Journal of Chromatography A, 652 (1993) 539–546
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 259

Ion-association capillary electrophoresis

New separation mode for equally and highly charged metal chelates

Nobuhiko Iki*, Hitoshi Hoshino and Takao Yotsuyanagi

Department of Molecular Chemistry and Engineering, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980 (Japan)

ABSTRACT

A new separation method for highly charged metal chelates based on ion association in capillary zone electrophoresis (CZE) has been developed. Metal chelates of Al(III), Co(III), Cr(III) and Fe(III) ions with 2,2′-dihydroxyazobenzene-5,5′-disulphonate could not be separated by a conventional CZE system (electrophoretic buffer: $[NaH_2PO_4]_T = 0.02$ *M*, pH 7.0, $V = 21.7$ kV, $i = 20$ μA, $l = 50$ cm), because they have fixed and identical (−5) charges. However, when 25 m*M* tetrabutylammonium bromide was added to the same electrophoretic buffer, each of the four chelates was well resolved and migrated in the order Fe(III), Co(III), Cr(III) and Al(III) with theoretical plate numbers of 210 000–250 000 per 50 cm within 11 min ($V = 21.3$ kV, $I = 40$ μA). The detection limits ($S/N = 3$) as determined by spectrophotometric detection ($\lambda = 494$ nm, I.D. = 50 μm) were 1.00, 1.89 and 3.11 fmol for Al, Co and Fe, respectively, in $6.0 \cdot 10^{-9}$ dm^3 of sample solution injected. The effect on the separation of the sizes, types and the concentrations of quaternary ammonium ions added to the electrophoretic buffer was also investigated. As a result, some evidence was obtained that the ion-association reaction between the chelates and ammonium ions probably took the major role in the separation mechanism.

INTRODUCTION

High-performance capillary electrophoresis (HPCE), which is a general term for capillary electrophoresis (CE) or capillary zone electrophoresis (CZE) and related methods such as micellar electrokinetic chromatography (MEKC), capillary gel electrophoresis and so on, has been intensively applied to bioorganic molecules in recent years [1]. By contrast, the application of HPCE to the separation of inorganic species such as metal chelates is still quite rare. In 1989 the first report on the separation of 4-(2-pyridylazo)resorcinolato (PAR) chelates by MEKC was published [2]. Also, in 1991, we reported MEKC of $\alpha,\beta,\gamma,\delta$-tetrakis(4-carboxyphenyl)porphinato chelates [3]. Since then CZE separation of metal chelates with 8-hydroxyquinoline-5-sulphonic acid (HQS) [4], ethylenediamine-N,N,N′,N′-tetraacetic acid (EDTA) [5] and 1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid (CyDTA) [6] has been performed. It should be noted that the separation principle of these CZE systems always lies in the complexation equilibria between the injected metal ions and the ligands in the electrophoretic buffer. In other words, a difference in the degree of complexation of each metal ion in the capillary produces individual electrophoretic mobilities, μ_{ep}. This separation principle is exactly the same one that has been widely employed in conventional electrophoretic separation of metal complexes such as paper electrophoresis [7] and isotachophoresis [8]. Although the migration behaviour is easily explained by

* Corresponding author.

0021-9673/93/$06.00

the formation constants and intrinsic μ_{ep} values of the aqueous ion and the complex(es), as shown by the HQS–Ca(II) and Mg(II) system [4], this separation strategy has two restrictions or problems. First, it can only be applied to kinetically labile chelates, *i.e.* unless the complexation reaction is faster than the electrophoretic process, peak broadening or splitting corresponding to each composition occurs. Second, the reagent stream always causes a background signal, which often damages the sensitivity of the system even when a highly sensitive detection method such as laser-induced fluorescence detection is used [4].

Compared with a reversed-phase high-performance liquid chromatography (RP-HPLC) system using a reagent stream, an RP-HPLC system that uses mobile phase without a chelating reagent is free from the background signal caused by the reagent. Therefore higher amplification of signal intensity by the detector is readily achievable and thus detection at the sub-ppb level is always feasible. In addition, since kinetically labile chelates are decomposed on-column and not detected, detection using this method is highly selective for inert metal chelates. In a sense, the separation column behaves as if it could differentiate the kinetic stability of the chelates [kinetic differentiation (KD) mode] [9].

When this mode is transferred to CZE, the system requires a distinct design strategy for pre-column derivatizing ligands and separation systems. Recently, we reported the CZE separation of five kinds of PAR chelates in the KD mode [10]. Only pH control was required in order to manipulate the migration and resolution of the chelates. It was advantageous that the acid dissociation constants of the 1-hydroxy groups of each PAR chelate as well as free PAR are different so that they migrated with different electrophoretic mobilities. This fact provides crucial information on the ligand design, *i.e.* the ligand should have at least one functional group to express the characteristics of the central metal ion by means of the magnitude of the acid dissociation constant.

