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# Chemical modification of capillary column for electrophoretic separations of transition metal ions

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## Abstract

The surface modification of a fused-capillary for the separation of transition metal ions is described. A comparison was made of the properties of capillaries for each reaction stage, containing  $\text{NH}_2$ ,  $\text{COOH}$  and  $\text{CONHOH}$  functional groups, with those of the unreacted capillary by electroosmotic flow measurements and its dependence on pH. Multi-component mixtures of transition metal complexes with 4-(2-pyridylazo)resorcinol were separated in modified capillaries containing  $\text{NH}_2$  and  $\text{COOH}$  functional groups with an ammonia–ammonium buffer of pH 9.0 at an applied voltage of 15 kV followed by direct UV–Vis detection at 520 nm. Others were separated using the treated capillary containing  $\text{CONHOH}$  groups with pH 4.0 imidazole (5 mM)– $\alpha$ -hydroxyisobutyric acid (8 mM) as electrophoresis buffer, followed by indirect UV detection at 214 nm. The effects of electrophoretic buffer parameters on the complexation reaction and migration behaviour are discussed. Experimental results indicated that incorporating the chelating functional group in the inner wall of the capillary markedly enhances the selectivity of the system. Further, the optimum separation efficiencies were obtained by employing capillaries containing  $\text{CONHOH}$  functional groups.

## 1. Introduction

Capillary electrophoresis (CE) has become a powerful separation technique during the past decade because of its separation efficiency and high resolution and the availability of several different separation modes. Capillary zone electrophoresis was initially applied primarily in biochemical analyses; in recent years, however, its applicability in all fields of chemical analysis has been demonstrated. CE was recently found to be capable of providing results equivalent to those reported by ion chromatography (IC) in a

shorter period of time, but with lower sensitivity [1]. Compared with IC, capillary electrophoresis provides a higher resolution with much less sample and lower costs of reagents and columns. Most of these investigations have involved separations of inorganic anions; however, a few applications of CE to inorganic cations have been published [2–6]. In the separation of inorganic cations by CE, close values of equivalent ionic conductivities imply a more challenging separation. The group IA cations are found to be more readily separated than the transition metals or the lanthanides.

A variety of column technologies for chromatographic approaches have already been developed. However, the technology for CE is

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relatively new. The properties of the capillary surface play an essential role in the success of any CE analysis. Different brands of commercially available quartz capillaries have been studied with respect to their surface properties [7]. The use of chemical modifications to the inner wall of conventional capillaries to reduce analyte adsorption and electroosmotic flow (EOF) continue to be a vital area of research activity [1]. Although several capillary surface modifications have been evaluated for the separation of proteins, peptides, oligosaccharides and nucleotides [8–15], no investigation has examined capillary surface modification via chemical bonding for the separation of transition metal ions. Hence a series of studies have been undertaken in our laboratory concerning the preparation of chelating resins as packing materials for chromatographic approaches. There is considerable interest in the development of new selective capillaries for the separation of metal ions by CE. In this paper, the incorporation of hydroxamate groups on the surface of fused silica through chemical bonding and the application of the capillary to the separation of transition metal ions is reported.

## 2. Experimental

### 2.1. Instrumentation

The capillary electrophoresis apparatus used in these studies employs a high-voltage power supply with a 30 kV capacity (Model 890-CE, Jasco Tokyo, Japan), a variable-wavelength UV-Vis detector (Jasco 870-CE) and an integrator (Jasco 807-IT). A chemically modified capillary made from an unmodified silica column (100  $\mu\text{m}$  I.D. and 375  $\mu\text{m}$  O.D.) with an external coating of polyimide (J&W Scientific, Folsom, CA, USA), 70 cm in total length and 50 cm from the point of sample introduction to the detector window, was used. The optical window was prepared by removing a small section of the polyimide coating from the fused-silica capillary.

