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# Separation selectivity of some ethylenediaminetetraacetic acid and cyclohexane-1,2-diaminetetraacetic acid complexes in column and ion electrokinetic chromatography

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## Abstract

The complexes of  $Mn^{2+}$ ,  $Cd^{2+}$ ,  $Fe^{3+}$ ,  $Pb^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$ ,  $Zn^{2+}$  and  $Cu^{2+}$  with EDTA and cyclohexane-1,2-diaminetetraacetic acid (CDTA) were separated and detected in column and ion electrokinetic chromatography with suppressed conductivity and direct UV detection, respectively. In column ion chromatography (IC) these complexes were separated on an IonPac AS4A anion-exchange column (Dionex, USA). Parameters of carrier electrolyte, which were examined in the ion electrokinetic chromatography (IEKC) mode, include polymer and sulfate concentrations. In IEKC separation selectivity of complexes with poly(diallyldimethylammonium) cation as modifier is similar as for an IonPac AS4A column both for EDTA and CDTA chelates. It was shown that the ion-exchange capacity of the electrokinetic system is more than 100-times lower than the capacity of the IC column for the same peak resolution. In comparison with column main advantages of electrokinetic version are high separation efficiency (220 000–390 000 theoretical plates) and the absence of the analyte interaction with the sorbent matrix. © 1999 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Electrokinetic chromatography; Selectivity; Metal chelates; Metal cations

## 1. Introduction

The popular approach based on complexation allows improving the ion chromatographic separation selectivity of metals ions [1]. Metal–ethylenediaminetetraacetic acid (EDTA) chelates are one of the most convenient for the metal ions separation and determination as anionic complexes with pre-column complexation in ion chromatography (IC) [2,3]. Capillary electrophoresis (CE) procedures for the determination of inorganic anions and cations widely utilize different aspects of column IC experience: the choice of detection mode, sample pretreatment, improvement of separation selectivity. Because of

dramatic difference in the separation mechanism of CE and IC, the last point is the most complicated and interesting. Our recent work introduced the ion-exchange interactions for the multi-component determination of metal chelates by CE [4].

The separation of metal–EDTA (or cyclohexane-1,2-diaminetetraacetic acid, CDTA) by CE chelates based on difference in own electrophoretic mobilities of the analytes. EDTA (CDTA) forms negatively charged anions with mono-, di- and trivalent metal cations. These complexes have similar structure, size and as consequence very similar electrophoretic mobilities. Separation selectivity of these complexes is very poor in the CE mode [5,6]. The first example of the separation utilizing an ion-exchange mechanism for mobility differentiation was demonstrated

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by Terabe and Isemura [7]. The addition of cationic water soluble polymers, such as poly-(diallyldimethylammonium chloride) (PDADMA) reverses electroosmotic flow (EOF), provides the condition for fast and selective separation of inorganic and organic anions, as well as of anionic chelates, including metal–EDTA, 4-(2-pyridyl-azo)resorcinolato (PAR) complexes [4,8,9]. The ion-exchange mechanism dominates in the separation of anions when high concentration of “pseudo-stationary” anion-exchange polymeric phase in carrier electrolyte used. Selectivity of such separation is very similar to IC [4]. That is why we named this variant of CE ion electrokinetic chromatography (IEKC).

The aim of this work is to compare the separation selectivity and efficiency of metal–EDTA and CDTA chelates in column and electrokinetic ion chromatography, using the capillary modified with water soluble cationic polymer PDADMA. The influence of carrier electrolyte parameters on the separation selectivity is also discussed.

## 2. Experimental

### 2.1. Instrumentation

IEKC experiments were carried out with a BioFocus3000 instrument (Bio-Rad, USA), equipped with a UV–Vis spectrophotometric detector, liquid thermostated User Assembled Cartridge, and a personal computer for processing and data handling. Fused-silica capillaries (GL Science, Japan) were 50 cm (45 cm to detector)  $\times$  375  $\mu$ m O.D.  $\times$  50  $\mu$ m I.D. Zones of metal–EDTA and CDTA complexes were detected in capillary at 200–220 nm. A negative polarity power supply (–20 kV, cathodic injections) was used. Injections were produced by applying a pressure of 3 p.s.i. s (1 p.s.i. = 6894.76 Pa).

