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Short communication

# Advanced possibilities on multi-element separation and detection of metal ions by capillary zone electrophoresis using precapillary complexation

## I. Separation aspects

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### Abstract

Many metal ions can be separated by capillary zone electrophoresis (CE) using cyclohexane-1,2-diaminetetraacetic acid (CDTA) as a complexing reagent for precapillary derivatization of analytes. Adding an organic solvent, such as ethylene glycol, permits a wide selection in separation resolution. The effect of this electrolyte additive, as well as that of tetraalkylammonium salts, anionic and cationic surfactants, varying borate buffer concentration and some operational variables (applied voltage, sample volume, etc.), was studied in detail with regard to electrophoretic and electroosmotic mobility. The gain in resolution is due to the enhancing effect of electrolyte changes on the migration range and peak efficiency. An impact of modest electrophoretic velocity changes is believed to occur in a much lesser degree. Under optimized conditions, excellent separations of 23 metal-CDTA complexes within 10 min were obtained. Aluminium(III), antimony(III), bismuth(III), chromium(III), mercury(II), palladium(II), silver(I), thallium(I), tin(IV), uranium(VI), vanadium(IV) and zirconium(IV), which are scarcely subject to conventional CE procedures, were separated, along with several other metal ions, using a 20 mM sodium borate buffer (pH 9.0) containing 5% ethylene glycol and 1 mM CDTA. The problem of improving the detectability for complexed metal ions was also addressed.

**Keywords:** Metals; Cyclohexane-1,2-diaminetetraacetic acid; Metal complexes

### 1. Introduction

Since its introduction in analytical practice, much of the research on capillary electrophoresis (CE) has

involved metal ion separation studies. Precapillary reactions using chelating reagents are increasingly being used to carry out such separations [1]. A number of complexing reagents have already been examined for the CE separation of metal cations. Amongst these, are 4-(2-pyridylazo)resorcinol [2–5], 8-hydroxyquinoline-5-sulfonic acid [6,7], Arsenazo III [4] and various polyaminocarboxylic acids such as EDTA [8,9], CDTA [10–12] and others [13–15]. Inorganic ligands, like cyanide [16–20] and chloride

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[21–23], are generally less applicable as they suppose more rigid control on complexation conditions.

The most encouraging separation examples based on the above principle and obtained in our laboratories include the resolution of a 13-component mixture of different group metals after complexation with 2,6-diacetylpyridine bis(N-methylenepyridiniohydrazone) [24] and the separation of fifteen rare earth–CDTA complexes [11]. To our mind, the latter complexing reagent appears to show a greater promise due to its proven ability to resolve alkaline earth and transition metal ions. In order to exploit the possibility of using CDTA in full, in this work the separation behaviour was investigated for the complexes of the most commonly determined metal ions as well as of those metals which present a problem for the current CE methodology.

Manipulation of selectivity in CE is guided by altering the electrophoretic mobilities of analytes. For negatively charged species such as metal–CDTA complexes of our interest, this can primarily be effected by (i) compositional changes of the electrolyte buffer (i.e., by choice of its type [4–6], concentration [6,11,25,26] and pH [2,3,6,8,11,13,17,21,25,26]); (ii) addition of organic solvents, e.g., acetone [10] or acetonitrile [20]; (iii) employment of ionic surfactant of either anionic [2,4,7,10,27] or cationic [9,28] nature and (iv) use of ion-pairing cationic additives such as tetraalkylammonium salts of relatively short chain lengths [7,17,28,29]. In fact, every electrolyte parameter mentioned controls the rate of electroosmotic flow (EOF), the regulation of which is another way to improve the separation ability in CE. In this paper, all of these options were therefore explored, briefly or in detail, to ascertain their effects on resolution.