For this reason it is impossible to separate the complexes of Al(III), Co(III), Cr(III) and Fe(III) with 2,2′-dihydroxyazobenzene-4,4′-disulphoate (DHABS, H_2L^{2-}) by CZE (KD mode) because they have no such functional groups. Therefore, various approaches to the resolution of these chelates by system design have been attempted. For instance, MEKC and ion-exchange EKC [11] systems were examined but did not afford resolution either. In this paper, a new effective separation method for DHABS chelates by CZE coupled and the ion-association reaction is reported. In addition, the effects of the sizes and types of ion-associating agents are discussed.

EXPERIMENTAL

The ligand for pre-column derivatization was the disodium salt of DHABS, Na_2H_2L, synthesized by the method of Süs [12], which was used as a 10 m*M* aqueous solution. Standard metal ion solutions were made by dissolving metal salts of chloride or nitrate, except for V(V) and Mo(VI), ammonium metavanadate and ammonium molybdate, in diluted hydrochloric acid. The buffer for complex formation was 1 *M* tris(hydroxymethyl)aminomethane (Tris)–HCl buffer (pH 8.0). Phosphate buffer was not used, as it interfered with the chelate formation of Al(III) and Fe(III). The electrophoretic buffer was 20 m*M* NaH_2PO_4 solution, which was adjusted to pH 7.0 by NaOH solution. The buffer modifiers used were tetramethylammonium bromide (TMABr) (Kanto Chemical, Japan), tetraethylammonium bromide (TEABr), tetrapropylammonium bromide (TPABr), tetrabutylammonium bromide (TBABr) tetraamylammonium bromide (TAABr) (Tokyo Kasei Kogyo, Japan) and tetraphenylphosphonium chloride (TPPCl) (Dojindo Lab., Japan). All other chemicals used were of analytical reagent grade. Triply distilled water was used throughout this study.

The capillary electrophoresis equipment, photometric detector and data processor were the same as described elsewhere [2]. Fused-silica capillary tubing (650–720 mm × 0.05 mm I.D.) was purchased from Scientific Glass Engineering (Australia). On-column detection at 490 or 494 nm was performed 150 mm from the negative

end. The temperature of the system was kept at 30 ± 1°C in a thermostated safety box with an interlocking system.

A typical procedure is as follows: sample solution containing eleven kinds of metal ions [Al(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Ni(II), Mn(II), Mo(VI), V(V) and Zn(II)] was added to 5 cm^3 of DHABS solution and 0.5 cm^3 of Tris–HCl buffer, then made up to 50 cm^3, and heated at 60°C for 15 min. After cooling to room temperature, $6.0 \cdot 10^{-9}$ dm^3 was injected into the positive end of the capillary by siphonic action. Electrophoresis was accomplished by applying about 20 kV by constant-current supply mode.

RESULTS AND DISCUSSION

The tridentate ligand, DHABS, formed metal complexes with ten kinds of metal ions, except Mo(VI) ion, under the recommended chelation conditions. It was, however, ambiguous whether or not Cr(III) chelate formed quantitatively, because the reaction was extremely slow. Most of the chelates had their absorption maxima around 490–510 nm, except Fe(III) and Mn(II), which had rather broad absorption bands.

Separation of these chelates was attempted by a normal CZE system using electrophoretic buffer of pH 7.0–9.0, which consisted of 0.02 *M* NaH_2PO_4 or a mixture of 0.01 *M* NaH_2PO_4 and 0.01 *M* $Na_2B_4O_7$ solutions. Although free DHABS, M(III), Cu(II), and V(V) chelates were separated, as typically shown in Fig. 1a, mutual separation of M(III)–DHABS chelates was impossible, where M(III) denotes Al(III), Co(III), Cr(III) and Fe(III) ions. The compositions of all M(III) chelates except Cr(III) chelate was confirmed to be 1:2 by the molar ratio method. Therefore these chelates are expressed as $[M(III)L_2]^{5-}$, have the identical charge of −5, and thus migrated at the same mobility. In addition, the chelates of Cd(II), Ni(II), Mn(II) and Zn(II) were not detected. This was probably due to the lack of kinetic stability of these chelates, which might result in their dissociation in the capillary and the absence of the peaks, just as with RP-HPLC separation in the KD mode [9].