### 2.2. Chemicals

Most chemicals were of analytical-reagent grade (Merck, Darmstadt, Germany). N-[3-(Trimethoxysilyl)propyl]ethylenediamine,  $\alpha$ -hydroxyisobutyric acid (HIBA) and imidazole were obtained from Aldrich (Milwaukee, WI, USA). Purified water (18 M $\Omega$  cm), obtained using a Milli-Q water purification system (Millipore) was used to prepare all solutions. 4-(2-Pyridylazo)resorcinol (PAR) reagent solutions were prepared by dissolving an appropriate amount of PAR (Merck) in ammonia–ammonium buffer (0.3 M, pH 10). All PAR solutions were stored in plastic vessels and in the dark. Metal salts were purchased from Merck.

### 2.3. Stock standard solutions

Stock standard solutions (ca. 0.1 M) of metal ions were prepared by dissolving an appropriate amount of metal salt in 0.1 M nitric acid and diluting to 250 ml with pure water, then adjusting the pH to 1.6 and storing the solutions in polyethylene bottles. The concentration of each metal ion was verified by EDTA complexometric titration.

### 2.4. Working standard solutions

The stock standard solutions were diluted to concentration levels that bracketed the concentration level of interest, just prior to analysis.

### 2.5. Surface modification of the capillary column

Fused-silica capillaries were first rinsed with 1 M NaOH for 15 min, followed by 1 M HCl for 15 min and finally with methanol for 15 min. The capillaries were dried at 110°C with a nitrogen purge overnight. A 2-ml volume of N-[3-(trimethoxysilyl)propyl] ethylenediamine–methanol (2:3, v/v) was placed in a small pressurizing chamber which was subsequently purged with nitrogen for 15 min. One end of the capillary tube was placed in the chamber to allow nitrogen

to flow through the capillary tube for several minutes. The end of the capillary tube was then lowered into the silylating agent while maintaining a constant flow of nitrogen into the sealed pressuring chamber, thereby forcing the silylating agent into the capillary tube. When the capillary tube was filled with the silylating agent, both ends of the capillary tube were sealed off using silicone-rubber seals. The capillary tube was maintained at 70°C by immersing it in a heating bath for 5 h. The silicon-rubber seals at both ends of the sealed silylation capillary were subsequently removed and purged with nitrogen to remove the excess reagent. The resulting product is called R-NH<sub>2</sub>.

By similar procedures, under a nitrogen purge, a saturated bromoacetic acid solution was used to fill the R-NH<sub>2</sub> capillary, then maintained at 100°C for 24 h. The silicone-rubber seals at the ends of the sealed capillary were removed and the capillary was purged with nitrogen to remove the excess reagent. The resultant product is called R-COOH. Hydrochloride-free hydroxylamine was prepared as in a previous investigation [16]. This solution was deaerated and used to fill the R-COOH capillary. The sealed capillary was maintained at 70°C for 72 h. Next, the excess reagent was purged with nitrogen and rinsed with water, leaving a chemically bonded layer of hydroxamic acid on the inner walls of the capillary. The resultant material is called R-CONHOH.

### 2.6. Procedures

For conditioning the capillary, the electric field was turned on and left on until the current through the capillary had stabilized, usually within 10–15 min. Buffer solutions were degassed immediately prior to use. Injection was achieved electrophoretically at 5 kV for 2 s or 10 kV for 10 s. With electromigration injection, the sample introduction end of the capillary was placed in the sample vial and a voltage was briefly applied, thereby causing a small band of sample to electromigrate into the capillary. After the sample had been drawn into the capillary

tube, the end of the capillary was placed back in the electrophoresis buffer, together with the electrode, and the electrophoretic voltage was applied. Detection was carried out by direct UV-Vis detection at 520 nm and indirect UV detection at 214 nm. No column temperature control was available.

