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Short communication

# On-column complexation of metal ions using 2,6-pyridinedicarboxylic acid and separation of their anionic complexes by capillary electrophoresis with direct UV detection

ZuLiang Chen\*, Ravendra Naidu

*CSIRO Land and Water, Adelaide Laboratory, PMB2, Glen Osmond, SA 5064, Australia*

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## Abstract

On-column complexation of metal ions with 2,6-pyridinedicarboxylate (2,6-PDC) to form anionic complexes enabled their separation by capillary zone electrophoresis with direct UV detection at 214 nm. Nine metal ions,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$ , were determined in less than 7 min using 10 mM 2,6-PDC solution containing 0.75 mM tetradecyltrimethylammonium bromide at pH 4.0. Satisfactory working ranges (20–300  $\mu\text{M}$ ), detection limits (3–10  $\mu\text{M}$ ) and good repeatability of the peak areas (RSD 2.1–4.2%,  $n=5$ ) were obtained using hydrodynamic injection (30 s). The proposed method was used successfully for the determination of  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$  in groundwaters.

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## 1. Introduction

Concern over the possible health and ecosystem effects of metals in soil has received much attention in recent years. The interactions between metal ions and dissolved organic solutes are of particular importance because of their role in mineral weathering and soil formation processes, as well as their potential role in heavy metal contamination of soil and groundwater [1]. There are a number of different methods currently used for the determination of metal ions, such as atomic absorption spectrometry

(AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) or mass spectrometry (ICP-MS) and ion chromatography (IC) with its various separation modes [2]. These methods are useful for the determination of metal ions with high efficiency, selectivity and sensitivity, and are thus suitable for the determination of metal ions in environmental samples.

Capillary zone electrophoresis (CZE) has the potential to become an alternative to ion chromatography for the analysis of ionic species. The use of CZE for the analysis of metal ions has developed rapidly over the past few years [3–8]. To enhance selectivity, the addition of a complexing reagent to the background electrolyte (BGE) to form metal complexes has been proposed to provide a greater

\*Corresponding author. Tel.: +61-8-8303-8421; fax: +61-8-8303-8565.

E-mail address: [zuliang.chen@csiro.au](mailto:zuliang.chen@csiro.au) (Z. Chen).

difference in electrophoretic mobility. In principle, two approaches are used in CE separation of metals. One is on-column complexation, where a soluble ligand is added to the running electrolyte and weak complexes are rapidly formed. Indirect UV detection is usually employed and carboxylic acids are usually used as the weak ligands [7,8]. Another approach is pre-column complexation, where an excess of strong ligand is added to the sample to form complexes prior to CE analysis [6]. This method allows for direct UV detection of the metal ions after chelation with suitable UV-absorbing ligands.

Many carboxylic acids have been used as the complexing reagents when using the on-column complexation approach, including acetic, glycolic, lactic,  $\alpha$ -hydroxyisobutyric (HIBA), oxalic, malonic, malic, tartaric, succinic and citric acids [9–11]. In addition, chromophore ions such as aromatic amines, imidazole, pyridine and  $\text{Cu}^{2+}$  [9–15] have been added to the BGE for the indirect UV detection of metals. However, there are only a few reports on direct UV detection using on-column complexation of metal ions. Previously, 2,6-pyridinedicarboxylate (2,6-PDC) was used as the mobile phase in ion chromatography for UV detection of inorganic anions, cations, and carboxylic acids [16–20]. Recently, 2,6-PDC has also been used as the BGE for on-column complexation in the separation of lanthanides [21], and some heavy metals [22]. Unfortunately, metal ions such as  $\text{Mn}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$ , which play important roles in geochemistry, were not included [23]. Hence, in this study, on-column complexation of metal ions with 2,6-PDC was investigated systematically to establish a new CZE method for the separation of metal ions. As part of the method development, studies included the following aspects: (1) optimisation of on-column complexation and co-electroosmotic flow (co-EOF) separation; and (2) demonstration of the determination of metal ions in groundwaters.

