



ELSEVIER

Journal of Chromatography A, 687 (1994) 343–349

JOURNAL OF  
CHROMATOGRAPHY A

# Separation of metallo–cyanide complexes by capillary zone electrophoresis

Wolfgang Buchberger, Paul R. Haddad\*

*Department of Chemistry, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia*

First received 26 May 1994; revised manuscript received 29 August 1994

## Abstract

The optimisation of electrolyte systems for the separation and detection of cyanide complexes of Au, Pt, Fe(II), Fe(III), Pd, Cu(I), Co, Ag, Cr and Ni using capillary zone electrophoresis is described. A phosphate–triethanolamine buffer at pH 8.5 was chosen for the separation. The factors affecting resolution and analysis time include the addition of ion-pairing reagents (which also act as electroosmotic mobility modifiers) as well as organic solvents to the carrier electrolyte. Less stable cyano complexes [such as those of copper(I)] require the presence of low concentrations of free cyanide in the electrolyte in order to achieve acceptable peak shapes. Direct UV detection at 214 nm is feasible for most of the metallo–cyanide complexes although some of them give higher response in an indirect mode at 254 nm using trimellitate as the carrier electrolyte. Approaches for preconcentration are discussed in order to achieve detection limits necessary for environmental samples and other applications.

## 1. Introduction

Cyanide is used extensively in the mining industry for extraction of gold from its ores as well as in the metal processing industry for electroplating. Furthermore, certain metallurgical processes can lead to the generation of cyanide as an undesired by-product. In all these cases, rigorous control of waste solutions as well as monitoring of cyanide in environmental samples taken from the vicinity of industrial plants is essential in order to protect the environment. Cyanide in environmental samples is likely to be

present in the form of metallo–cyanide complexes exhibiting varying persistence and toxicology. During recent years, ion–interaction chromatography has emerged as an efficient technique for separation of metallo–cyanides and has been employed for speciation analysis at  $\mu\text{g/l}$  levels [1].

Currently, capillary zone electrophoresis (CZE) is growing in significance as an analytical method for the separation of low-molecular-mass ionic species. It appears to be an attractive complementary technique to ion chromatography with promising features such as high separation efficiency, short analysis time and unique separation selectivity. CZE separation

\* Corresponding author.

procedures for a variety of small inorganic and organic anions and cations are already well documented in the literature, but its use for the separation of metallo–cyanides has not yet been fully exploited.

Aguilar et al. [2] have used CZE to separate hexacyanoferrate(II) and hexacyanoferrate(III) and have applied this method to the determination of iron cyanide complexes in zinc electroplating solutions. In another paper, Aguilar et al. [3] reported the separation of gold and silver cyanides in leaching solutions from ore. In both cases, a counterelectroosmotic mode was employed. The vectors of the electrophoretic mobilities of iron cyanides oppose the direction of the electroosmotic flow and exceed its magnitude. Therefore, these complexes were injected at the cathode and detected at the anode. Silver and gold cyanides show much lower electrophoretic mobilities and were injected at the anode and detected at the cathode.

In an attempt to improve the separation of metal ions by CZE, we have reported a pre-capillary derivatisation using cyanide with subsequent separation of the metallo–cyanides in a cyanide-containing carrier electrolyte [4]. This approach yielded satisfactory results for a range of metal ions, but the conditions are not suitable for the determination of metallo–cyanides in environmental samples since cyanide present in the carrier electrolyte could react with free metal ions in the sample, leading to erroneous results.

The aim of the work presented in this paper was the separation of a range of metallo–cyanides, preferably in a co-electroosmotic CZE mode, which would be applicable to complexes having either low or high electrophoretic mobility. Co-electroosmotic CZE of anionic species can only be carried out if the direction of the electroosmotic flow is reversed and directed to the anode. This reversal is generally accomplished by the addition of hydrophobic quaternary ammonium ions to the carrier electrolyte [5,6]. These electroosmotic flow modifiers can also act as ion-pairing reagents, and for this reason investigations into the potential of ion-pairing for manipulation of separation selectivity of metallo–cyanides have been included in our work.

