The Use of N,N-Bis(hydroxybenzyl)ethylenediamine-N,N-diacetic Acid (HBED) for the Determination of Trivalent Metal Ions by Capillary Electrophoresis

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The separation of Bi(III), Sc(III), In(III), Co(III), Ga(III), Al(III), Cr(III), Mn(III), and Fe(III) ions with HBED (Figure 1) by an ion exchange variant of electrokinetic chromatography was demonstrated. The use of high concentration of cationic polymer and a careful choice of counter ions added in carrier electrolyte allowed to stretch selectivity scale for the complete baseline resolution of all studied chelates with efficiency 40-300 thousands theoretical plates. Favorable arrangement of donor groups in HBED structure made possible the first separation of Ga(III) and In(III) chelates by CE.

The pioneer study devoted to the separation of negatively charged metal-chelates by MECK has been performed ten years ago for 4-(2-pyridylazo)resorcinol.[1] Now this approach takes a leading role in CE analysis of metal ions.[2]

Polyaminocarboxylate ligands is most convenient for multi-component metal determination. However, poor separation selectivity of equally charged EDTA-type chelates minimizes this advantage in the CE applications. Several attempts have been made for enhancement of separation selectivity of metal - polyaminocarboxylate chelates. However, neither introduction of tetraalkylammonium salts,[3] nor use of organic modifier like ethylene glycol improved the situation essentially.[4] This was the reason why Krokhin et al.[5] introduced ion-exchange interaction into the CE separation of EDTA chelates. An ion-exchange variant of electrokinetic chromatography was first introduced by Terabe et al.[6] for the mobility differentiation of isomers of organic acids. The separation of EDTA-type chelates requires the addition of very high concentration of cationic polymer in carrier electrolyte. Separation selectivity in such a kind of CE system completely controlled by ion exchange and coincides with Ion Chromatographic (IC) separation of metal-EDTA and CDTA chelates. That is why we proposed the term Ion Electrokinetic Chromatography (IEKC) for this separation scheme.[7]

There are a restricted number of separations of several M(III)-polyaminocarboxylates. Timerbaev et al. used ethylene glycol for mobility differentiation of metal-CDTA chelates.[4] In the list of resolved peaks were five M(III)CDTA⁻ complexes (Ce, Bi, Fe, Co, Cr) within 2 min migration window. The same research group reported separation of 14 lanthanoid ions

as complexes with CDTA; the mutual selectivity was achieved with the aid of the formation of the mixed hydroxo complexes as M(III)(OH)CDTA²⁻ at pH 11.[8] Padarauskas and Shwedt[9] optimized the conditions for the separation of Co, Bi, Fe, Cr and 6 divalent cations as M^{n}DTPA$^{(5-n)-}$. Polyaminocarboxylate ligands with aromatic moiety were applied for the enhancement of the detection sensitivity of this approach. Motomizu et al.[10] introduced HBED for the analysis of metal ions by CE. The use of Quin2 ligand allowed to decrease 5 - 6 times the detection limits for Fe(III) and Ni(II) in comparison with EDTA.[5] High stability constants for M(III) cations (Table 1) were found to be due to presence of two phenolate groups in the ionized HBED ligand.[11] The affinity of such kind of ligands to Ga and In has been demonstrated for EDDHA (Ethylenediaminedi-(o-hydroxyphenylacetic acid)) (LogK$_{ML}$ 33.89 and 26.68, respectively)[12]. We expected the similar behavior of HBED, because of more favorable arrangement of donor groups in its structure[11]. The aim of this work was to combine high complexation ability of HBED and separation power of IEKC for the determination of M(III) cations.

Table 1. Stability constants of metal-HBED chelates and their electrophoretic mobility under different carrier electrolyte conditions

Cations	LogK$_{ML}$	μ_{ep} $(cm^2/kV^{-1}s^{-1})$		
		Cl-form[c] 1×10^{-3} M	Cl-form[d] 5×10^{-2} M	Ac-form[e] 5×10^{-2} M
Co(II),(III)		-0.133	-0.044	0.064
Al(III)	24.78[a]	-0.135	0.025	0.124
Mn(II),(III)		-0.132	0.026	0.134
Fe(III)	39.68[b]	-0.129	0.070	0.221
EOF		-0.224	-0.254	-0.342

[a]-ref.[13], [b]-ref.[11], [c]-buffer: 1×10^{-3} M PDADMA-Cl, [d]-buffer: 5×10^{-2} M PDADMA-Cl, other conditions see Figure 2, [e]-buffer: 5×10^{-2} M PDADMA-Ac, other conditions see Figure 3.

The CE electrolyte system proposed in our previous work contained a polymeric modifier – PDADMA in chloride form (Figure 1). Similar composition of carrier electrolyte was used for the preliminary experiments which were focussed upon oxidation of Co(II) and Mn(II) during complexation with HBED. Electrophoretic mobilities of the HBED species mainly determine the separation selectivity at low concentrations of polymer modifier. The electrophoretic mobilities of Fe (III), Al (III), Co(II), Mn(II) chelates at 1×10^{-3} M of PDADMACl in a carrier electrolyte were found very close (Table 1). This fact arises from the same charge number of the complexes. The complexes transformation of Mn(II) and Co(II) into Mn(III) (brown) and Co(III) (yellow) was confirmed
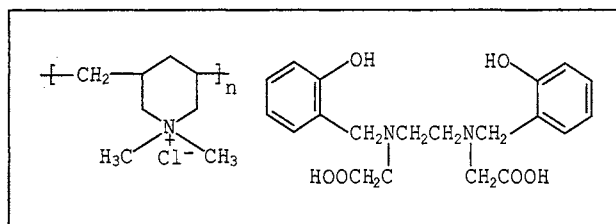