## 2. Experimental

### 2.1. Chemicals and solutions

All reagents (analytical grade) were obtained from Sigma–Aldrich (Sydney, Australia) and dissolved in

Milli-Q water without further purification. Standard solutions of the metal ions were diluted daily from 10 mM stock solutions using Milli-Q water and adjusted to pH 3.0 with 1 mM HCl. BGEs required for CE were prepared by dissolution of appropriate amounts of 2,6-PDC in Milli-Q water containing appropriate amounts of cationic surfactants. All electrolytes were filtered through a Millipore 0.45  $\mu\text{m}$  membrane filter. The pH of the BGE was adjusted with 0.1 M NaOH or 0.1 M HCl solution. Groundwater samples were filtered through a 0.45  $\mu\text{m}$  membrane filter.

### 2.2. Instrumentation

All CE experiments were performed using a Quanta 4000 instrument (Waters, Milford, USA). The system was controlled by Millennium (Waters) software. Separation was carried out using a 56 cm fused-silica capillary [56 cm (49.5 cm effective length)  $\times$  50  $\mu\text{m}$  I.D.]. The UV wavelength was set at 214 nm.

### 2.3. Electrophoretic procedures

Prior to use, the capillary was pre-treated with the following cycle: 0.1 M NaOH for 20 min, 0.01 M NaOH for 20 min, deionized water for 20 min and 10 mM electrolyte for 30 min. The capillary was pre-conditioned with the electrolyte for 2 min before each run. Samples were injected in the hydrostatic mode for 30 s. The capillary was held at 25  $^{\circ}\text{C}$ , and the applied constant voltage was  $-20$  kV. Identification of each of the solutes was based on the migration time and was verified by spiking samples with known standards. Benzyl alcohol (0.05%, v/v) was used as a neutral marker for the determination of the electrophoretic mobility.

## 3. Results and discussion

### 3.1. On-column complexation and separation conditions

Work on 2,6-PDC as an eluent in ion chromatography [24–26] has shown that the eluent pH plays an important role in the formation of anionic complex-

es. Similarly, the conversion of metal ion to anionic complex on the column is highly dependent upon on the electrolyte pH and the concentration of 2,6-PDC [27]:



As 2,6-PDC is an ionisable compound ( $pK_{a1}=2.16$ ,  $pK_{a2}=6.92$ ), its ligand concentration in the BGE depends on the pH, as shown in Eq. (2). The protonated ligand concentration is favoured with increasing pH, and consequently the degree of metal complexation with 2,6-PDC is increased. However, increasing the electrolyte pH not only influences metal hydrolysis, as shown in Eq. (3), but also the EOF, and, consequently, pH changes affect both the separation selectivity and the detection sensitivity. Therefore, optimisation of the electrolyte pH is required. A 10 mM 2,6-PDC solution containing 0.75 mM tetradecyltrimethylammonium bromide (TTAB) was used as the BGE. It was found that the optimum electrolyte pH was 4.0 and this pH was used in subsequent studies.

In order to obtain fast CZE separation of the metal complexes, cationic surfactants can also be added to the electrolyte so that the EOF moves in the same direction as the metal under co-EOF mode [27]. However, faster migration often results in poorer resolution. Hence, different cationic surfactants were added to the electrolyte containing 10 mM 2,6-PDC at pH 4.0 to obtain a reasonable optimal resolution in a reasonable time. Fig. 1 shows a comparison of the electropherograms obtained using (A) no surfactant, (B) 0.75 mM cetyltrimethylammonium bromide (CTAB), (C) 0.75 mM TTAB and (D) 5 mM tetramethylammonium hydroxide (TMAOH). Without surfactant in the electrolyte, a longer migration time was observed for the separation of the metal complexes, because the EOF direction was opposite to that of the electrophoretic migration of the metal complexes (Fig. 1A). However, when CTAB and TTAB (Fig. 1B and C) were added to the BGE, the migration times of the metal complexes were dramatically reduced. This was because the EOF

now moved in the same direction as the metal complexes. In addition, the EOF was larger when CTAB (EOF:  $-2.64 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) and TTAB (EOF:  $-2.33 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) were present in the electrolyte. However, a better resolution was obtained using TTAB as the EOF modifier. This can be attributed to a lower EOF in the presence of TTAB in the electrolyte, which leads to a broader separation window. Similar results were obtained in our previous studies of the separation of organic anions using the co-EOF mode [28,29]. In contrast to these cationic surfactants, only a few CE studies have discussed the role of ion-pairing reagents in improving selectivity [30,31]. Recently, Liu et al. [32] demonstrated the improved pre-column separation resolution of metal complexes using different ion-pairing reagents. Fig. 1D was obtained using 10 mM 2,6-PDC electrolyte with 5 mM TMAOH at pH 4.0. A longer migration and broader separation window was achieved using TMAOH due to a lower EOF value ( $-1.01 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ ). The resolution was improved between most metal complexes with the exception of  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ . The addition of an ion-pairing reagent can yield changes in selectivity due to ion-pair interactions between the ion-pairing reagent and the solute. This interaction results in alterations of the zeta potential at the capillary wall and possibly prevents solute-wall interactions that lead to increased dispersion [31].

