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Potential of ethylenediaminedi(*o*-hydroxyphenylacetic acid) and *N*,*N*′-bis(hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid for the determination of metal ions by capillary electrophoresis☆

O.V. Krokhin[a,*], O.V. Kuzina[a], H. Hoshino[b], O.A. Shpigun[a], T. Yotsuyanagi[b]

[a]*Chemistry Department, Lomonosov Moscow State University, 119899 Moscow, Russia*

[b]*Department of Applied Chemistry, Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai 980-77, Japan*

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Abstract

Two aromatic polyaminocarboxylate ligands, ethylenediaminedi(*o*-hydroxyphenylacetic acid) (EDDHA) and *N*,*N*′-bis(hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED), were applied for the separation of transition and heavy metal ions by the ion-exchange variant of electrokinetic chromatography. EDDHA structure contains two chiral carbon centers. It makes it impossible to use the commercially available ligand. All the studied metal ions showed two peaks, which correspond to *meso* and *rac* forms of the ligand. The separation of metal–HBED chelates was performed using poly(diallyldimethylammonium) polycations in mixed acetate–hydroxide form. Simultaneous separation of nine single- and nine double-charged HBED chelates, including In(III), Ga(III), Co(II)–(III) and Mn(II)–(III) pairs demonstrated the efficiency of 40 000–400 000 theoretical plates. The separation of Co(III), Fe(III) complexes with different arrangements of donor groups and oxidation of Co(II), Mn(II), Fe(II) ions in reaction with HBED have been discussed.

Keywords: Ion electrokinetic chromatography; Electrokinetic chromatography; Ethylenediaminedi-(*o*-hydroxyphenylacetic acid); Bis(hydroxybenzyl)ethylenediaminediacetic acid; Metal cations; Metal chelates

1. Introduction

The synthesis of ethylenediaminedi(*o*-hydroxyphenylacetic acid) (EDDHA) [or *N*,*N*′-ethylenebis-2-(*o*-hydroxyphenyl)glycine (EHPG)] and *N*,*N*′-bis-(hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED) (Fig. 1), which take particular interest of chemists, were reported in 1957 [1] and 1967 [2], respectively. Initially, EDDHA was proposed for correcting iron deficiency in plants grown on alkaline soils and as a promising reagent for ferric ion. Much later, EDDHA was used to understand the role and transport of many metal ions in biological systems [3,4]. The great potentiality of these reagents for routine analyses of iron determined the interest of analytical chemists in these types of ligands.

The chelating tendency of EDDHA and HBED was carefully studied by Martell and co-workers [2,5]. These hexadentate complexants have two phenolic groups replacing two of the carboxylates of EDTA. Hard phenolate donors impart a high thermodynamic stability of the metal complexes with hard

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*Corresponding author.

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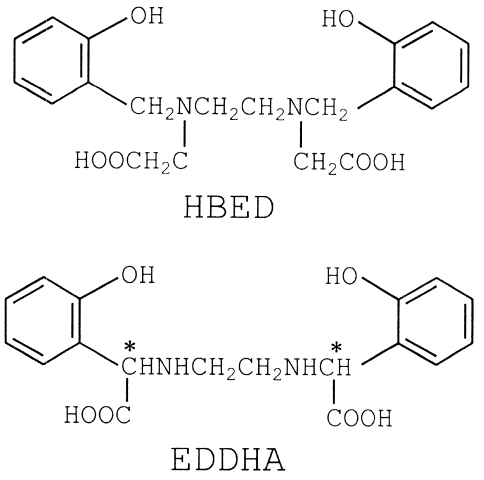

Fig. 1. The structures of used ligands.

metal ions. Both substances have the same set of donor groups and show a similar behavior in reactions with metal ions. One significant difference has been found: the presence of two chiral carbon atoms in the EDDHA structure (marked with an asterisk in Fig. 1) leads to the formation of *meso* and *rac* forms of the chelates, which have different stability constants due to changes in the coordination geometry [6]. Thermodynamic stability of EDDHA and HBED complexes for many metal ions was found [2] to be higher than for EDTA and increasing in the order: *meso*-EDDHA<*rac*-EDDHA<HBED with the exception of Ni^{2+} and Cu^{2+}, which have smaller stability of their HBED chelates.