## 2. Experimental

### 2.1. Instrumentation

The CZE instrument employed was a Quanta 4000 (Waters, Milford, MA, USA) interfaced to a Maxima 820 data station (Waters). Separations were carried out using an AccuSep (Waters) fused-silica capillary (60 cm  $\times$  75  $\mu$ m I.D., effective length 52 cm). Injection was performed hydrostatically by elevating the sample at 10 cm for a specified time. Direct UV detection at 214 nm or indirect UV detection at 254 nm was used.

### 2.2. Carrier electrolytes

The following carrier electrolytes were used for direct UV detection: phosphate buffer at pH 11 (prepared from 5 mM  $\text{Na}_3\text{PO}_4$  adjusted to pH 11 with phosphoric acid); phosphate buffer at pH 8 (prepared from 5 mM  $\text{K}_2\text{HPO}_4$  adjusted to pH 8 with phosphoric acid); phosphate–triethanolamine buffer at pH 8.5 (prepared from 5 mM  $\text{K}_2\text{HPO}_4$  and 5 mM triethanolamine, both adjusted to pH 8.5 with phosphoric acid). The carrier electrolyte for indirect UV detection consisted of 2.5 mM trimellitic acid adjusted to pH 9.5 with sodium hydroxide. The electroosmotic flow was adjusted by addition of varying amounts of hexamethonium bromide (Sigma, St. Louis, MO, USA). All carrier electrolytes were prepared from analytical-reagent grade chemicals using water purified with a Millipore (Bedford, MA, USA) Milli-Q water-treatment system.

### 2.3. Metallo–cyanide complexes

The following analytical-reagent grade metallo–cyanides were purchased:  $\text{KAu}(\text{CN})_2$  (Fluka, Buchs, Switzerland),  $\text{K}_2\text{Pt}(\text{CN})_4$  (Pfaltz & Bauer, Waterbury, CT, USA),  $\text{K}_3\text{Fe}(\text{CN})_6$  (Fluka),  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$  (Fluka). Other metallo–cyanides, namely  $\text{K}_2\text{Pd}(\text{CN})_4$ ,  $\text{K}_3\text{Cu}(\text{CN})_4$ ,  $\text{K}_3\text{Co}(\text{CN})_6$ ,  $\text{KAg}(\text{CN})_2$ ,  $\text{K}_3\text{Cr}(\text{CN})_6$  and  $\text{K}_2\text{Ni}(\text{CN})_4$  were synthesised using previously reported procedures [7].

### 3. Results and discussion

#### 3.1. Control of the electroosmotic flow

The addition of hydrophobic trimethylalkylammonium salts is generally recommended for applications requiring suppression or reversal of the electroosmotic flow [5]. Unfortunately, attempts to use these salts for the separation of metallo-cyanides were unsuccessful because they tended to form precipitates with some of the complexes. Recently, 1,6-bis(trimethylammonium)hexane (hexamethonium bromide) has been introduced for control of the electroosmotic flow [6]. Our investigations indicated that carrier electrolytes consisting of phosphate buffers at pH 8 or 11 and up to 0.8 mM hexamethonium bromide can avoid the problem of precipitation. On the other hand, the electroosmotic flow was found not to be reversed but only decreased to a certain extent. Data for the electroosmotic mobility are shown in Fig. 1. These data suggest that a co-electroosmotic CZE mode cannot be employed as originally intended but the decrease in the electroosmotic flow might be sufficient to allow the separation of a broad range of metallo-cyanides using the anodic side for detection.