### 3.2. Analytical performance of the method

The above experiments suggest that optimal complexation and separation can be achieved using an electrolyte containing 10 mM 2,6-PDC and 0.75 mM TTAB at pH 4.0. These conditions allowed the simultaneous separation of nine metal ions. However, co-migration of  $\text{Co}^{2+}$  with  $\text{Zn}^{2+}$  was observed. Other metal ions, including  $\text{Mg}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Ag}^+$ , were also tested under the same conditions for the possibility of interference. It was found that either no, or only small, peaks were obtained under these conditions and the peaks did not interfere with the separation. The migration order in Fig. 1C is  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$ . The migration order reflects differences in both the charge and the size of the anionic metal complexes based on the change in

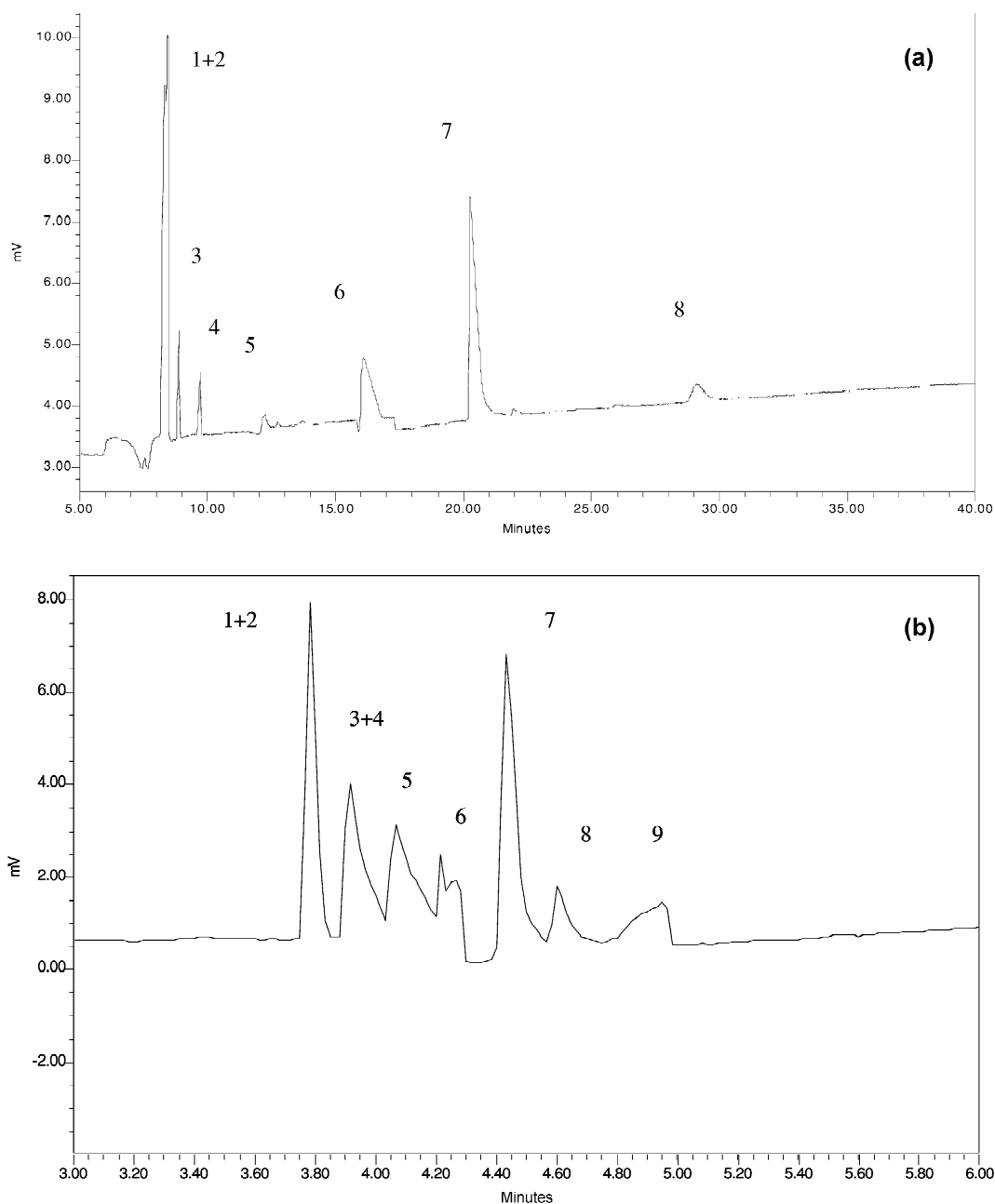


Fig. 1. Electropherograms of nine metal ions (0.10 mM) obtained using 10 mM 2,6-PDC electrolyte at pH 4.0, containing: (A) no surfactant, (B) 0.75 mM CTAB, (C) 0.75 mM TTAB, and (D) 5 mM TMAOH. (1)  $\text{Cu}^{2+}$ ; (2)  $\text{Zn}^{2+}$ ; (3)  $\text{Ni}^{2+}$ ; (4)  $\text{Cd}^{2+}$ ; (5)  $\text{Mn}^{2+}$ ; (6)  $\text{Pb}^{2+}$ ; (7)  $\text{Fe}^{3+}$ ; (8)  $\text{Al}^{3+}$ ; (9)  $\text{Ca}^{2+}$ . Conditions: capillary, fused-silica capillary 55 cm (effective length 48.5 cm)  $\times$  50  $\mu\text{m}$ ; electrolyte, 10 mM PDC; applied potential, -20 kV; hydrostatic injection, 30 s; UV detection at 214 nm; capillary temperature, 25  $^{\circ}\text{C}$ ; Concentration of each metal, 0.1 mM.