A higher detection sensitivity (in comparison with aliphatic polyaminocarboxylates) and the stability of the chelates provide the advantages of these ligands with respect to multicomponent determination of metal ions by modern separation techniques like capillary electrophoresis (CE). Motomizu et al. [7] introduced HBED for CE analysis of metal ions and applied it for the determination of Ca and Mg in river water and serum samples. However, the values of electrophoretic mobility were found to be very close for metal cations with the same charge. Poor separation selectivity is a common problem for CE separation of polyaminocarboxylate chelates due to a negligible difference in their charge-to-size ratio, e.g., electrophoretic mobility. An ion-exchange mechanism has been proposed recently to solve this problem. This approach utilizes an addition of cationic polymers like poly(diallyldimethylammonium chloride) into the carrier electrolyte [8,9]. This reverses the electroosmotic flow and provides the condition for the fast determination of anions with a negative-polarity power supply. The amount of ion-exchange sites in the separation media can be varied continuously for optimizing the separation selectivity resulting from changes in free/polymer-bonded analyte ratio. Such kinds of systems have many analogies with ion chromatography (IC), show a similar to IC separation selectivity for small inorganic anions and metal–EDTA (CDTA) chelates. That is why the term ''ion electrokinetic chromatography'' (IEKC) was proposed for this scheme [9].

The aim of this work was to estimate the potentiality of two aromatic polyaminocarboxylate ligands, EDDHA and HBED, for multicomponent determination of metal ions by IEKC.

2. Experimental

All the experiments were performed on a Bio-Focus 3000 (Bio-Rad) instrument with a user assembled cartridge and a variable-wavelength spectrophotometric detector. Fused-silica capillaries (GL Science, Japan) were 50 cm (45.5 cm to detector)× 375 μm O.D.×50 μm I.D. The negative connection of a power supply (−20 kV, cathodic injections) was used. Zones of metal–EDDHA and –HBED complexes were detected in the capillary at 242 nm. Injections were produced by applying a pressure of 3–15 p.s.i.×s (1 p.s.i.=6894.76 Pa). Poly(diallyldimethylammonium chloride) (PDADMA-Cl) as a 20% solution in water, and ligands were purchased from Aldrich (USA) and Dojindo (Japan), respectively. The carrier electrolytes were prepared using sodium tetraborate, sulfate and acetate (all reagent grade). They were filtered, degassed prior to use and placed in vials, which were stored in dessicator under CO_2-free conditions. Stock solutions of the ligands were prepared in 10 m*M* NaOH. Metal–HBED (EDDHA) chelates were prepared by adding a desired amount of a metal ion solution to 1 m*M* HBED (EDDHA) at pH 9.2. The Cr(III) complex was prepared separately in a methanol solution. PDADMA-Cl was converted into the –OH form using the procedure described by Stathakis and

Cassidy [10]. All the pH measurements were performed with a Horiba, M-13 pH meter (Horiba, Japan). Deionized water (Elgastat UHQPC) was used throughout.

3. Results and discussion

3.1. The comparison of EDDHA and HBED

Motomizu et al. [7] used borate buffer for the CE separation of HBED chelates and showed that electrophoretic mobilities of metal(II) complexes decrease with a pH shift from 10 to 6. This decrease is caused by protonation of $M^{II}HBED^{2-}$ chelates (Table 1). We choose pH 9.2 (higher than log K^{H}_{MHL} for double-charged metal ions) to provide a difference in the charge between $M^{II}HBED^{2-}$ and $M^{III}HBED^{-}$ separated species. This difference should be important for the optimization of the ion-exchange separation selectivity. Since the basicities and complexing abilities of the two ligands are essentially similar, the same pH was used for the separation of EDDHA chelates. The choice of other parameters of the carrier electrolyte were described elsewhere [8].