$\text{Ag}(\text{CN})_2^-$  exhibits the lowest electrophoretic mobility of the metallo-cyanides included in this study. Its electrophoretic mobility was found to be  $-5.75 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ . The observed mobility (being the vector sum of the electrophoretic and the electroosmotic mobility) of

$\text{Ag}(\text{CN})_2^-$  must exceed  $-1.30 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  in order to obtain an analysis time of less than 20 min using the anode as the detection side. Therefore, the electroosmotic mobility must be decreased to at least  $4.45 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ . Fig. 1 indicates that the necessary extent of decrease can be accomplished at pH 8 but not at pH 11, although the latter pH would be more favourable with respect to the stability of the metallo-cyanides.

#### 3.2. Optimisation of separation selectivity

Initial experiments on optimisation of separation selectivity were performed employing a phosphate buffer at pH 8 as the carrier electrolyte. Under these conditions reproducible peaks could be obtained only if the electrolyte in the vial at the (anodic) detection side was replaced after each run. This behaviour was attributed to the fact that a phosphate buffer at pH 8 has a relatively low buffer capacity, so that after two or more consecutive runs hydrogen ions generated at the anode lower the pH to such an extent that considerable dissociation of metallo-cyanides occurs in the capillary during the run. Therefore, all further experiments were performed using a phosphate-triethanolamine buffer at pH 8.5 as the carrier electrolyte, since this mixture exhibits a much higher buffer capacity and avoids the necessity of frequent buffer replacement.

Fig. 2 shows a typical electropherogram of a mixture of metallo-cyanides using 0.8 mM hexamethonium bromide for controlling the electroosmotic flow. The solute concentrations used were in the range 5–15  $\mu\text{g}/\text{ml}$  (calculated as the metal). Separation selectivity can easily be manipulated by varying the concentration of the hexamethonium bromide. Fig. 3 demonstrates the dependence of observed mobilities upon the concentration of hexamethonium bromide. It can be assumed that the observed changes in migration order are due predominantly to ion-pairing effects. Fig. 3 also indicates that different separation selectivities can be achieved at lower concentrations of hexamethonium bromide only if analysis time is sacrificed. A solution to this problem might be the use of electrolyte gra-

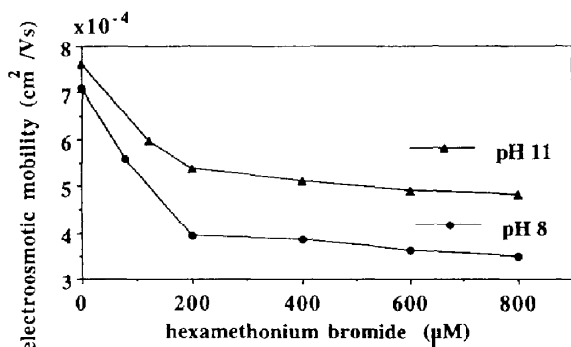


Fig. 1. Dependence of electroosmotic mobilities in phosphate buffers on the concentration of hexamethonium bromide.

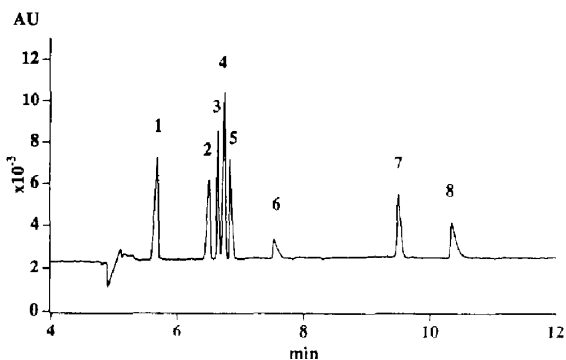


Fig. 2. Electropherogram of a mixture of metallo-cyanides. Carrier electrolyte: phosphate-triethanolamine buffer at pH 8.5 containing 0.8 mM hexamethonium bromide. Voltage: -20 kV. Injection time: 20 s. Detection: direct UV at 214 nm. Peaks: 1 = Fe(II); 2 = Pd; 3 = Co; 4 = Pt; 5 = Fe(III); 6 = Cr; 7 = Au; 8 = Ag.