### 3. Results and discussion

Hydroxamic acid is an important and very useful analytical reagent. A series of hydroxamic acid or hydroxamate resins have been prepared in our laboratory. In our experience, a hydroxamate-containing capillary is easily and reproducibly prepared. The reaction steps are shown in Fig. 1. The scheme consists of three separate stages: (1) silylation with the silanol groups of the silica surface; (2) reaction of the bromoacetic acid with the silylating silica, resulting in attachment of the carboxylic acid to the silica surface; and (3) reaction of the bonded

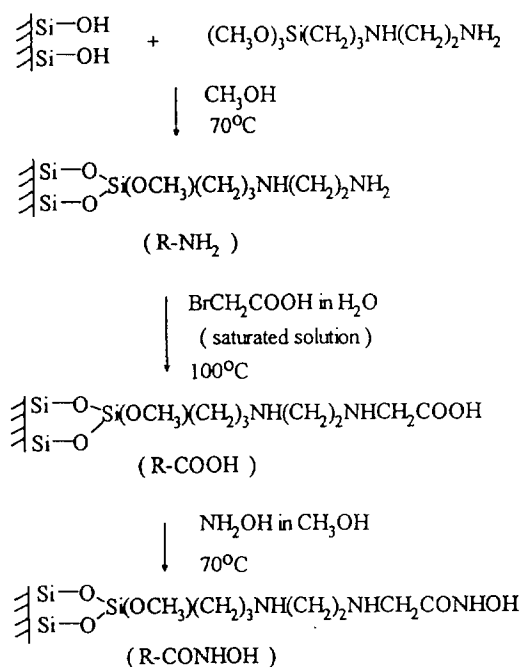


Fig. 1. Scheme of surface modification of fused silica.

carboxylic acid with hydroxylamine to yield the hydroxamic acid coating.

### 3.1. Characterization of surface modification of capillaries

The effectiveness of bonding procedures was measured by CE via the variation of the EOF. As in CE the EOF is superimposed on the electrophoretic migration of charged species, the magnitude of the EOF depends on the applied electric field,  $E$ , and on the distribution of charges in the interfacial layer. The EOF velocity was determined according to the migration time of benzyl alcohol, which served as a neutral marker [7]. The EOF was studied with the four different capillaries in the pH range 7.5–10. The results are given in Table 1. At  $\text{pH} > 8$ , the surface silanols on the fused-silica capillary wall should be totally dissociated. Consequently, the magnitude of the EOF is very large and the migration of benzyl alcohol increases as the pH increases. By chemical modification, the number of surface silanols is decreased and, consequently, the direction of EOF with capillary bonding with amino groups is reversed and the migration velocity increases as the pH value decreases. Hence the sample injection was made from the negative end. This can be accounted for by the finding from silica modification that mainly the amino groups are protonated at a lower pH value. With the incorporation carboxylate groups, the direction of the EOF is the same as

that for uncoated fused silica. The migration time decreased as the pH increased; however, the magnitude of EOF is smaller than that for the uncoated fused silica. As hydroxamate groups were incorporated on the inner wall of the fused silica, both the EOF and adsorption sites on the capillary walls decreased significantly.

### 3.2. Optimization of separation

The first application of micellar electrokinetic capillary chromatography to the separation of PAR metal chelates was demonstrated by Saitoh et al. [17]. In this investigation, only ammonia–ammonium chloride and not SDS eluent was used as the background buffer for separation of transition metal ions as PAR chelates with the R-NH<sub>2</sub> and R-COOH capillary columns, and detection was carried out by direct UV–Vis spectrophotometry at 520 nm. Moreover, the experimental results indicated that imidazole–HIBA mixture would be well suited as the background buffer for the R-CONHOH capillary column; the metal analytes were separated directly and indirect UV detection was achieved at 214 nm. Although Beck and Engelhardt [18] had applied imidazole as a background electrolyte for the separation of alkali and alkaline earth metal ions and also of aliphatic amines and amino alcohols, they used sulfuric acid to adjust the pH of the solution. Moreover, HIBA, a weak complexing agent, which exhibits better separation