For the preliminary experiments, a carrier electrolyte with a low concentration (10 m*M*) of a polymeric modifier was used. It was found that EDDHA shows two peaks for all the studied metal ions [Cu, Fe(III), Al, Co(II) and Mn(II)]. Peaks of *meso*- and *rac*-$CuEDDHA^{2-}$ were identified using complex formation at different metal:ligand ratios (Fig. 2). Owing to a higher stability of the *rac* form, its total amount in the reaction mixture increased with an increase in the ligand concentration. The HBED ligand does not have chiral carbon atoms and shows a single peak of the complex on the electropherogram. This is why HBED was chosen for the following experiments.

Polyaminocarboxylate ligands with hard phenolate donor atoms have the essential preference from the point of view of pH of the carrier electrolyte (or eluent in high-performance liquid chromatography, HPLC) used. In the case of $FeEDTA^{-}$, pH range has an upper limit (about 7) due to formation of hydroxo complex. The carrier electrolyte with pH up to 11 can be applied for the iron determination as HBED chelate. This result coincides with the data, that for

Table 1
Stability constants of some metal–EDDHA and –HBED chelates

Cation	EDDHA[a] (log K^{M}_{ML})		HBED[b]		
	Meso	*Rac*	Log K^{M}_{ML}	Log K^{H}_{MHL}	Charge at pH 9.2
H^{+}	$K_{a1}=6.36$	$K_{a1}=6.33$	$K_{a1}=4.64$		
	$K_{a2}=8.76$	$K_{a2}=8.79$	$K_{a2}=8.78$		
	$K_{a3}=10.85$	$K_{a3}=10.87$	$K_{a3}=10.56$		
	$K_{a4}=11.90$	$K_{a4}=12.05$	$K_{a4}=11.85$		
Mn^{2+}			14.78	7.66	−1[e]
Co^{2+}			19.89	7.77	−1[e]
Ni^{2+}	19.42	21.33	19.31	8.51	−2
Cu^{2+}	23.68	25.25	21.38	8.63	−2
Zn^{2+}	16.88	18.66	18.37	8.27	−2
Cd^{2+}			17.52	8.11	−2
Pb^{2+}			18.24	8.98	−2
Al^{3+}			24.78[c]		−1
Fe^{3+}	33.28	35.54	39.01[d]		−1
In^{3+}	25.26	26.68	27.76[d]		−1
Ga^{3+}	32.40	33.89	38.51[d]		−1

[a] [6].
[b] [2].
[c] [11].
[d] [12].
[e] Oxidized in complex with HBED.

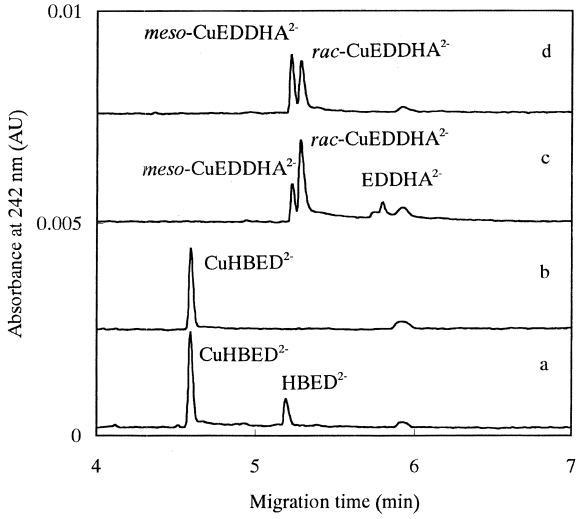

Fig. 2. Electropherogram of Cu–HBED and –EDDHA chelates at different metal:ligand ratios. Carrier electrolyte: 10 m*M* PDADMA-Cl, 10 m*M* sodium acetate, 5 m*M* Na_2SO_4, 10 m*M* $Na_2B_4O_7$, pH 9.2; conditions: −20 kV; detection 242 mn; sample injection: 5 p.s.i.×s; $[Cu]_T$=0.1 m*M*; (a) Cu:HBED (1:2), (b) Cu:HBED (1:1), (c) Cu:EDDHA (1:2), (d) Cu:EDDHA (1:1).