dients. This technique is not yet widely used in CZE but its benefits have already been demonstrated by the work of Yeung and coworkers [8,9] who have employed a dynamic flow gradient induced by a step change in the concentration of cetyltrimethylammonium bromide (CTAB) in the carrier electrolyte. This step

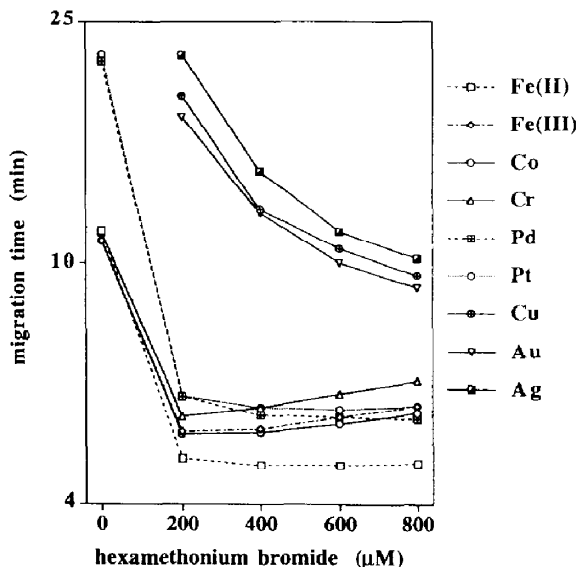


Fig. 3. Dependence of observed mobilities on the concentration of hexamethonium bromide (the y-axis is on a logarithmic scale).

change was created by injecting the sample into the capillary containing a low concentration of CTAB, then running with buffer electrolyte containing a high concentration of CTAB. In this case, the electroosmotic flow, initially directed to the cathode, is monotonically reduced to zero. We have used a similar approach for the separation of metallo-cyanides, although in this case the electroosmotic flow is not reduced to zero because unlike CTAB, hexamethonium bromide does not reverse the electroosmotic flow (see above).

An electropherogram obtained in the gradient mode from 0.1 to 0.8 mM hexamethonium bromide is shown in Fig. 4. The changes in selectivity are in accordance with the trends shown in Fig. 3. The step change in the hexamethonium bromide concentration causes a step in the baseline at approximately 5.5–6 min. Therefore, the baseline obtained from a blank run was subtracted from the electropherogram given in Fig. 4, and some minor peaks appearing in this electropherogram are artefacts from the subtraction process. These baseline interferences can be avoided by using hexamethonium chloride instead of the bromide salt since the former does not show significant absorbance at the detection wavelength.

A further approach to manipulation of separation selectivity consists of employing carrier electrolytes containing organic solvents. Fig. 5 illustrates the dependence of observed mobilities

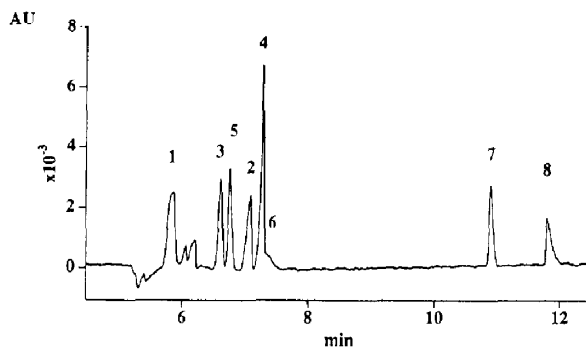


Fig. 4. Electropherogram of a mixture of metallo-cyanides using a gradient from 0.1 to 0.8 mM hexamethonium bromide. All other conditions and peaks as in Fig. 2.