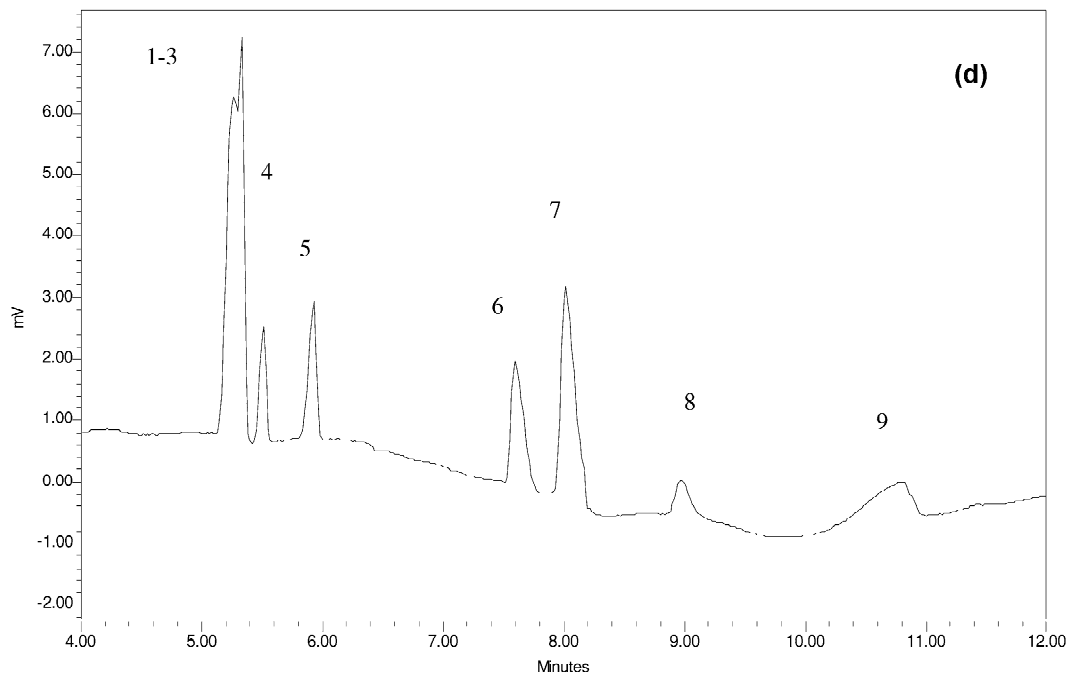
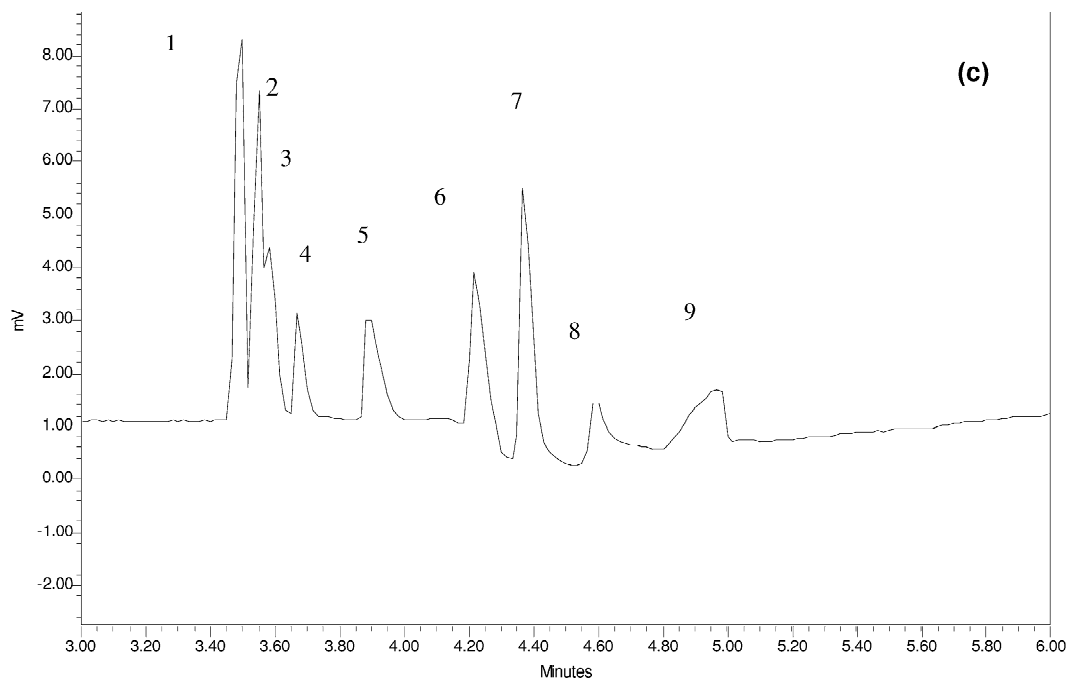


Fig. 1. (continued)

Table 1

Performance characteristics of the proposed method for the determination of metal ions

Metal ion	Regression line	Coefficient	Detection limit ( $\mu M$ )	Reproducibility (peak area, $n=5$ , %)
Cu <sup>2+</sup>	$y = 3.73 \cdot 10^4 x + 1.0 \cdot 10^2$	0.998	3.0	2.1
Zn <sup>2+</sup>	$y = 2.95 \cdot 10^4 x + 1.4 \cdot 10^2$	0.998	5.0	2.7
Ni <sup>2+</sup>	$y = 1.96 \cdot 10^4 x + 2.0 \cdot 10^2$	0.996	8.0	3.9
Cd <sup>2+</sup>	$y = 8.35 \cdot 10^3 x + 9.3 \cdot 10^2$	0.994	9.0	4.2
Mn <sup>2+</sup>	$y = 1.65 \cdot 10^4 x + 1.6 \cdot 10$	1.000	9.0	3.4
Pb <sup>2+</sup>	$y = 2.65 \cdot 10^4 x - 6.4 \cdot 10^2$	0.997	7.0	4.1
Fe <sup>3+</sup>	$y = 3.33 \cdot 10^4 x - 7.07 \cdot 10^2$	1.000	6.0	2.8
Al <sup>3+</sup>	$y = 1.24 \cdot 10^4 x - 1.38 \cdot 10^2$	1.000	10.0	3.4
Ca <sup>2+</sup>	$y = 1.87 \cdot 10^4 x - 2.51 \cdot 10^2$	1.000	10.0	3.9

Conditions as in Fig. 1C.

selectivity brought about by the addition of 2,6-PDC to the electrolyte, which can be related to their conditional stability constants [18–20]. However, poor resolution was observed between the Zn<sup>2+</sup>, Ni<sup>2+</sup> and Co<sup>2+</sup> complexes due to their similar mobility.