In order to resolve these M(III) chelates, the separation system was investigated by modifying the electrophoretic buffers with, for instance, cationic micelles of dodecyltrimethylammonium bromide (DTMABr) or polyelectrolyes of hexadimethrine bromide (HDBr). These HPCE systems of micellar and ion-exchange EKC [11] modes were, however, incapable of resolving M(III)–DHABS. This was probably because the

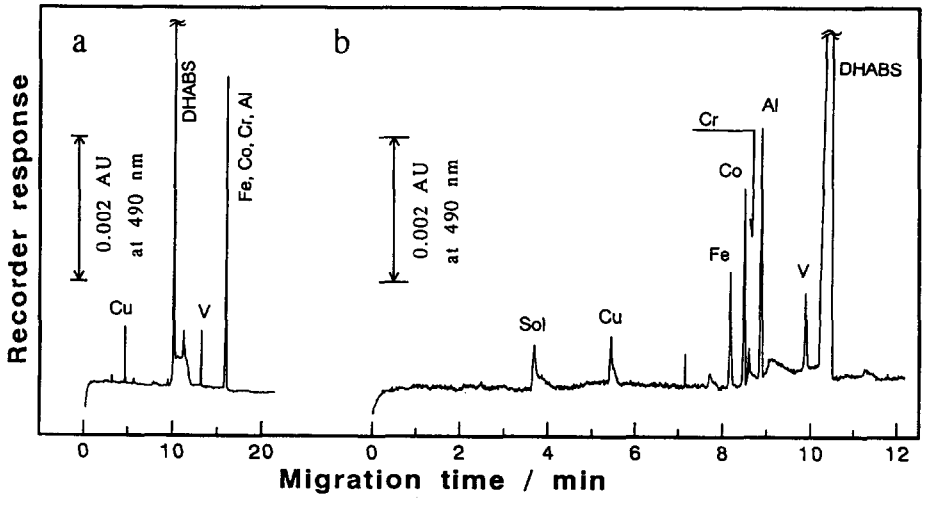

Fig. 1. Typical electropherograms for DHABS chelates. (a) $[NaH_2PO_4]_T = 0.02$ *M*, pH 7.0, $V = 21.7$ kV, $I = 20$ μA. (b) $[NaH_2PO_4]_T = 0.02$ *M*, pH 7.0, [TBABr] = 25 mM, $V = 21.7$ kV, $I = 20$ μA. Total capillary length, $L = 65$ cm, effective capillary length, $l = 50$ cm.

electrostatic attraction between these modifiers and the solutes was too strong.

Interestingly, when the modifier was changed to TBABr, a rather smaller quaternary ammonium salt than DTMABr and HDBr, M(III) chelates were easily resolved. A typical electropherogram is shown in Fig. 1b. Separations were performed within 12 min with theoretical plate numbers, N, described by eqn. 1, of Al, 253 000; Co, 243 000; and Fe, 214 000 per 50 cm:

$$N = 5.54\left(\frac{t_m}{W_{1/2}}\right)^2 \quad (1)$$

where t_m and $W_{1/2}$ are migration time and full-width at half-height for each solute, respectively. The effect of TBA concentration in the electrophoretic buffer on the electrophoretic mobility, μ_{ep}, given by eqn. 2, is shown in Fig. 2:

$$\mu_{ep} = \mu_{obs} - \mu_{eo} = -\left(\frac{1}{t_m} - \frac{1}{t_0}\right)\left(\frac{L \cdot l}{V}\right) \quad (2)$$

where μ_{obs} is apparent electrophoretic mobility, $-(1/t_m)(L \cdot l/V)$, and μ_{eo} is electroosmotic mobility, $-(1/t_0)(L \cdot l/V)$; t_m and t_0 are the migration times of solute and solvent peak; l is the effective capillary length; L is the total capillary length; and V is the applied voltage. When the direction of electrophoretic velocity and electroosmotic flow is toward positive end, the value of μ_{ep} and μ_{eo} are defined as positive.

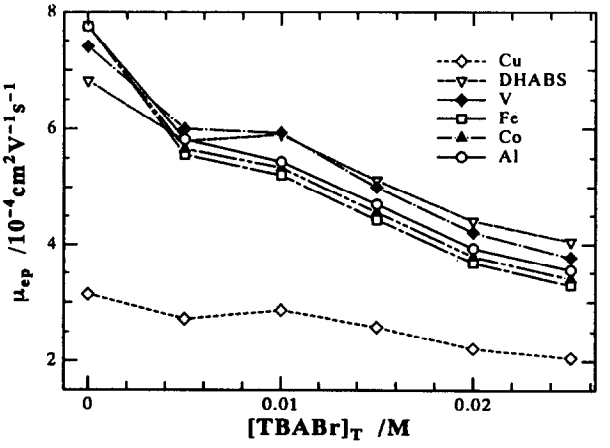

Fig. 2. The effect of TBA ion on the migration of DHABS chelates. Buffer: $[NaH_2PO_4]_T = 0.02$ M, [TBABr] = 0–25 mM, pH 7.0. $L = 72$ cm, $l = 57$ cm.