IC experiments were carried out with an ion chromatograph DX-120 (Dionex, USA) with one or two coupled IonPac AS4A columns (250  $\times$  4 mm) and ASRS (4 mm) suppressor of background conductivity. A Chromatopac CR-3A (Shimadzu, Japan) instrument was used for chromatogram recording. All the pH measurements were performed with a Horiba M-13 pH meter (Horiba, Japan).

### 2.2. Chemicals and procedure

The polymer solutions of PDADMA (Aldrich, Milwaukee, WI, USA) with a concentration 200 mM of functional groups were prepared by dilution of the initial product. PDADMA was used as received and not converted to other ionic forms. Sodium sulfate, acetate (all analytical-reagent grade) were used for carrier electrolyte solution preparations. Non-modified capillaries were purged with water (5 min), 0.1 M NaOH (10 min), water (10 min), and a carrier electrolyte solution (30 min). Carrier electrolytes were filtered through a 0.45- $\mu$ m membrane filter (Advantec, Tokyo, Japan) and degassed. Between each run the capillary was washed with 0.1 M NaOH (1 min), water (1 min) and the carrier electrolyte solution (5 min). Metal cations stock solutions were prepared from acetate salts. Samples were prepared by the addition of metal stock solution to 5 mM EDTA or CDTA (pH 7.0). The polyaminocarboxylic acids used were purchased from Dojindo (Japan). Ethanol–water (1:9, v/v) sample was used for the measurement of EOF.

Five mM Na<sub>2</sub>CO<sub>3</sub> and 2.5 mM Na<sub>2</sub>CO<sub>3</sub>–2.5 mM NaHCO<sub>3</sub> eluents for IC were prepared from reagent grade salt, filtered and degassed. Flow-rate 0.75 ml/min and 25  $\mu$ l sample injection were used for all separations. Samples were prepared by the addition of metal stock solution to 1 mM EDTA or CDTA solution (pH 7.0). CaCl<sub>2</sub> solution was added to EDTA containing samples with concentration of 1 mM so that to prevent the influence of EDTA peak tailing. Doubly distilled water was used for the preparation of all the solutions.

## 3. Results and discussion

### 3.1. The separation with column IC

The elution order of EDTA and CDTA complexes in the IC mode depends on their ion-exchange affinity to the sorbents. Retention times for one IonPac AS4A column and 5 mM Na<sub>2</sub>CO<sub>3</sub> eluent are presented in Table 1. Fe(III) chelate was excluded from IC experiments because of high pH of the eluents used. Retention times for doubly charged chelates increase in the following order:

Table 1  
Retention (migration) times for IC and IEKC separation modes (min)

Analyte	EDTA (IC) <sup>a</sup>	CDTA (IC) <sup>a</sup>	EDTA (IEKC) <sup>b</sup>	CDTA (IEKC) <sup>b</sup>
Cl <sup>-</sup>	2.09	2.09		
NO <sub>3</sub> <sup>-</sup>	4.1	4.1	3.80	3.80
Mn	3.46	4.04	5.33	5.47
Cd	3.68	4.08	5.39	5.54
Pb	4.1	4.99	5.41	5.72
Ni	5.23	6.4	7.85	9.19
Co	5.78	5.94	8.07	8.48
Zn	7.5	7.01	8.37	9.55
Cu	10.7	7.05	9.25	9.83

<sup>a</sup> Column: IonPac AS4A column (250×4 mm); eluent: 5 mM Na<sub>2</sub>CO<sub>3</sub>; flow-rate 0.75 ml/min; sample volume 25 μl, 0.05 mM of each metal ion; suppressed conductivity detection.

<sup>b</sup> Carrier electrolyte: 3 mM sodium sulfate, 50 mM PDADMA, 10 mM sodium acetate, pH 7.0; capillary 50 cm (45 cm to detector)×375 μm O.D.×50 μm I.D.; conditions -20 kV, sample injections 3 p.s.i. s, detection 210 nm.