Table 1  
Effect of pH on electroosmotic flow mobility in various modified capillary materials

| Capillary <sup>a</sup>                        | $\mu_{\text{EOF}}$ ( $10^{-4}$ cm <sup>2</sup> /V·s) |        |        |        |
|---|--|--------|--------|--------|
|   | pH 8.0   | pH 8.5 | pH 9.0 | pH 9.5 |
| Fused silica (R) <sup>b</sup>                 | 13.20  | 14.10  | 17.20  | 18.00  |
| R-NH <sub>2</sub> (intermediate) <sup>c</sup> | 7.01   | 6.84   | 5.07   | 2.84   |
| R-COOH (intermediate) <sup>b</sup>            | 5.58   | 6.14   | 6.48   | 7.58   |
| R-CONHOH (final product) <sup>b</sup>         | 3.90   | 3.98   | 4.39   | 4.50   |

<sup>a</sup> Total capillary length, 70 cm; effective capillary length (to the detector), 50 cm.

<sup>b</sup> Applied voltage 15 kV.

<sup>c</sup> Applied voltage 10 kV.

efficiency than sulfuric acid or acetic acid, was used to adjust the pH of the imidazole solution in this investigation. The reason might be due to the enhancement of the variation in the electrophoretic mobilities of the analytes in the R-CONHOH column. As the formation constants of acetic acids are slightly larger than that of HIBA, this probably suppresses the differences in the electrophoretic mobilities. Sulfuric acid cannot act in the same way as HIBA, probably owing to the poor complexation ability.

The effects of buffer pH and concentration on the migration times were also investigated. The optimum conditions are given in Table 2. The results indicate that the type of column, the pH and concentration of buffer solutions and the applied voltage affect the selectivity of CE. With the R-CONHOH capillary, the EOF was essentially eliminated (Table 1), so higher voltages were used to promote faster migration of metal ions than the R-NH<sub>2</sub> and R-COOH capillary columns.

### 3.3. Analytical application

The electropherograms of metal ions as PAR chelates with the R-NH<sub>2</sub> and R-COOH columns are shown in Fig. 2a and b, respectively. With the R-NH<sub>2</sub> column, only Ni(II), Co(II), Fe(II) and Zn(II) could be separated under the conditions applied (Fig. 2a). With the R-COOH column, Cu(II), Co(II), Zn(II), Mn(II), Ni(II)