## 2. Experimental

A Waters Quanta 4000 CE system (Millipore Waters, Milford, MA, USA), equipped with a negative power supply was employed to generate all electropherograms. Polyimide-coated fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA) were of 50 cm in length with an I.D. of 75  $\mu\text{m}$  and a distance of 42.5 cm from the point of injection to the window for on-column detection. Direct UV

detection was carried out at 214 nm. A voltage of 15 kV was applied for separations, unless stated otherwise. The time of hydrodynamic injection was 15 s for most separations. Electropherograms were recorded and processed with a Hewlett-Packard 3359 data acquisition system. The dip of the water peak, originated from the background absorbance of CDTA-containing electrolytes, was used as a neutral marker for the EOF measurements. The electrophoretic mobility ( $\mu_{\text{ep}}$ ) was calculated from  $\mu_{\text{ep}} = \mu_{\text{eo}} - \mu_{\text{ob}}$ , where  $\mu_{\text{eo}}$  and  $\mu_{\text{ob}}$  are the electroosmotic and observed mobility, respectively.

New capillaries were conditioned by rinsing with 0.1 M NaOH for approximately 1 h, followed by a 20-min rinse with water. The 2-min rinses with 0.05 M NaOH and then with water were used to wash the capillary between runs with different electrolyte solutions. In addition, the capillary was purged with electrolyte solution for 2 min before each run.

All electrolytes were prepared with analytical-reagent grade chemicals and doubly distilled water and contained  $1 \times 10^{-3}$  M CDTA to prevent (i) the in-capillary dissociation of the complexes and (ii) interferences from metal ions present as impurities which could bind to silanol groups on the capillary wall and influence separations. Metal ion standard solutions were prepared as described elsewhere [22]. To prepare metal complexes, sufficient reagent, as a  $5 \times 10^{-3}$  M solution in 0.01 M sodium tetraborate, was added to metal stock solution to give a 2.5-fold molar excess in the final sample.

## 3. Results and discussion

In all previous studies [10–12,14], CE separations of metal–CDTA complexes were performed with borate buffer electrolytes recognized to be the optimum electrolyte system. Therefore initial experiments on the optimization of separation selectivity were attempted employing a simple sodium borate buffer at pH 9.0 as the carrier electrolyte. It should be noted that the selected pH value provides a constancy of both the charge (ionization degree) and chemical composition of the complexes [7,12,14]. Under these conditions, rather good CE separations could be obtained, as can be judged from Fig. 1 showing the separation of a test mixture, comprised

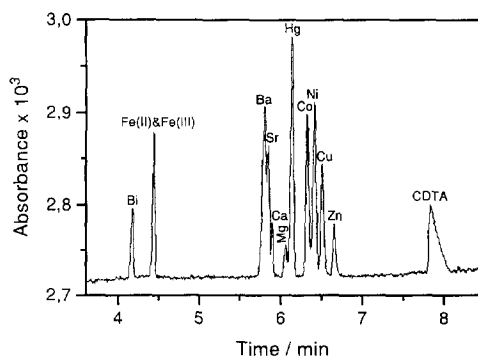


Fig. 1. Separation of metal complexes with non-modified borate electrolyte. Carrier electrolyte: 10 mM sodium borate containing 1 mM CDTA (pH 9.0). Metal ion concentration:  $5 \cdot 10^{-5}$  M Fe(II), Fe(III) and  $1 \cdot 10^{-4}$  M other metals.

of 12 different group metal ions, when there is no additives present in a 10 mM borate buffer. Obviously, to extend the multi-elemental ability, various challenges on regulating the resolution must have been investigated. These were directed toward altering either electrophoretic mobilities or the EOF or separation efficiency.

### 3.1. Effect of cationic additives

#### 3.1.1. Tetraalkylammonium salts

Iki et al. [28] found that incorporation of tetraalkylammonium salts into the electrolyte improved the separation of anionic complexes in CE by virtue of an ion-association effect. In earlier papers [4,7] we evaluated ion-pair formation in micellar-mediated CE systems and found that it contributes to selectivity only when metal complexes are sufficiently hydrophobic.

In the present investigation addition of tetrabutylammonium chloride or perchlorate led to only minor changes in selectivity. Over the range studied (up to 12 mM), the electrophoretic mobilities remained virtually constant. Resolution of iron(II) and iron(III) complexes became evident when the concentration of tetrabutylammonium chloride increased to 6 mM, and then, at 12 mM, was baseline completed, but this was accompanied by overlap of some of the slowly migrating peaks of transition metal complexes.