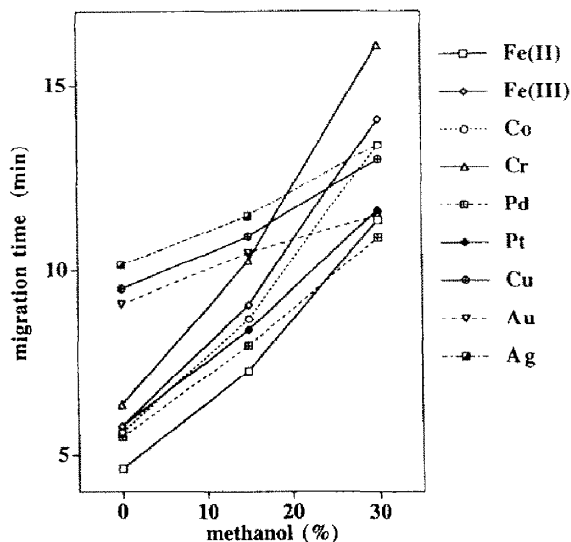


Fig. 5. Dependence of observed mobilities on the amount of methanol in the carrier electrolyte.

on the amount of methanol in the carrier electrolyte. The addition of methanol causes a general increase in the migration times of all complexes as a result of changes in the electroosmotic flow. Besides this general trend, there are several changes in migration order, as the slopes of the lines in Fig. 5 are lower for complexes with a low charge and higher for complexes with a high charge. The observed changes in separation selectivity may be a result of the change in hydration of the charged species in the presence of methanol.

Injection of the tetracyano complex of copper(I) resulted in a peak migrating between the cyanide complexes of gold and silver, but the peak shape was poor. Copper(I) forms cyano complexes containing 2 to 4 cyanide ions, so that during migration through the capillary different copper cyano species will be formed according to the respective dissociation constants and kinetics. The peak shape could be improved considerably by adding cyanide (0.02 to 0.3 mM) to the carrier electrolyte. Increasing the cyanide concentration in the carrier electrolyte also caused an increase in the electrophoretic mobility of the copper cyano complex. A speciation calculation based on stability constants from the

literature [10,11] indicated that under the conditions chosen Cu(I) should form cyano complexes containing between 2 and 3 cyanide ligands and should therefore have an effective charge between  $-1$  and  $-2$ . The increase in electrophoretic mobility is due mainly to the increase in charge as can be seen from Fig. 6. Slight deviations from linearity in Fig. 6 may be due to changes in the size and shape of the species.

### 3.3. Optimisation of detection

Most of the metallo-cyanide complexes investigated in this study exhibit UV absorption maxima in the wavelength range from 200 to 220 nm [1]. Therefore, a fixed-wavelength UV detector at 214 nm was employed. A 20-s hydrostatic injection yielded detection limits (defined as three times the signal-to-noise ratio and calculated as the metal) of  $0.16 \mu\text{g/ml}$  for Fe(II),  $0.64 \mu\text{g/ml}$  for Pd,  $0.15 \mu\text{g/ml}$  for Co,  $0.39 \mu\text{g/ml}$  for Pt,  $0.21 \mu\text{g/ml}$  for Fe(III),  $1.1 \mu\text{g/ml}$  for Cr,  $1.2 \mu\text{g/ml}$  for Au and  $2.5 \mu\text{g/ml}$  for Ag. Unfortunately, at 214 nm the chromium cyanide (which may be important in several environmental applications) has only a moderate molar extinction coefficient compared with cyano complexes of e.g. Fe(II) and Fe(III). For this reason, electrolytes were investigated which would permit the use of indirect UV detection as a more universal detection technique. Best results were obtained using 5 mM trimellitic acid adjusted to pH 9.5 as the carrier electrolyte, with

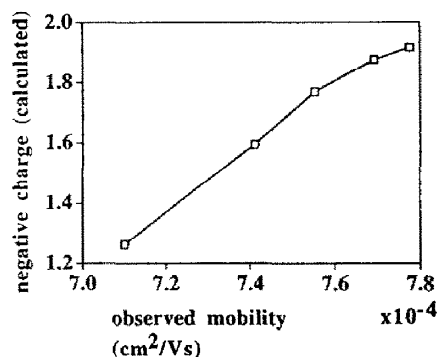


Fig. 6. Dependence of the observed mobility of the copper cyanide complex on the calculated charge.