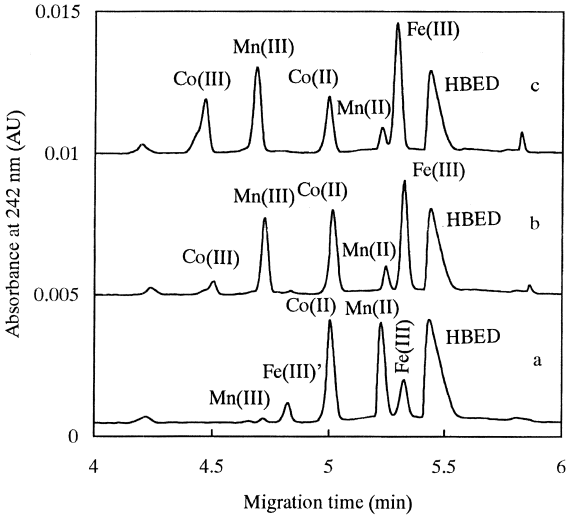

Fig. 3. The oxidation of Fe(II), Mn(II), Co(II) in reaction with HBED. $[Co]_T=[Mn]_T=[Fe]_T$=0.1 m*M*, $[HBED]_T$=1 m*M*; (a) 2 min after sample preparation, (b) 24 h, (c) 48 h; other conditions see Fig. 2.

$FeHBED^-$ no formation of hydrogen or hydroxo complexes is apparent from pH 2.5 to 11 [2].

3.2. Oxidation of Fe(II), Mn(II) and Co(II) metal ions in the reaction with HBED

The addition of Fe(II), Mn(II) and Co(II) solutions to colorless 1 m*M* HBED at pH 9.2 leads to a significant change in its color (red, brown and yellow, respectively). However, the rate of this transformation is different and decreases in the order: Fe(II)>Mn(II)>Co(II). The reaction of Fe(II) ion with HBED ligand yields the red $Fe^{III}HBED^-$ chelate immediately after mixing, whereas the oxidation of Co(II) with dissolved oxygen needs several days at room temperature for complete formation of yellow $Co^{III}HBED^-$. Electrophoretic mobilities of Co(II) and Mn(II) chelates were found to be very close to those for Al(III) and Fe(III) [7]. This fact also indicates that the oxidation takes place due to a difference in the stability constants for M(II) and M(III) chelates:

$$M^{II}HBED^{2-} \rightarrow M^{III}HBED^-$$

Simultaneous IEKC experiments confirmed this suggestion. Fig. 3 shows electropherograms of the same Fe(II)–, Mn(II)–, Co(II)–HBED chelates sample for different times after preparation. Mn and Co exhibit a gradual increase in the percentage of $M^{III}HBED^-$ forms and a decrease in $M^{II}HBED^{2-}$. Fe(II) also gives two peaks immediately after the sample preparation. However, the peak with the migration time of 4.8 min [Fe(III)′, Fig. 3a] was not identified as $Fe^{II}HBED^{2-}$, because the addition of Fe(III) to the reaction mixture gives the same picture. A relatively low rate of Fe(III) complex formation, as a result of a competition between the ligand and hydroxyl ion at pH 9.2, should be noted.

3.3. Separation of HBED complexes with different spatial arrangements of the donor groups

Six donor groups of the ligand occupy six the most favorable positions in the octahedral coordination polyhedron of chelate. HBED does not have optical isomers but can give three spatial arrangements of the donor groups during complexation, which can be denoted as (5,5,5), (6,5,5) and (6,5,6) depending on the number of atoms in the three equatorial chelate rings [12]. In this case, nitrogen

atoms work as the asymmetric center. Arrangement (5,5,5) corresponds to the chelate with a plane formed by two nitrogen atoms and two carboxylic oxygens, (6,5,5) by two nitrogen atoms, carboxylate and phenolate, (6,5,6) by two nitrogens and two phenolates (Fig. 4). All the arrangements are in equilibrium, but the stability of complexes should probably increase in the order (5,5,5)<(6,5,5)< (6,5,6) due to a more comfortable arrangement of atoms in the six-membered rings.