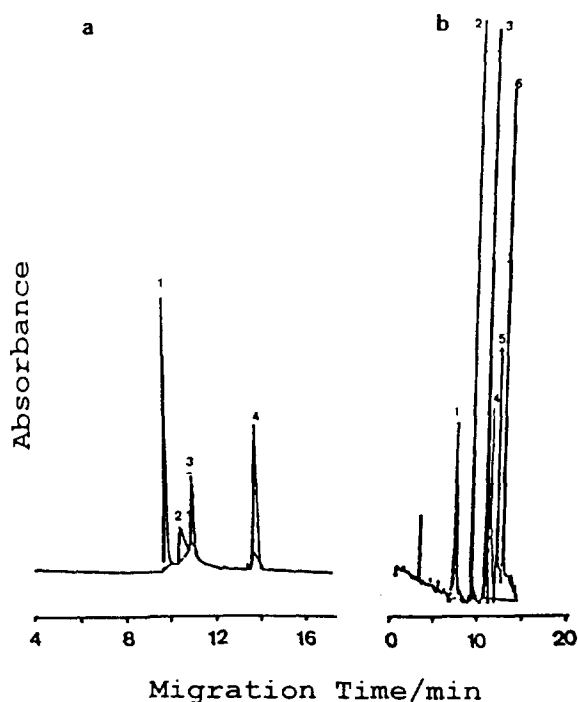


Fig. 2. Electropherogram of metal ions as PAR chelates. Column, 100  $\mu$ m I.D.  $\times$  70 cm (50 cm to detector); electromigration injection, 2 s with 5 kV; applied field, 15 kV; detection wavelength, 520 nm. (a) R-NH<sub>2</sub> column: NH<sub>3</sub>-NH<sub>4</sub>Cl (pH 9.0, 15 mM) as electrophoresis buffer; PAR,  $1.5 \times 10^{-3}$  M; metal ion concentrations, Ni, Co, Fe and Zn  $1 \times 10^{-4}$  M each. Peaks: 1 = Ni; 2 = Zn; 3 = Fe; 4 = Co. (b) R-COOH column: NH<sub>3</sub>-NH<sub>4</sub>Cl (pH 8.5, 20 mM) as electrophoresis buffer; PAR,  $3 \times 10^{-4}$  M; metal ion concentrations, Cu, Co, Mn, Ni and Zn  $1 \times 10^{-5}$  M, Fe  $5 \times 10^{-5}$  M. Peaks: 1 = Cu; 2 = Co; 3 = Fe; 4 = Mn; 5 = Zn; 6 = Ni.

Table 2  
Comparison of column efficiencies

| Column            | Voltage  | Buffer solution   | Analyte   | <i>N</i> <sup>a</sup> |
|-------------------|--|---|---|-----------------------|
| R-NH <sub>2</sub> | S <sup>b</sup> : 5 kV, 2 s<br>A <sup>c</sup> : 15 kV | NH <sub>3</sub> -NH <sub>4</sub> Cl<br>(0.02 M, pH 9.0) | Ni, Zn, Fe(III),<br>Co                              | 10931                 |
| R-COOH            | S: 5 kV, 2 s<br>A: 15 kV                             | NH <sub>3</sub> -NH <sub>4</sub> Cl<br>(0.02 M, pH 8.5) | Cu, Co, Fe(III),<br>Mn, Zn, Ni                      | 13023                 |
| R-CONHOH          | S: 10 kV, 10 s<br>A: 20 kV                           | Imidazole (5 mM)<br>-HIBA (8 mM)<br>(pH 4.0)            | K, Ba, Ca, Mg,<br>Na, Mn, Cd, Co,<br>Fe(II), Ni, Zn | 10441                 |

<sup>a</sup> Average number of theoretical plates.

<sup>b</sup> Voltage for electrokinetic injection.

<sup>c</sup> Applied voltage.

and Fe(II) were well separated and the calibration graphs of peak area against metal ion concentration are given in Table 3. Under the optimum conditions, the simultaneous determination of eleven metal ions can be achieved within 7 min with pH 4.0 imidazole (5 mM)–HIBA (8 mM) as the electrophoresis buffer with the R-CONHOH column. The electropherogram is shown in Fig. 3a; the migration time increased in the order  $K < Ba < Ca < Mg < Na < Mn < Cd < Fe(II) < Co < Ni < Zn$ . According to the values of the limiting ionic equivalent conductances ( $\times 10^{-4} \text{ m}^2 \text{ S mol}^{-1}$ ) of the metal ions studied, the order is  $K (73.48) > Ba (63.6) > Ca (59.47) > Co (55) > Cd (54) > Fe(II)(54) > Mn (53.5) > Mg (53.0) > Zn (52.8) > Na (50.8) > Ni (50)$  [19]. The stability constants ( $\log K$ ) of transition metal ions towards acetohydroxamic acid increase in the order  $Mn (10.9) < Cd (12.3) < Fe(II) (13.3) < Co (14.0) < Ni (14.6) <$