detection at 254 nm. It must be taken into account that even at 254 nm some metallo-cyanides show molar extinction coefficients considerably higher than trimellitate. Therefore, the injection of a mixture of different metallo-cyanides may result in electropherograms comprising both negative and positive peaks. A typical example is given in Fig. 7 which shows the separation of the cyano complexes of Ni, Pd, Pt, Cr and Au. Here, the Pt peak is due to an increase in the absorbance resulting from the high absorptivity of the platinum complex, whereas all other peaks are caused by a decrease in the background absorbance. As one would anticipate, indirect detection improved the detectability of the chromium complex relative to other metallo-cyanides. Unfortunately, the absolute detection limit could not be improved significantly for chromium and was worse for other metallo-cyanides (1.0  $\mu\text{g}/\text{ml}$  for Cr, 1.3  $\mu\text{g}/\text{ml}$  for Pd, 1.6  $\mu\text{g}/\text{ml}$  for Pt, 3.6  $\mu\text{g}/\text{ml}$  for Au). The overall performance of the trimellitate carrier electrolyte in terms of resolution and efficiency was not as good as the carrier electrolyte described earlier for use with direct detection.

### 3.4. Approaches to sample preconcentration

The detection limits obtained with the proposed method are such that some environmental

applications may require the use of preconcentration procedures in order to achieve sufficient sensitivity. Fortunately, CZE itself includes a preconcentration step (known as electrostacking) when the ionic strength of the introduced sample plug is lower than that of the carrier electrolyte. Under these conditions, solute ions from the injected sample are focused into a short band due to the higher electric field in the sample region. Nevertheless, a prediction of the influence of sample volume on separation efficiency for a given separation is difficult so that an experimental verification is appropriate. Fig. 8 shows the number of theoretical plates (measured for the Au-cyano complex in an aqueous standard) as a function of the injection time. An increase in the injection time from 20 to 200 s results in a loss of separation efficiency by approximately 40%. In some applications this decreased efficiency may be offset by an approximately tenfold increase in sensitivity (e.g. the detection limit for Au is decreased to 0.16  $\mu\text{g}/\text{ml}$ ). Larger volumes will sooner or later lead to losses of some of the injected species. This phenomenon can be explained by the fact that the hexamethonium bromide adsorbed to the capillary wall will dissolve into the water of the sample plug, so the  $\zeta$  potential and the electroosmotic flow are increased in the sample region. Slow metal-cyano complexes cannot migrate fast enough to the boundary between the sample plug and the carrier electrolyte and are pumped

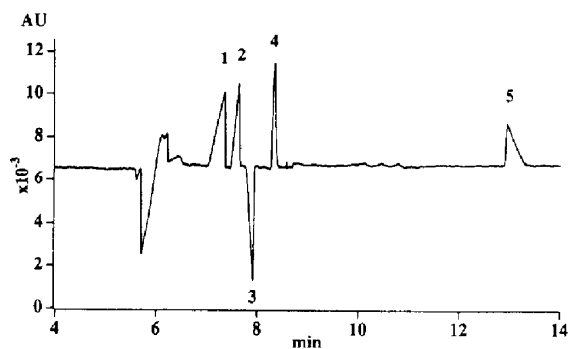


Fig. 7. Electropherogram of a mixture of metallo-cyanides using indirect UV detection at 254 nm. Carrier electrolyte: 2.5 mM trimellitate pH 9.5 containing 0.8 mM hexamethonium bromide. Voltage: -20 kV. Injection time: 30 s. Peaks: 1 = Ni; 2 = Pd; 3 = Pt; 4 = Cr; 5 = Au.