Chelation of such kinds of ligands with Fe^{3+} was studied by Lucena et al. [13] for ion-pair HPLC determination of iron chelates in commercial fertilizers. They found two peaks on a $Fe^{III}HBED^{-}$ chromatogram. The second peak was more than tenfold smaller than the first one. Very likely, the largest peak corresponds to a more stable (6,5,6) arrangement. We studied [14] multicomponent separation of metal–HBED chelates in an ion-pair reversed-phase HPLC mode on a Mightysil PR-18 column. The same phenomena were found for Co(III), Al(III), and Fe(III) chelates. In all the cases, less stable complexes with the (6,5,5) arrangement were eluted later. A gradual decrease in these peaks was observed for Al(III) and Fe(III), whereas the ratio of peak areas for Co(III) remained essentially constant.

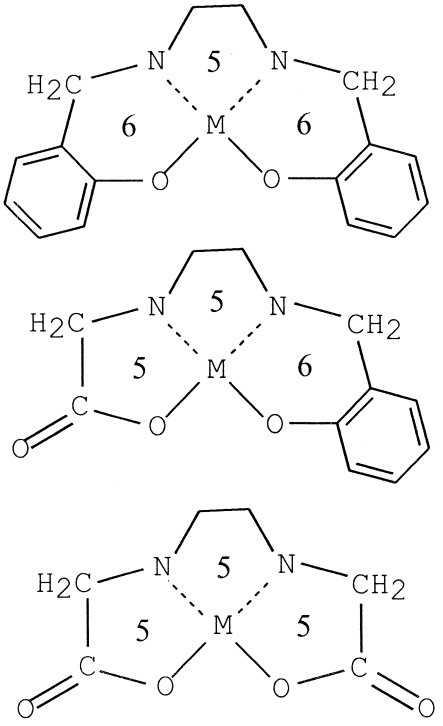

Fig. 4. The structure of equatorial coordination plane with different arrangements of the donor groups.

According to HPLC data and Fig. 3a, we can identify two peaks of iron [Fe(III) and Fe(III)′] as $Fe^{III}HBED^{-}$ chelates with different arrangements of donor groups (probably): (6,5,6) and (6,5,5), respectively. The area of the latter decreases to a negligible value and should not influence quantitative determination of iron. The behavior of the Co(III) chelate corresponds to our HPLC results as well. Thus, the peak of Co(III) chelate (4.5 min in Fig. 3b and c) has a shoulder, whereas it is symmetrical at low (1 m*M*) concentration of the polymeric modifier. It means that two $Co^{III}HBED^{-}$ complexes with a different arrangement of the donor groups have the same electrophoretic mobility but different ion-exchange affinities to PDADMA polycation. We used IC approaches for optimizing IEKC separation selectivity to separate the forms of Co(III) chelate (see Section 3.4).

3.4. Multicomponent separation of metal–HBED chelates

Increasing the column capacity and selecting a weakly retained competing anion is a common approach for improving peak resolution in IC. An increase in the PDADMA-Cl concentration in the carrier electrolyte up to 50 m*M* expands the migration time window of the studied chelates. However, it was not enough for a complete separation of 18 complexes that were selected for these experiments. Thus, Al(III) and Mn(III) complexes migrate almost together at this concentration of the polymer. Competing chloride anion in the carrier electrolyte was replaced by passing the PDADMA-Cl solution through an anion exchanger in the –OH form and neutralizing it with acetic acid. Because of the decomposition of some kinetically labile chelates was possible, a small amount of the ligand (0.1 m*M*) was added to the run buffer.

Fig. 5 shows the separation of 18 M^{2+}, M^{3+}, and $(VO)^{2+}$ chelates using a carrier electrolyte with a high PDADMA concentration (55 m*M* of functional groups) in the mixed acetate–hydroxide form. Two