$Zn (15.0)$  [20]. In this system, separations are clearly based not only on the differences in the electrophoretic mobilities of solutes in the presence of an electric field, which is dependent on the charge and size of the solutes, but also on the differences in the complexation ability of solutes with the functional groups of the capillary. In addition, better separation efficiencies were achieved with this column than with the untreated fused-silica column (Fig. 3b) and the R-NH<sub>2</sub> and R-COOH capillary columns (Figs. 2a and b), in spite of the lower average number of theoretical plate in the R-CONHOH column than in the R-NH<sub>2</sub> and R-COOH columns (Table 2). These results can be explained by the selectivity of hydroxamic acids towards transition metal ions usually being larger than that of compounds containing amino or carboxylic acid groups. The experimental results revealed that the surface modification of the fused silica obvi-

Table 3  
Summary of results for the determination of transition metal ions

| Column                | Metal ion | Linear equation                                       | Dynamic range <sup>a</sup><br>( $10^{-6} M$ ) | Detection limit |
|-----------------------|-----------|---|---|-----------------|
| R-COOH <sup>b</sup>   | Co(II)    | $y = 3.85 \times 10^9 x - 128.06$<br>( $r = 0.9979$ ) | 0.4–4.0                                       | 6.3 pmol        |
|                       | Cu(II)    | $y = 6.49 \times 10^9 x - 7802.6$<br>( $r = 0.9990$ ) | 1.2–12.0                                      | 24.4 pmol       |
|                       | Fe(II)    | $y = 0.54 \times 10^9 x + 210.5$<br>( $r = 0.9996$ )  | 2.0–20.0                                      | 21.8 pmol       |
|                       | Ni(II)    | $y = 3.68 \times 10^9 x - 421.7$<br>( $r = 0.9982$ )  | 0.4–4.0                                       | 4.5 pmol        |
|                       | Zn(II)    | $y = 1.16 \times 10^9 x + 2221.7$<br>( $r = 0.9995$ ) | 1.2–12.0                                      | 15.5 pmol       |
| R-CONHOH <sup>c</sup> | Co(II)    | $y = 3.12 \times 10^5 x + 1.18$<br>( $r = 0.9752$ )   | 2.0–10.0                                      | 6.83 nmol       |
|                       | Cd(II)    | $y = 3.95 \times 10^5 x + 1.19$<br>( $r = 0.9841$ )   | 2.0–10.0                                      | 7.11 nmol       |
|                       | Fe(II)    | $y = 2.97 \times 10^5 x + 0.62$<br>( $r = 0.9867$ )   | 2.0–10.0                                      | 6.97 nmol       |
|                       | Ni(II)    | $y = 2.50 \times 10^5 x + 1.12$<br>( $r = 0.9673$ )   | 2.0–10.0                                      | 5.92 nmol       |
|                       | Zn(II)    | $y = 2.14 \times 10^5 x + 1.04$<br>( $r = 0.9770$ )   | 2.0–10.0                                      | 5.83 nmol       |
|                       | Mn(II)    | $y = 5.90 \times 10^5 x + 1.23$<br>( $r = 0.9961$ )   | 2.0–10.0                                      | 8.08 nmol       |

<sup>a</sup> The upper limit corresponds to the maximum concentration studied.

<sup>b</sup> Determined by peak area. Conditions as in Fig. 2b.

<sup>c</sup> Determined by peak height. Conditions as in Fig. 3a.