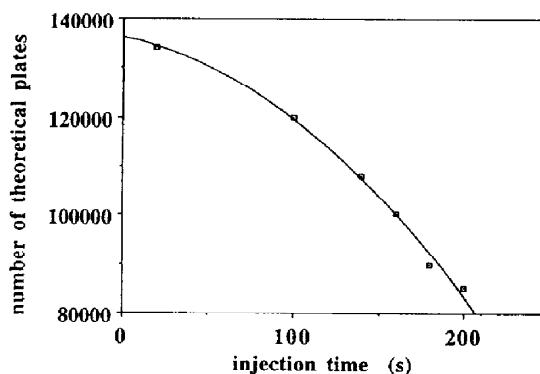


Fig. 8. Dependence of the number of theoretical plates (measured for the gold-cyanide complex) on the injection time.

out of the capillary by the increased electro-osmotic flow and are therefore lost. These aspects of large volume electrostacking have also been discussed recently by Burgi [12].

Preconcentration of some metallo–cyanides can also be achieved using off-line solid-phase extraction on reversed-phase  $C_{18}$  silica. The complexes can be enriched efficiently from aqueous solutions if an ion-interaction mode is applied. The preconcentrated complexes may be eluted by a small volume of an organic solvent such as methanol. Preliminary experiments were carried out for the determination of the Au–cyano complex in leaching solutions from a gold mine. A Sep-Pak  $C_{18}$  cartridge was preconditioned with 2 ml of 5 mM PIC A (Waters) as the ion-interaction reagent. A 20-ml aliquot of the sample was passed through the cartridge and then eluted with 1 ml methanol and 1 ml water, and the eluate was used for CE without any further treatment. This procedure yielded a recovery of 103.6% for an aqueous standard containing 4  $\mu\text{g/ml}$  Au (standard deviation 2.7%;  $n = 6$ ). A real mine sample containing 1.3  $\mu\text{g/ml}$  Au gave a recovery of 83%. This decreased recovery can be explained by the fact that the sample contained relatively high concentrations of anions, such as chloride and sulfate, which compete for the preconcentration sites on the Sep-Pak cartridge. Further investigations will have to include a careful optimisation of all steps involved in the sample preparation procedure, especially for samples with high ionic strengths.

#### 4. Conclusions

The results obtained in this work indicate that CZE is an attractive technique for the separation of various metal–cyanide complexes. High efficiencies and short analysis times are some of

the advantages of this method compared with the well-established technique of ion-interaction chromatography. The separation selectivity can be optimised for different applications by using ion-interaction reagents and/or organic solvents in the carrier electrolyte. Samples of high ionic strength are still a challenge for CZE as overloading effects are more pronounced than in chromatography. Adequate sample preparation procedures including preconcentration steps are crucial and will be the focus of further studies.

#### Acknowledgements

Financial support from Waters Corporation and the Australian Research Council are gratefully acknowledged.

#### References

- [1] P.R. Haddad and C. Kalambaheti, *Anal. Chim. Acta*, 250 (1991) 21.
- [2] M. Aguilar, X. Huang and R.N. Zare, *J. Chromatogr.*, 480 (1989) 427.
- [3] M. Aguilar, A. Farran and M. Martinez, *J. Chromatogr.*, 635 (1993) 127.
- [4] W. Buchberger, O.P. Semenova and A.R. Timerbaev, *J. High Resolut. Chromatogr.*, 16 (1993) 155.
- [5] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
- [6] M.P. Harrold, M.J. Wojtusik, J. Riviello and P. Henson, *J. Chromatogr.*, 640 (1993) 463.
- [7] D.F. Hilton and P.R. Haddad, *J. Chromatogr.*, 361 (1986) 141.
- [8] C.W. Whang and E.S. Yeung, *Anal. Chem.*, 64 (1992) 502.
- [9] H.-T. Chang and E.S. Yeung, *J. Chromatogr.*, 608 (1992) 65.
- [10] R.D. Hancock, N.P. Finkelstein and A. Evers, *J. Inorg. Nucl. Chem.*, 34 (1972) 3737.
- [11] R.M. Izatt, G.D. Watt, D. Eatough and J.J. Christensen, *J. Chem. Soc. (A)*, (1967) 1304.
- [12] D.S. Burgi, *Anal. Chem.*, 65 (1993) 3726.