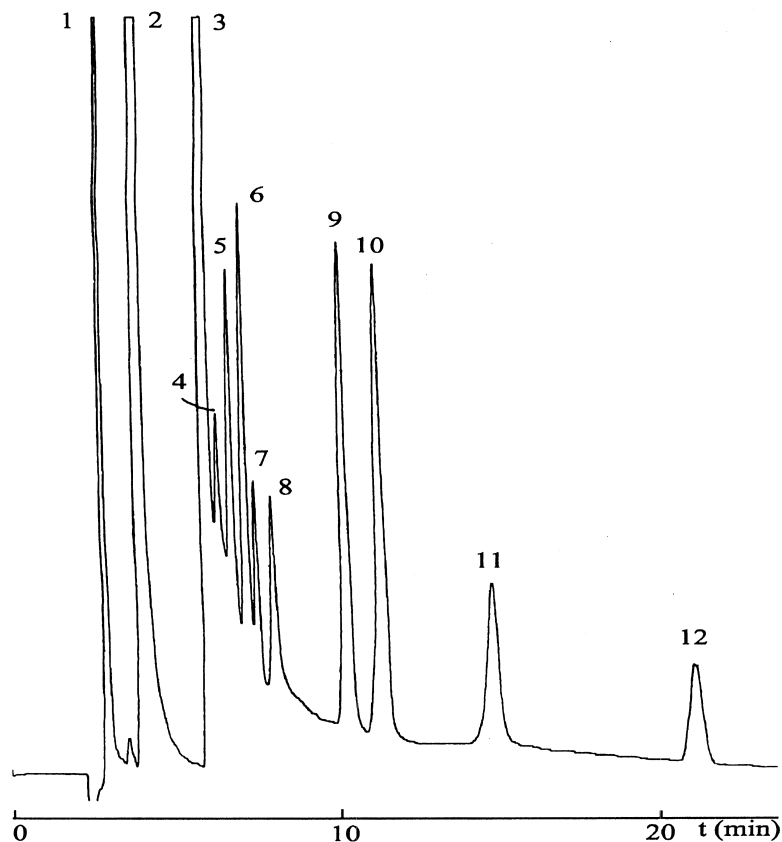


Fig. 1. IC separation of eight metal-EDTA chelates. Column: couple of IonPac AS4A columns (250×4 mm); eluent: 5 mM Na<sub>2</sub>CO<sub>3</sub>; flow-rate 0.75 ml/min; sample volume 25 μl; suppressed conductivity detection. Peak identification: 1=acetate, 2=chloride, 3=CaEDTA<sup>2-</sup>, 4=unknown, 5=MnEDTA<sup>2-</sup>, 6=CdEDTA<sup>2-</sup>, 7=NO<sub>3</sub><sup>-</sup>, 8=PbEDTA<sup>2-</sup>, 9=NiEDTA<sup>2-</sup>, 10=CoEDTA<sup>2-</sup>, 11=ZnEDTA<sup>2-</sup>, 12=CuEDTA<sup>2-</sup>; 0.05 mM of each metal ion, [EDTA]=[Ca<sup>2+</sup>]=1 mM.

$\text{MnEDTA}^{2-} < \text{CdEDTA}^{2-} < \text{PbEDTA}^{2-} < \text{NiEDTA}^{2-} < \text{CoEDTA}^{2-} < \text{ZnEDTA}^{2-} < \text{CuEDTA}^{2-}$ ; (1)  
 $\text{MnCDTA}^{2-} < \text{CdCDTA}^{2-} < \text{PbCDTA}^{2-} < \text{CoCDTA}^{2-} < \text{NiCDTA}^{2-} < \text{ZnCDTA}^{2-} < \text{CuCDTA}^{2-}$  (2).

There is one essential difference for EDTA and CDTA chelates: Ni(II) and Co(II) change elution order. Selectivity scale obtained is in a good correlation with the IonPac AS9 Dionex anion-exchange column [3]:  $\text{MnEDTA}^{2-} < \text{CdEDTA}^{2-} < \text{NiEDTA}^{2-} < \text{CoEDTA}^{2-}$ .