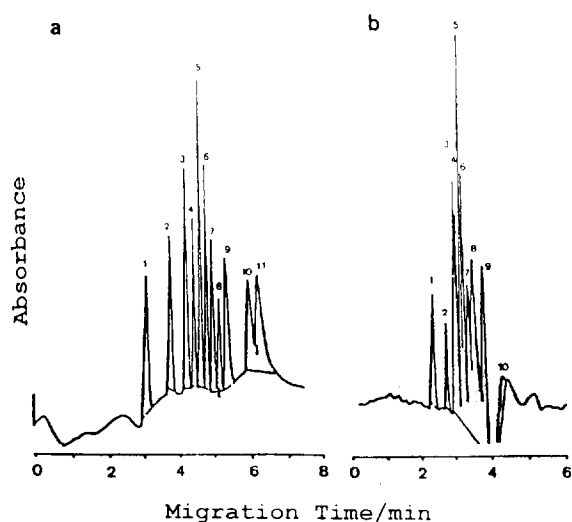


Fig. 3. Electropherograms of metal ion mixtures. Column, (a) R-CONHOH and (b) fused silica, both  $100\ \mu\text{m}$  I.D.  $\times$  70 cm (50 cm to detector); electromigration injection, 10 s with 10 kV; applied field, 20 kV; electrophoresis buffer, pH 4.0 imidazole (5 mM)–HIBA (8 mM); detection: wavelength, 214 nm; metal ion concentrations,  $5 \times 10^{-5}$  M each. Peaks: 1 = K; 2 = Ba; 3 = Ca; 4 = Mg; 5 = Na; 6 = Mn; 7 = Cd; 8 = Fe(II); 9 = Co; 10 = Ni; 11 = Zn.

ously alters the apparent mobilities of the cations sufficiently to permit the separation of more elements. The calibration graphs of peak height against metal ion concentration are given in Table 3.

The reproducibility of the technique for five consecutive injections is displayed in Table 4. Despite the fact that the reproducibility decreases with increasing dilution and increases at higher concentrations, the reproducibility in terms of peak area was between 1.0 and 3.2% and that in terms of peak height was between 0.6 and 4.2% [relative standard deviation (R.S.D.)], depending on the cation. Stability was reflected in the fact that the migration times remained fairly consistent from day to day (less than 2% R.S.D. for a 7-day period).

The applicability of the proposed method to the determination of trace elements in natural water and also biological, botanical and geological materials was studied. These samples generally have high levels of alkali and alkaline earth metals or other chelating agents with trace levels

Table 4  
Reproducibility of CE method

| Cation | R.S.D. (%) ( $n = 5$ ) |           |             |
|--------|------------------------|-----------|-------------|
|        | Migration time         | Peak area | Peak height |
| K      | 0.032                  | 2.40      | 1.72        |
| Ba     | 0.037                  | 2.14      | 1.92        |
| Ca     | 0.037                  | 1.21      | 0.64        |
| Mg     | 0.037                  | 0.95      | 0.69        |
| Na     | 0.041                  | 2.09      | 0.84        |
| Mn     | 0.042                  | 2.69      | 0.66        |
| Cd     | 0.041                  | 2.54      | 1.56        |
| Fe     | 0.045                  | 3.17      | 4.20        |
| Co     | 0.046                  | 1.59      | 2.71        |
| Ni     | 0.044                  | 1.63      | 3.65        |
| Zn     | 0.045                  | 2.73      | 3.04        |

Conditions as in Fig. 3a.

of the transition elements. High levels of alkali and alkaline earth metals or other chelating agents cause significant interferences and/or sensitivity losses for most analytical techniques used for trace metal determinations. As alkali and alkaline earth metal ions can be efficiently separated from the transition metals with the R-CONHOH column (Fig. 3a), only the effects of chelating agents on the separation of transition metal ions were studied in more detail in this work. EDTA and ascorbic acid were used to simulate a powerful chelating agent and reducing agent, respectively, and humic acid was taken as a representative of colloidal substances with a complicated structure and unknown molecular mass. In the presence of ascorbic acid ( $10^{-3}$  M), the peak height decreased significantly, but the effect was reduced distinctly as the concentration of ascorbic acid decreased (Table 5 and Fig. 4a). The most pronounced effects are with EDTA (Fig. 4b) and humic acid (Fig. 4c). The reason might be due to the complicated properties of the latter and the strong chelation properties of these substances. With varying concentrations of these chelating agents, the results indicate that even in the presence of a strong chelating agent,  $10^{-6}$  M EDTA, the recovery is more than 90% (Table 4). In general, the concentration of EDTA in natural water is not higher than  $10^{-7}$  M [21]. Hence the proposed method might be