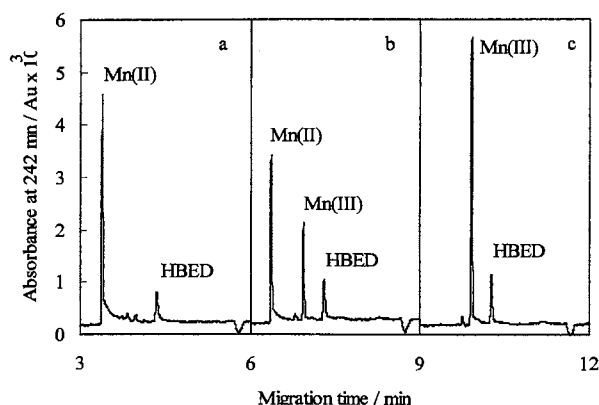**Figure 1.** The structure of polymer and ligand used.

Figure 2. Gradual oxidation of Mn(II) - Mn(III) in the HBED complex after preparation. a – 2 min, b – 90 min, c – 24 h. Capillary: 50 cm (effective length 45.5 cm) × 50 μm I.D.; carrier electrolyte: 5×10^{-4} M PDADMA-Cl, 1×10^{-2} M $Na_2B_4O_7$, 5×10^{-3} M Na_2SO_4, 1×10^{-2} M sodium acetate, pH 9.2; conditions: -20 kV; detection 242 nm; sampling: pressure 3 psi × s.; $[Mn]_T = 2 \times 10^{-4}$ M, $[HBED]_T = 4 \times 10^{-4}$ M.

by the parallel IEKC experiments (Figure 2). Under these conditions (at a low concentration of PDADMA-Cl) the doubly charged chelates migrate faster than singly ones.

The increasing PDADMA-Cl concentrations in a carrier electrolyte up to 5×10^{-2} M of functional groups expanded the migration time window of studied chelates. However, Al and Mn chelates had almost the same mobility (Table 1). The
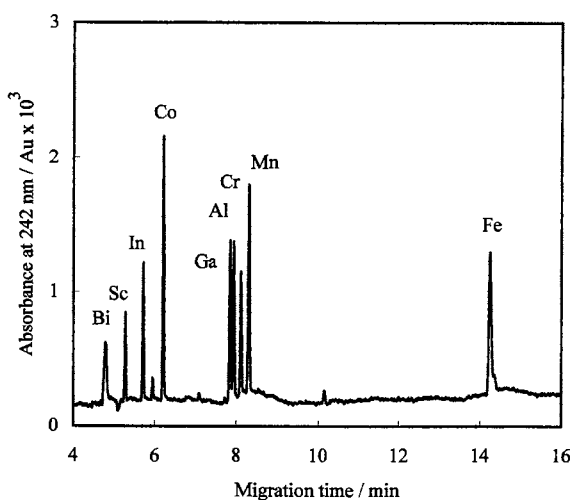

Figure 3. Separation of nine M(III)-HBED chelates. Carrier electrolyte: 5×10^{-2} M PDADMA-Ac, 1×10^{-2} M $Na_2B_4O_7$, 1×10^{-4} M HBED, pH 9.2; sampling: pressure 15 psi × s.; $[Bi]_T = [Sc]_T = 2.5 \times 10^{-5}$ M, $[M]_T = 5 \times 10^{-5}$ M, $[HBED]_T = 1 \times 10^{-3}$ M. Other conditions see Figure 2.

choice of eluting ion with low affinity to ion exchangers improves resolution of weakly retained species in IC, thus is a convenient procedure to apply to the IEKC. The polymer was transformed into the acetate form by passing through an anion exchanger column in -OH form followed by neutralization with acetic acid. The ligand was added to the run buffer to prevent possible dissociation of some separated chelates (Figure 3). The mobility difference between Co and Fe – HBED complexes (Table1) increased with changing counter anions; 3.6 and 8 minutes for Cl⁻ and acetate forms (at the same concentration of functional groups (5×10^{-2} M)), respectively. In and Ga gave good peaks shape and reproducibe detector response. Calibration graphs were linear for these metals in the range $(0.01 - 0.5) \times 10^{-3}$ M. The detection limits for a 7.5 nl injection under sampling pressure of 15 psi × s were estimated 4×10^{-6} M and 3×10^{-6} M for In and Ga, respectively. The combination of highly selective ion-exchange separation with CE efficiency allows to evaluate IEKC as a powerful technique for the separation of ionic metal chelates and the great potential of HBED for multi-component determination of metal species.

References and Notes

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14   The CE equipment was Bio-Focus 3000 (Bio-Rad). Deionized water with resistance 18 MΩ (ELGASTAT UHQPC) was used for the solutions preparation. PDADMA-Cl (as 20% solution in water) and HBED were purchased from Aldrich (USA) and Dojindo (Japan), respectively. The samples of metal-chelates were prepared by addition of desired amount of metal stock solutions to 1×10^{-3} M HBED (borate buffer pH 9.2) and storage at room temperature until complete complex formation. Cr(III) chelate was prepared separately in methanol solution.