#### 3.1.2. Alkyltrimethylammonium salts

Next, an attempt was made to exploit alkylammonium salts such as 1,6-bis(trimethylammonium)hexane, or hexamethonium bromide (HMB). Recently, HMB was found to work well for manipulating the separation selectivity of anionic complexes [20], presumably owing to its 2+ charge. Although the main function of this electrolyte additive was supposed to reduce the EOF, the authors observed simultaneously certain changes in migration order of metal cyanides by varying the concentration of HMB.

The effect of HMB on the separation of metal-CDTA complexes was studied by measuring the electrophoretic mobilities and EOF at increasing HMB concentrations. Although values of  $\mu_{ep}$  were largely unaffected, resolution of alkaline-earth metal complexes improved slightly. This suggested the use of electrolyte gradients, as proposed by Buchberger and Haddad [20]. The step change in HMB concentration was created using different electrolytes for injecting the sample (i.e., within the capillary) and running (buffer vial). After trying several electrolyte combinations, the optimum gradient mode was achieved when the concentration of HMB changed from 0.1 to 0.5 mM. These conditions provided nearly-fully resolved alkaline earths complexes (the electropherogram is not shown), but some of the next migrating peaks tended to be superimposed.

### 3.2. Effect of anionic surfactant

Since the electrophoretic mobility of metal-CDTA complexes hardly changed in the presence of positively charged compounds the separation selectivity was further evaluated by introducing sodium dodecylsulphate (SDS) into the migrating electrolyte solution. It is well established [4,7] that SDS has a large effect on the electroosmotic mobility of anionic complexes. Furthermore, under certain conditions it can also affect their electrophoretic velocity via micellar solubilization of the solutes [2,4].

In fact, increasing the SDS concentration produced a marked decrease in the magnitude of the EOF; the ionic strength effects and hydrophobic interactions on the capillary wall may account for this result. At the same time, effective mobilities decreased considerably up to ca. 15 mM SDS as depicted in Fig. 2.

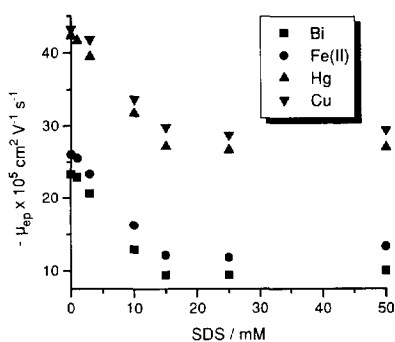


Fig. 2. Effect of SDS concentration on electrophoretic mobilities of metal complexes.

In view of the previous results, ion interaction between sodium cations and the complexed anions is unlikely to occur. Therefore, the most plausible mechanism to explain the variation in  $\mu_{ep}$  in Fig. 2 is one in which micellar partitioning overwhelms the electrostatic repulsion between similarly charged metal complexes and micelles. This mechanism is supported by the fact that these variations are only observed in the range of lower surfactant concentrations where the packing density of the capillary with micelles is rather low. When actual separations are viewed, the effect of using SDS, however, is not that impressive because the changes in electrophoretic mobility, far from being selective themselves, are compensated by a pronounced reduction of the oppositely directed EOF.

### 3.3. Effect of organic solvents

It was essential for optimization of the separation to investigate the effect of using various organic solvents. Organic solvents have successfully been used in adjusting the selectivity in CE for both fully complexed [10,20], partially complexed [30–32] and free metal ions [33,34], and observed effects on resolution have been attributed to changes in solvation, degree of complexation, effective charge or viscosity.

#### 3.3.1. Methanol

First, methanol was added to the electrolyte in an attempt to improve the resolution. Both  $\mu_{ep}$  and  $\mu_{eo}$  decreased while the fraction of methanol changed

from 0 to 20% (v/v). Variations in viscosity are an obvious reason of both reduced electroosmotic and electrophoretic velocities. The changes in analyte solvation may also play a role for decreased electrophoretic mobilities. Evidence of such interactions can be found in a rather strong solvating capability of methanol with respect to anionic species [35]. With respect to migration times, the general trend is that they increase as the methanol concentration increases. However, the corresponding changes in the migration range were not sufficiently pronounced to influence the separation.