Table 5  
Recoveries of metal ions in the presence of complexing agents

| Interference               | Recovery (%) |        |        |        |        |        |
|----------------------------|--------------|--------|--------|--------|--------|--------|
|                            | Mn(II)       | Cd(II) | Fe(II) | Co(II) | Ni(II) | Zn(II) |
| Ascorbic acid <sup>a</sup> | 36.40        | 41.17  | 45.31  | 44.25  | 38.94  | 51.85  |
| Ascorbic acid <sup>b</sup> | 76.44        | 87.71  | 89.45  | 81.73  | 80.68  | 90.12  |
| EDTA <sup>c</sup>          | 74.07        | 74.04  | 69.60  | 74.99  | 74.58  | 74.28  |
| EDTA <sup>d</sup>          | 99.85        | 97.55  | 91.40  | 97.57  | 96.52  | 98.41  |
| Humic acid <sup>e</sup>    | 77.99        | 84.68  | 91.87  | 75.68  | 70.36  | 88.59  |

Conditions as in Fig. 4.

<sup>a</sup> [Ascorbic acid] =  $10^{-3}$  M.

<sup>b</sup> [Ascorbic acid] =  $10^{-4}$  M.

<sup>c</sup> [EDTA] =  $10^{-5}$  M.

<sup>d</sup> [EDTA] =  $10^{-6}$  M.

<sup>e</sup> [Humic acid] = 10 ppm.

applicable to the determination of trace metal ions in natural water.

#### 4. Conclusion

CE is a versatile and efficient method for the separation of inorganic cations. Selective and sensitive detection in the low ppm range is made possible by proper modification of the silica surface and UV-absorbing buffer component.

Compared with IC, the greatest advantage is the lower cost of the separation system, higher efficiencies and shorter separation time. In this investigation, the complexation took place heterogeneously at the wall and the separation mechanism may be more complicated than that only based on CE. The chromatographic behaviour of the open-tubular type of LC column, with associated mass transfer dispersion, might also be involved in tubes of 100  $\mu$ m I.D. However, this is the first attempt to effect surface modification of fused silica by chemical bonding of chelating groups for the separation of transition metal ions. The use of a smaller diameter tube for the surface modification is now in progress. Moreover, the most significant feature of these capillary columns is their stability and performance in complex matrices. With only a dilution required for the sample preparation, an environmental sample, a fermentation broth or biological sample could be analysed for metal cations with these capillary columns, especially the R-CONHOH column.

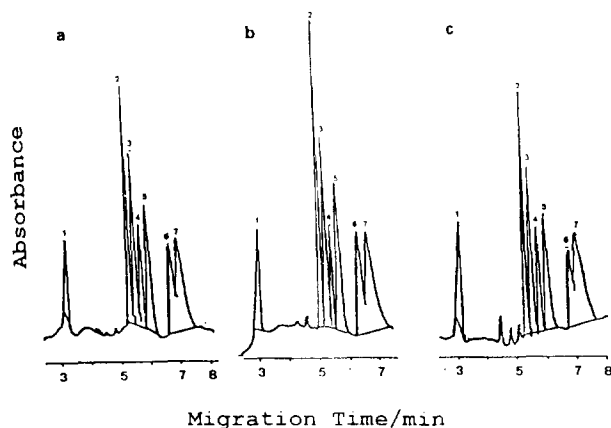


Fig. 4. Separation of metal ion mixtures in the presence of chelating agents. Column, R-CONHOH; conditions as Fig. 3. Sample: (a) K, Cd, Co, Fe, Mn, Ni and Zn ( $5 \cdot 10^{-5}$  M each) in  $10^{-4}$  M ascorbic acid; (b) sample (a) in  $10^{-6}$  M EDTA; (c) sample (a) in 10 ppm humic acid. Peaks: 1 = K; 2 = Mn; 3 = Cd; 4 = Fe(II); 5 = Co; 6 = Ni; 7 = Zn.

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