For increasing of the separation power a couple of IonPac AS4A columns were used. Fig. 1 shows the separation of eight EDTA chelates in used conditions. Another eluent, 2.5 mM  $\text{Na}_2\text{CO}_3$ –2.5 mM  $\text{NaHCO}_3$  has been used for metal–CDTA chelate separation. Nevertheless the separation of Mn(II)–Cd(II) and Zn(II)–Cu(II) pairs has not been obtained (Fig. 2).

### 3.2. Separation in IEKC

The choice of carrier electrolyte parameters for metal–EDTA chelates separation was described elsewhere [4]: 5 mM sodium sulfate, 50 mM PDADMA, 10 mM sodium acetate, pH 7.0. The amounts of added  $\text{Na}_2\text{SO}_4$  and cationic polymer have evident analogs in column IC: eluent concentration and ion-exchange capacity of separation system, respectively. The acetate anion also has an influence on the ion-exchange interactions in this system. However its ion-exchange affinity is much smaller than for sulfate. The capacity of IEKC separation media can be changed continuously, whereas for IC this parameter is constant. Fig. 3 shows the effect of the polymer concentration in carrier electrolyte on EOF and the migration time for the EDTA complexes. Concentrations of all components were constant with exception of polymer (75, 50, 25, 10, 5, 0 mM). The change of the amount of polymer in carrier electrolyte affects analyte mobilities through ratio of free and polymer-bonded anions. The increase of PDADMA concentration leads to decrease in electrophoretic mobilities of anions due to association with cationic polymer chains. The similar behavior observed in IC:

$$\log k' = (a/b) \log C - (a/b) \log E + \text{constant} \quad (1)$$

where capacity factor ( $k'$ ) can be calculated from the

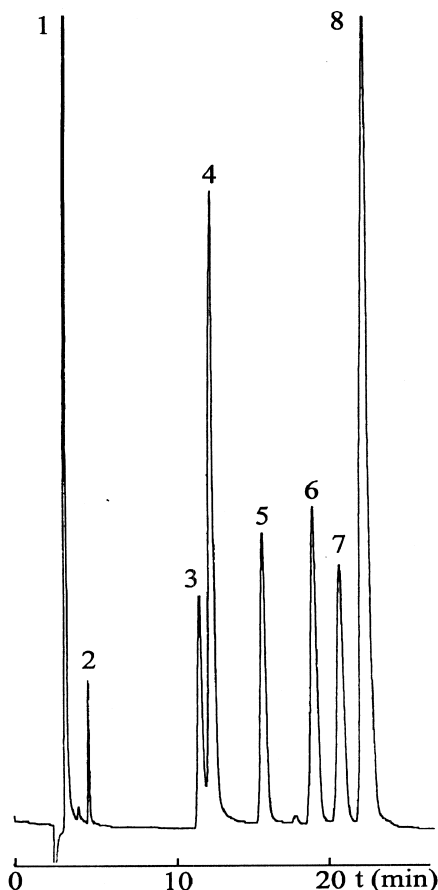


Fig. 2. IC separation of seven metal–CDTA chelates. Column: couple of IonPac AS4A columns (250×4 mm); eluent: 2.5 mM  $\text{Na}_2\text{CO}_3$ , 2.5 mM  $\text{NaHCO}_3$ ; flow-rate 0.75 ml/min; sample volume 25  $\mu\text{l}$ ; suppressed conductivity detection. Peak identification: 1=acetate, 2=chloride, 3= $\text{CDTA}^{2-}$ , 4= $\text{MnCDTA}^{2-}$  and  $\text{CdCDTA}^{2-}$ , 5= $\text{PbCDTA}^{2-}$ , 6= $\text{CoCDTA}^{2-}$ , 7= $\text{NiCDTA}^{2-}$ , 8= $\text{ZnCDTA}^{2-}$  and  $\text{CuCDTA}^{2-}$ ; 0.05 mM of each metal ion,  $[\text{CDTA}] = 1 \text{ mM}$ .

distribution coefficient of analyte between stationary and mobile phases;  $a$ ,  $b$ ,  $C$ , and  $E$  are the charges of analyte, eluting anions, column capacity and eluent concentration, respectively. In the case of IEKC polymer concentration is equivalent to column capacity in IC. Because of difference in  $a/b$  ratio, the slope of migration times vs. PDADMA concentration should be higher for  $\text{MeEDTA}^{2-}$  than for  $\text{Fe(III)EDTA}^-$ . This situation takes place in the range 0–25 mM of the polymer. The dependencies of