#### 3.3.2. Ethylene glycol

When using ethylene glycol instead of methanol, metal complexes displayed similar migration behaviour in terms of electrophoretic mobilities and migration times. Increasing the ethylene glycol concentration produces a monotonous decrease in migration times (with no changes of migration order) and extends to a certain extent the migration range owing to reducing the EOF. As a result, the baseline resolved separation of virtually all the components of the test mixture was quite successfully achieved (Fig. 3). However, the main reason of the resolution improvements with ethylene glycol-containing electrolytes is assumed to be due to enhanced plate efficiencies resulted from lowered diffusion coefficients of analytes in rather viscous electrolyte medium. On the other hand, one should avoid too high a fraction of ethylene glycol since, for the same

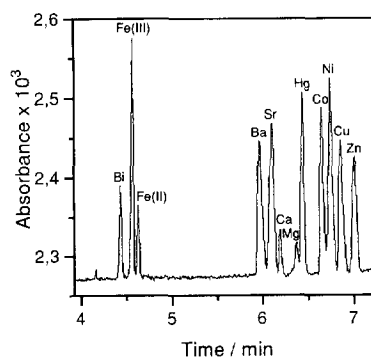


Fig. 3. Separation of metal complexes using ethylene glycol as an electrolyte additive. Electrolyte: 10 mM sodium borate, 1 mM CDTA and 5% ethylene glycol.

reason, the performance of the CE system is deteriorated (high and erratic baseline, poor peak shapes), as was observed at 10% (v/v) ethylene glycol (the highest concentration studied). Therefore, the electrolyte containing 5% ethylene glycol was found to give the best compromise between peak separation, analysis time and baseline noises. The migration times and electrophoretic mobilities measured with this electrolyte solution are summarized in Table 1. Also given in Table 1 are the migration characteristics obtained with a 10 mM borate buffer used at the initial stage of optimization procedure.

Table 1  
Migration times (min) and electrophoretic mobilities ( $\text{cm}^2/\text{V s}$ ) of metal complexes

Complex	10 mM sodium borate		10 mM sodium borate +5% ethylene glycol	
	$-\mu_{\text{ep}} \times 10^5$	$t_{\text{M}}$	$-\mu_{\text{ep}} \times 10^5$	$t_{\text{M}}$
Ag(I)	26.7	4.56	24.5	4.77
Al(III)	41.1	6.31	–	–
Ba(II)	37.4	5.74	34.7	6.02
Bi(III)	23.3	4.28	20.1	4.31
Ca(II)	38.3	5.87	35.5	6.15
Cd(II)	39.4	6.04	36.3	6.28
Ce(III)	15.8	3.76	12.9	3.87
Co(II)	41.0	6.29	37.9	6.56
Co(III)	26.1	4.50	22.9	4.63
Cr(III)	28.9	4.76	26.7	5.00
Cu(II)	41.6	6.40	39.3	6.82
Fe(II)	24.0	4.33	21.6	4.51
Fe(III)	24.5	4.37	22.4	4.58
Hg(II)	39.9	6.11	36.9	6.38
La(III)	42.1	6.48	–	–
Mg(II)	39.4	6.04	36.4	6.29
Mn(II)	38.7	5.93	36.1	6.24
Mo(V)	35.7	5.51	32.8	5.74
Ni(II)	41.3	6.34	38.4	6.65
Pb(II)	39.4	6.04	–	–
Pd(II)	43.7	6.78	–	–
Sb(III)	20.7	4.08	17.9	4.21
Sn(IV)	30.5	4.92	28.2	5.16
Sr(II)	37.9	5.81	35.2	6.10
Tl(I)	8.9	3.39	6.9	3.52
U(VI)	46.5	7.38	–	–
V(IV)	43.7	6.78	–	–
V(V)	26.7	4.56	–	–
W(VI)	35.4	5.48	–	–
Zn(II)	43.7	6.78	40.3	7.02
Zr(IV)	28.1	4.68	25.9	4.92

### 3.4. Effect of borate buffer concentration and voltage

Variation in the electrolyte buffer concentration and applied voltage can be used to finetune the separation.

Increasing the buffer concentration from 10 to 10 mM provides additional resolution improvement for metal-CDTA complexes. As expected, additional electrolyte decreases the EOF, which leads to enlarged migration times of the complexes. The improved resolution for several pairs of the complexes, such as Bi/Fe(III), Fe(II)/Fe(III) or Cu/Zn, indicates a combined favourable effect of the higher efficiencies and of an increase in the elution range generated with increased electrolyte concentrations. The concentrations higher than 20 mM were not applied to prevent excessive Joule heating due to a higher carrier electrolyte ionic strength.