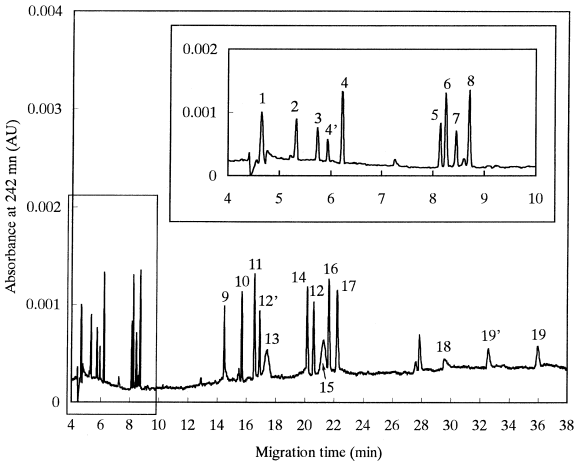

Fig. 5. Separation of 18 metal–HBED chelates. Carrier electrolyte: 0.1 m*M* HBED, 55 m*M* PDADMA-OH, 10 m*M* $Na_2B_4O_7$, pH 10 adjusted with acetic acid; sample injection: 5 p.s.i.×s; other conditions see Fig. 2; $[M]_T = 0.03$ m*M*, $[Bi]_T = [V]_T = 0.04$ m*M*, $[HBED]_T = 1$ m*M*. Peak identification: 1=Bi(III), 2=Sc(III), 3=In(III), 4, 4′=Co(III), 5=Ga(III), 6=Al(III), 7=Cr(III), 8=Mn(III), 9=Pb(II), 10=Fe(III), 11=Cd(II), 12, 12′=Pd(II), 13=Cu(II), 14=Co(II), 15=Zn(II), 16=Ni(II), 17=Mn(II), 18=HBED, 19, 19′=VO^{2+}.

different solutions of Co (Mn) chelates were used for the preparation of this sample. The first one contained freshly prepared $M^{II}HBED^{2-}$. The second one was stored for several days to allow the complete formation of $M^{III}HBED^{-}$. All the analytes were divided into two groups depending on the charge of their anionic chelates with the exception of $Fe^{III}HBED^{-}$, which migrated between Pb(II) and Cd(II). This deviation can be accounted for its highest stability constant ($\log K^{M}_{ML} = 39.01$), the most compact complex structure, and, consequently, the highest ion-exchange affinity among $M^{III}HBED^{-}$ chelates. Under these separation conditions, the shoulder of $Co^{III}HBED^{-}$ (Fig. 3b and c) transformed into a well-resolved peak. In addition to Co(III) (peaks at 5.9 and 6.2 min), only Pd(II) and V(IV) showed significant peak doubling. For both cases, the ratio of peak areas was found to be approximately 1:1. Under these conditions, a less stable (6,5,5) $Fe^{III}HBED^{-}$ chelate (4.8 min in Fig. 3a) migrates in the group of singly charged analytes (10.3 min, not shown in Fig. 5). For both Co(III) and Fe(III), more stable (6,5,6) spatial arrangements of donor groups provide a higher ion-exchange affinity of the complex, whereas the ion-pair HPLC selectivity of Co(III) and Fe(III) chelates forms is opposite [13,14].

Particular attention should be focused on preventing the adsorption of CO_2 from air. The transformation of carbon dioxide into HCO_3^- or CO_3^{2-} at pH 9–10 in the carrier electrolyte leads to a slow decrease in analyte migration times due to the appearance of additional competing ions in the polymer solution. Storing buffer vials in a dessicator with granulated NaOH was proved to be appropriate for providing a good reproducibility of migration times (Sr – relative standard deviation (RSD) = 0.02, $n = 10$).

4. Conclusion

The potential of EDDHA and HBED ligands for determining metal ions by IEKC was estimated. The IEKC separation mode provides a high separation efficiency due to a plug profile of the movement of the liquid and an easy access to ion-exchange sites in the polymer solution. High concentrations of the polymeric modifier and competing ion with a low ion-exchange affinity expanded the selectivity scale for the separation of 18 double- and single-charged anionic HBED chelates up to 31 min. IEKC turned out to be a powerful technique for the separation of anionic chelates, HBED appears very convenient for multicomponent determination of metal ions due to high stability of the complexes with hard metal ion and about a tenfold increase in the detection sensitivity over previously studied EDTA [9]. The formation of more than one peak of $FeHBED^{-}$ and $CoHBED^{-}$ will affect the determination of Fe and Co in the case of samples of unknown composition. To solve this problem, we plan to carefully optimize the conditions for complex formation with HBED for the determination of each group of elements.

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