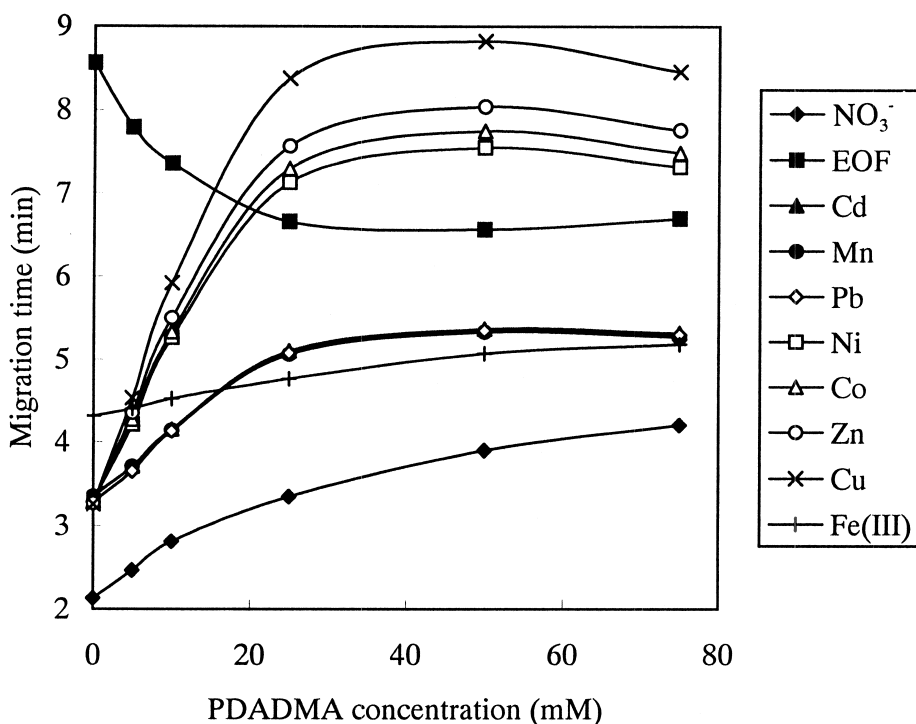


Fig. 3. Dependence of migration times of metal-EDTA chelates from PDADMA concentration. Carrier electrolyte: 5 mM sodium sulfate, 10 mM sodium acetate,  $x$  mM PDADMA, pH 7.0; capillary 50 cm (45 cm to detector)  $\times$  375  $\mu$ m O.D.  $\times$  50  $\mu$ m I.D.; conditions -20 kV, sample injections 3 p.s.i. s, detection 210 nm.

migration time for doubly charged metal chelates have a maximum at 50 mM concentration of the polymer. Very likely, the effect of saturation of ion-exchange capacity takes place, whereas content of  $\text{Cl}^-$  counter anion increases. Migration times of singly charged  $\text{NO}_3^-$  and  $\text{Fe(III)EDTA}^-$  increase in all studied concentration ranges.

As can be seen from Eq. (1) the influence of sulfate concentration should be equivalent to polymer concentration, though with opposite sign (Fig. 4). The carrier electrolyte composition was varied as follows: 50 mM PDADMA, 10 mM sodium acetate,  $x$  (15, 10, 7, 5, 3) mM sodium sulfate. Migration times of doubly charged complexes decrease rapidly than those of  $\text{FeEDTA}^-$  with increase of sulfate concentration in the carrier electrolyte.

The dependencies of electrophoretic mobilities of CDTA complexes from the concentrations of sulfate and PDADMA in the carrier electrolyte are similar to those for EDTA.