The detection limits ( $S/N=3$ ) were determined using hydrostatic injection for 30 s and were in the range from 3 to 10  $\mu M$ . All metal ions tested gave a

linear relationship between peak and concentration over a range of at least two orders of magnitude and correlation coefficients ( $n=5$ ) ranged from 0.994 to 1.000. The reproducibility was determined from five consecutive runs of a mixed standard solution at a concentration of 50  $\mu M$  for each metal and showed that the RSDs (relative standard deviations) of the migration times and peak areas were less than 1.05 and 3.9%, respectively. The performance characteris-

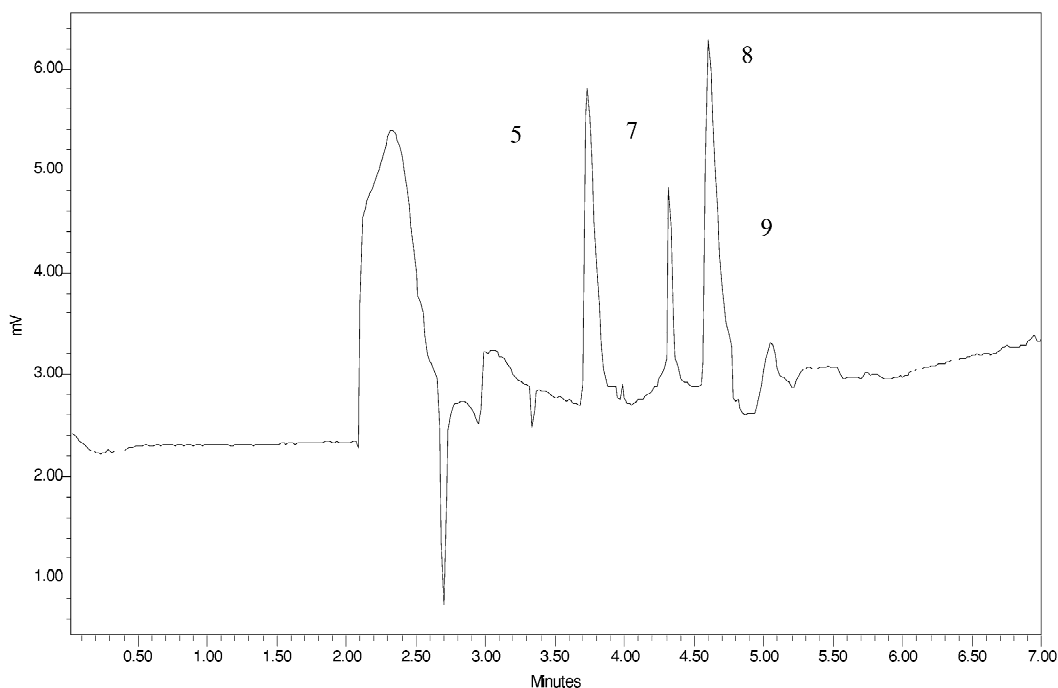


Fig. 2. Typical electropherogram obtained from underground waters. Other conditions as in Fig. 1C.

tics of the proposed method are summarised in Table 1.

The proposed method was applied to metal determination in groundwaters. Fig. 2 shows the electropherograms of groundwater sample. All samples were analysed in triplicate with satisfactory reproducibility of the peak areas (1.5–4.5% RSD). Recoveries for the metal ions were 90.5 to 102.5% for groundwater spiked with a 0.1 mM mixed standard. Results were obtained from the analysis of five groundwater samples from different sites (Newcastle, Australia). Higher concentrations (0.031–1.69 mM) of  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  and  $\text{Ca}^{2+}$  were found in all tested samples. Other metal ions of interest were not present in the samples. Even though other metal ions were not detected in the samples, it is possible that the concentrations of these metals in these groundwaters were too low to be detected by the proposed method (detection limits: 3.0–10  $\mu\text{M}$ ). However, the proposed method offers the advantages of fast analysis and requires minimal sample treatment apart from sample dilution.

