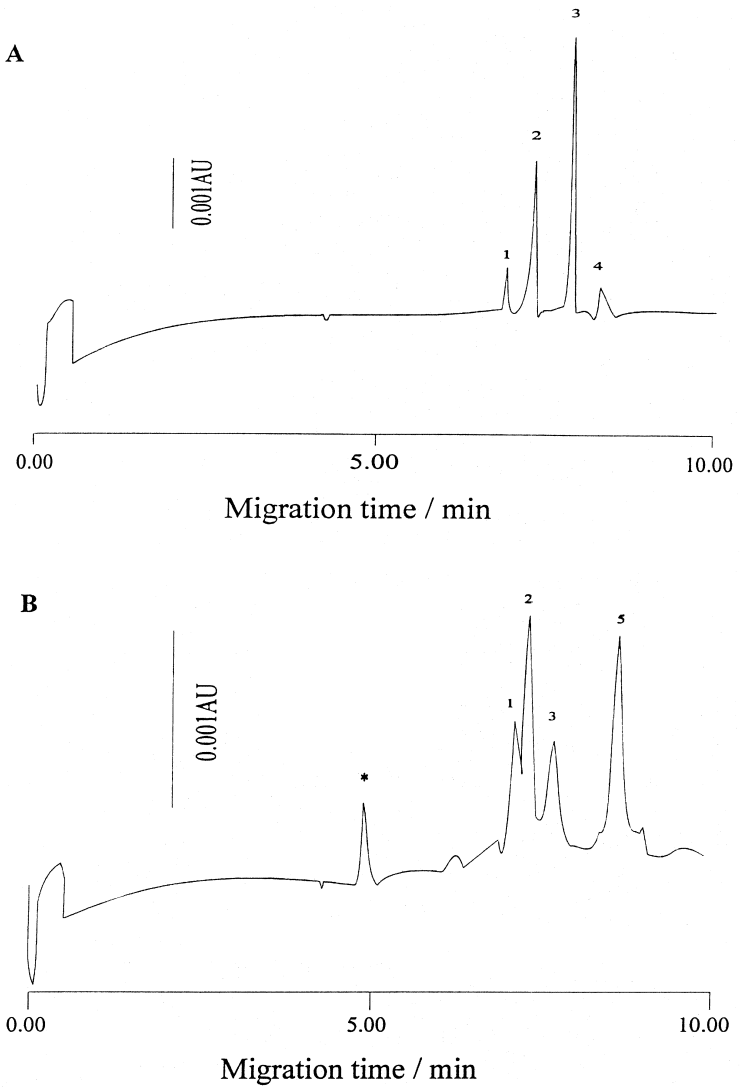

Fig. 8. Electropherogram of assaying samples. Peaks: 1=PAR, 2=Cu(II), 3=Fe(II), 4=Zn(II), 5=Ni(II).

Under the optimum conditions, the five complexes were completely separated in 9 min as shown in Fig. 7.

3.5. Quantification

Calibration curves which were established from seven concentration levels of mixed sample exhibited a linear dynamic range of two orders of magnitude between peaks areas and sample concentrations (see Table 1). Table 1 also gives the detection limits which were calculated based on a peak height of three times of baseline noise (S/N=3). The relative standard deviation of the peak areas for Co(II), Cu(II), Fe(II), Zn(II) and Ni(II) were found to be 0.4, 2.1, 0.7, 1.2, 0.9%, respectively, with five duplicate injections of standard sample containing $8 \cdot 10^{-6} M$ of each metal ion.

Table 2
Results from the assay of the pharmaceutical sample (Super-VATA)

Metal ion	Average value $\cdot 10^{-6}$ M	Added $\cdot 10^{-6}$ M	Found $\cdot 10^{-6}$M	Recovery (%)
Cu(II)	7.76			
		5.00	12.62	99.14
		10.00	17.54	98.76
		20.00	27.88	100.4
Fe(II)	44.27			
		5.00	49.05	99.55
		10.00	54.82	102.9
		20.00	63.99	99.56
Zn(II)	2.01			
		5.00	7.24	103.2
		10.00	12.26	102.1
		20.00	21.96	99.77

3.6. Applications

The method was applied to assaying a pharmaceutical sample (Super-VATA) and an anode slime sample for evaluating the qualitative and the quantitative performance Fig. 8 gives the typical electropherograms for these two samples. Three essential metal ions for human nutrition Cu(II), Fe(II) and Zn(II) were detected from the Super-VATA sample as shown in Fig. 8A, and the results are listed in Table 2. Fig. 8B illustrates the qualitative result of the anode slime sample.

4. Conclusion

This paper demonstrates the separation of metal complexes by CE in the presence of the mixed ion pairing agents. The interaction between the complexes and various ionic additives has been investigated. It was concluded that the ion pairing techniques could be a convenient alternative methodology for the analyses of metal ions as their complexes by CE. The employment of multiple or mixed ion paring agents could help achieve the desired separation selectivity.

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