The values of the own electrophoretic mobilities of double-charged complexes of Mn(II), Cd(II), Pb(II), Co(II), Ni(II), Zn(II), Cu(II) are very close both for EDTA [10] and CDTA chelates [6]. However, the addition of large amount of polymeric modifier divides this group into two parts: Cd(II), Mn(II) and Pb(II) chelates migrate faster than Co(II), Ni(II), Zn(II) and Cu(II) ones. The similar situation takes place in the IC mode with an IonPac AS4A column. This phenomenon can be explained from the point of view of ion-exchange mechanism of polymer-analyte interaction. Mn(II), Cd(II) and Pb(II) have coordination number 7 in complexes with the polyaminocarboxylates, whereas Ni(II), Co(II), Zn(II) and Cu(II) have 6-fold coordination [11]. Because of coordination of extra molecule of water, the size of the complexes increases. It leads to decreasing of their ion-exchange affinity.

Examples of IEKC separations of EDTA and CDTA chelates are presented in Fig. 5. Migration

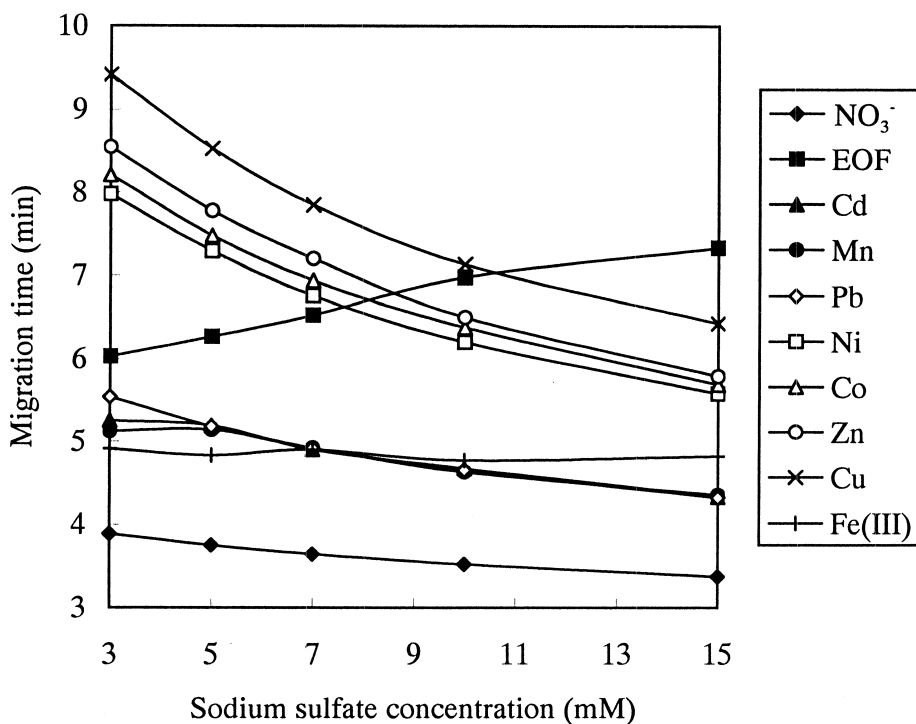


Fig. 4. Dependence of migration times of metal–EDTA chelates from sulfate concentration. Carrier electrolyte: 10 mM sodium acetate, 25 mM PDADMA,  $x$  mM sodium sulfate, pH 7.0; other conditions as in Fig. 3.

times of doubly charged chelates for carrier electrolyte: 5 mM sodium sulfate, 50 mM PDADMA, 10 mM sodium acetate, pH 7.0 are shown in Table 1. The migration order of complexes is identical to elution in column IC variant. Selectivity inversion for Co(II) and Ni(II) was also found. However the resolution of Mn(II)–Cd(II) and Zn(II)–Cu(II) pairs of CDTA chelates in IEKC is much better.

### 3.3. Comparison of IC and IEKC

The separation efficiency, capacity of separation

systems and detection limits of metal–EDTA chelates in IC and IEKC are listed in Table 2. The IEKC system provides higher separation efficiency and needs a smaller amount (more than two-orders of magnitude) of functional groups in the separation media. Such a difference takes place owing to plug profile of liquid movement in IEKC and easy access to exchange sites in polymer solution (in comparison with ion-exchange resin). The absence of the interaction of analyte with the sorbent matrix seems also very important for high efficiency in IEKC. Besides, EDTA excess in sample solution does not influence

Table 2  
The comparison of column and electrokinetic IC separation modes

Method	Separation efficiency (theoretical plates)	Capacity of separation system ( $\mu\text{equiv.}$ )	Detection limits (mol/l)
IC <sup>a</sup>	7500–9000	40	$n \cdot 10^{-6}$
IEKC <sup>b</sup>	220 000–390 000	0.05	$n \cdot 10^{-5}$

<sup>a</sup> Couple of IonPac AS4A columns, 25  $\mu\text{l}$  sample volume.