#### 4. Conclusions

The findings of this study demonstrate that on-column complexation can be used for the separation of metal ions. On-column complexation is a simple procedure compared to that of pre-column complexation. Separation of heavy metals was possible using a new 2,6-PDC electrolyte containing 0.75 mM TTAB at pH 4.0, and cationic surfactants were used to manipulate the separation selectivity. The proposed method was demonstrated for the determination of metal ions in groundwaters and exhibited rapidity and direct injection of sample to CE. The potential applications of this method may include other environmental samples such as soil solutions and plant extracts, which could provide information on bioavailability. To increase the utility of the method, work on improving the detection limits using a sample-stacking technique is in progress.

#### References

- [1] R.D. Harter, R. Naidu, *Adv. Agron.* 55 (1995) 219.
- [2] C. Sarzanini, *J. Chromatogr. A* 850 (1999) 213.
- [3] A.R. Timerbaev, *Talanta* 52 (2000) 573.
- [4] A.R. Timerbaev, O.A. Shipgun, *Electrophoresis* 21 (2000) 4179.
- [5] C. Vogt, G.L. Klunder, *Fresenius J. Anal. Chem.* 370 (2001) 316.
- [6] B.F. Liu, L.B. Liu, J.K. Cheng, *J. Chromatogr. A* 834 (1999) 277.
- [7] M. Chiari, *J. Chromatogr. A* 905 (1998) 1.
- [8] V. Pacakova, P. Coufal, K. Stulik, *J. Chromatogr. A* 834 (1999) 257.
- [9] Y. Shi, J.S. Fritz, *J. Chromatogr.* 640 (1993) 473.
- [10] T.I. Lin, Y.H. Lee, Y.C. Chen, *J. Chromatogr. A* 654 (1993) 167.
- [11] Y.H. Lee, T.I. Lin, *J. Chromatogr. A* 675 (1994) 227.
- [12] C. Quang, M.G. Khaledi, *J. Chromatogr. A* 659 (1994) 459.
- [13] L. Arce, A. Rios, M. Valcarcel, *J. Chromatogr. A* 791 (1997) 279.
- [14] J.M. Xu, Z.L. Chen, C. Tang, J.C. Yu, *J. High Resolut. Chromatogr.* 23 (2000) 511.
- [15] N. Shakulashvili, T. Faller, H. Engelhardt, *J. Chromatogr. A* 895 (2000) 205.
- [16] Z.L. Chen, M.A. Adams, *Chromatographia* 49 (1999) 496.
- [17] Z.L. Chen, C. Tang, J. Xu, *J. Chromatogr. A* 859 (1999) 173.
- [18] X.J. Ding, S.F. Mou, K.N. Liu, A. Siriaks, J. Riviello, *Anal. Chim. Acta* 407 (2000) 319.
- [19] K.A. Ruth, R.W. Shaw, *J. Chromatogr.* 546 (1991) 243.
- [20] S. Motellier, H. Pitsch, *J. Chromatogr. A* 739 (1996) 119.
- [21] N. Oztekin, F.B. Erim, *J. Chromatogr. A* 924 (2001) 541.
- [22] T. Soga, G.A. Ross, *J. Chromatogr. A* 834 (1999) 65.
- [23] Y. Ge, P. Murray, W.H. Hendershot, *Environ. Pollut.* 107 (2000) 137.
- [24] N. Cardellicchio, S. Cavalli, J.M. Riviello, *J. Chromatogr. A* 770 (1997) 185.
- [25] C. Bonn, S. Reiffenstuhel, *J. Chromatogr.* 499 (1990) 669.
- [26] C.A. Lucy, H.N. Dinh, *Anal. Chem.* 66 (1994) 793.
- [27] Z.L. Chen, R. Naidu, A. Subramanian, *J. Chromatogr. A* 927 (2001) 219.
- [28] R. Naidu, Z.L. Chen, *Chromatographia* 54 (2001) 495.
- [29] Z.L. Chen, G.S.R. Krishnamurti, R. Naidu, *Chromatographia* 53 (2001) 179.
- [30] C.M. Shelton, J.T. Koch, N. Desai, J.F. Wheeler, *J. Chromatogr. A* 792 (1997) 455.
- [31] M.K. Weldon, C.M. Arrington, P.L. Runnels, J.F. Wheeler, *J. Chromatogr. A* 758 (1997) 293.
- [32] B.F. Liu, L.B. Liu, J.K. Cheng, *J. Chromatogr. A* 848 (1999) 473.