All above separations were obtained using an operating voltage of 15 kV. In principle the use of higher electric field can reduce analysis time and enhance separation efficiency. However, with essentially aqueous electrolytes high ionic strengths can cause appreciable Joule heating. Indeed, the dependence of electroosmotic mobility on field strength (the graph is not shown) revealed a certain deviation from linearity at an electric field of  $\approx 260$  V/cm. This corresponds to an applied voltage of  $\approx 13$  kV for the capillary of 50 cm total length. Therefore in order to eliminate the possibility of Joule heating effects, an operating voltage of 12.5 kV was selected for the separation when a large number of analytes are present in a sample. With this voltage, the efficiencies are not significantly altered, and this allows the larger elution range to offset the minor loss in efficiency in terms of resolution.

The findings of this section suggested that an optimal separation could be achieved using an electrolyte, containing 20 mM sodium borate and 5% ethylene glycol, and adjusting the applied voltage to 12.5 kV. These conditions permit a number of excellent CE separations, the best of which with as much as 23 metal ions simultaneously resolved is presented in Fig. 4. Apart from metal complexes shown in Fig. 4, Al and La co-migrate with Ni and Cu, respectively, whereas Mn, Cd and Pb

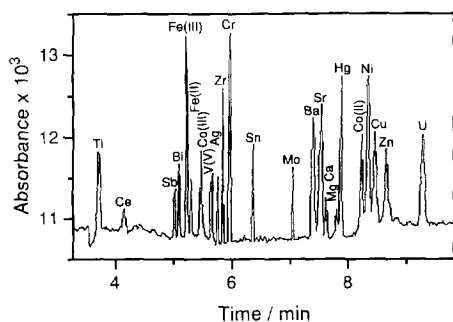


Fig. 4. Separation of metal-CDTA complexes under optimised conditions. Electrolyte: 20 mM sodium borate, 1 mM CDTA and 5% ethylene glycol; voltage: 12.5 kV.

complexes migrate with mobilities close to that of Mg. Palladium(II) and vanadium(IV) produce peaks with the same migration time as the zinc peak. Finally, the peak due to the tungsten complex can be just partially resolved from that of molybdenum chelate.

Under optimized separation conditions, the limits of detection were estimated as based on hydrostatic injection at 100 mm for 20 s. The detection limits (defined as three times the signal-to-noise ratio) range from  $1 \times 10^{-7}$  M (Fe(III)) to  $4 \times 10^{-6}$  M (Ca(II), Hg(II)) and, on average, are ca.  $10^{-6}$  M. This corresponds to analysis of metal ion mixtures in the mid-ppb range. Evidently, the sensitivity achieved for the large majority of metal ions presented in the study is not sufficient for environmental applications. One more comment regarding the detectability is that the presence of various additives in the electrolyte may affect adversely the background absorbance and thereby present a disadvantage for detection. For instance, the electrolytes containing 5% ethylene glycol, 10% methanol and 50 mM SDS decrease the respective absorbance signals of metal complexes up to 98, 70 and 50% of the signal intensity of pure borate buffer electrolyte. Therefore, the selected electrolyte composition must be a subtle compromise between detectability and attainment of the desired resolution.

The detection aspects and the means towards enhancing the detection limits for metal ions com-

plexed with CDTA have been studied in detail and will be reported in a subsequent publication.

#### 4. Conclusions

We believe that this is the first report of the CE separation of metal complexes at the level of more than 20 components. The advantage of several electrolyte compositional changes tried was manifested by the possibility to achieve the appropriate resolution not only by altering the EOF velocity but also by improving the separation efficiency. The best separation occurs when ethylene glycol is present in sodium borate electrolyte at a concentration of few percents, although some metal complexes co-migrate.

The CE method reported in this study is not optimised at this time for quantitation of metal ions due to moderate detectability with direct UV detection at 214 nm. Further studies will therefore include the use of other detection wavelengths and the electromigration method of injection. Ultimately, alternative modes of detection, such as indirect photometric detection in the visible range, will be investigated.

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