<sup>b</sup> Ref. [4], 6 nl sample volume.

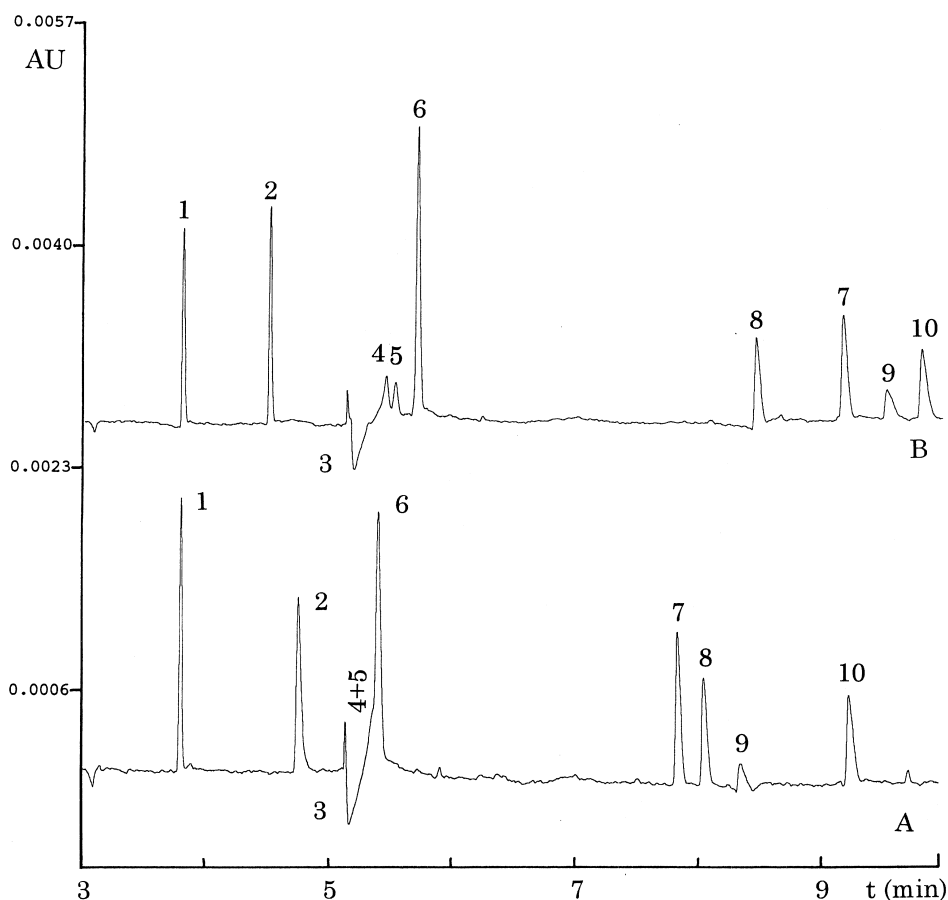


Fig. 5. IEKC separation of eight metal–EDTA (a) and CDTA (b) complexes. Carrier electrolyte: 3 mM sodium sulfate, 50 mM PDADMA, 10 mM sodium acetate, pH 7.0. Peak identification: 1=NO<sub>3</sub><sup>-</sup> (0.25 mM), 2=Fe(III) (0.25 mM), 3=system peak, 4=Mn(II) (0.5 mM), 5=Cd(II) (0.5 mM), 6=Pb(II) (0.25 mM), 7=Ni(II) (0.25 mM), 8=Co(II) (0.25 mM), 9=Zn(II) (0.5 mM), 10=Cu(II) (0.25 mM). Other conditions as in Fig. 3.

on separation of chelates in IEKC. Thus, IEKC has some advantages in comparison with IC for the determination of metal–EDTA and CDTA complexes. However lower detection sensitivity of direct UV detection in IEKC should be noted.

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