When the concentration of TBA ion increased from 0 to 25 mM, the direction of electroosmotic flow was unchanged but the value of μ_{eo} simply varied from $-9.99 \cdot 10^{-4}$ to $-7.68 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}. This indicates that there was specific adsorption of the TBA ion onto the capillary wall to decrease the ζ potential. Although the resolution was not good enough, addition of only 5 mM TBA ion made the μ_{ep} values of the three M(III)–DHABS chelates different, as shown in Fig. 2. Optimum separation was obtained at $[TBA]_T = 25$ mM, as shown in Figs. 1b and 2. The decrease in μ_{ep} might originate from the formation of ion associates of higher degree between each solute and TBA ion to reduce the total charge of the associate. Therefore, the sequence of the peaks of M(III) chelates indicates the capacity for ion association to be Fe(III) > Co(III) > [Cr(III)] > Al(III) chelates.

The detectability of Al(III), Co(III) and Fe(III) ions of the CZE system using 25 mM TBA ion was investigated by decreasing the concentration of each M(III) ion from $2 \cdot 10^{-5}$ to $1 \cdot 10^{-7}$ M in samples. Calibration curves in the log–log scale, obtained by the least squares method, are given by eqns. 3–5, where PHA, r and n are peak-height absorbance at 494 nm, correlation coefficient and number of experimental points, respectively:

$$\log (PHA - 7.01 \cdot 10^{-5}) = 0.940 \log [Al]_T + 2.03$$
$$r = 0.9984, \; n = 10 \quad (3)$$

$$\log (PHA) = 0.996 \log [Co]_T + 2.16$$
$$r = 0.9984, \; n = 9 \quad (4)$$

$$\log (PHA) = 0.953 \log [Fe]_T + 1.68$$
$$r = 0.9982, \; n = 7 \quad (5)$$

Linear correlations between the concentrations of Co(III) and Fe(III) ions and peak-height absorbance were obtained. However, the calibration curve of Al(III) ion deviated upwards and finally reached its blank peak height ($7.01 \cdot 10^{-5}$ AU) as the concentration decreased. This is because of the contamination from the reagents and/or environment. The detection limits (D.L.), defined by $S/N = 3$, were for Al $1.67 \cdot 10^{-7}$ M, Co for $3.16 \cdot 10^{-7}$ M and for Fe $5.19 \cdot$

10^{-7} m, where noise levels were $\sigma_{baseline} = 1.62 \cdot 10^{-5}$ AU and $\sigma_{Al\ blank} = 1.52 \cdot 10^{-5}$ AU ($n = 8$). The absolute values of D.L. in a $6.0 \cdot 10^{-9}$ dm^3 injected sample solution were for Al 1.00 fmol, for Co 1.89 fmol and for Fe 3.11 fmol. These values are the smallest absolute detection limits by HPLC or CZE with spectrophotometric detection ever reported.

The effect of the sizes or types and concentrations (5–25 m*M*) of the ion-association agents in the electrophoretic buffer of 0.02 *M* NaH_2PO_4 (pH 7.0) were examined. Representative electropherograms for 5 m*M* and 25 m*M* are shown in Figs. 3–7. Separation of M(III) chelates by using TMA ion, the smallest ion, was impossible (Fig. 3). The significant peak broadening of M(III) chelates at 25 m*M* might be the results of adsorption of the ion associate onto the capillary wall. Separation was just beginning in the case of the 5 m*M* TEA system (Fig. 4a), but baseline resolution was not achieved. When using 25 m*M* TEA, each M(III) peak was separated but the peaks were slightly broad and on the tail of DHABS peak (Fig. 4b), while the TPA system exhibited sharper peaks of M(III) chelates (Fig. 5). However, peak overlap between V(V) and

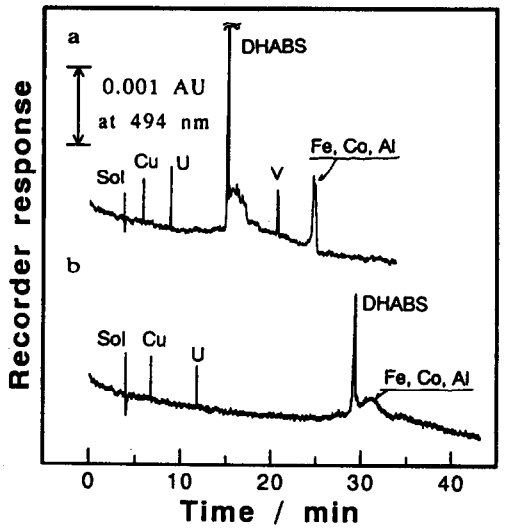

Fig. 3. Electropherograms for DHABS chelates in the TMA system. Buffer: $[NaH_2PO_4]_T = 0.02$ *M*, pH 7.0, [TMABr] = 5 and 25 m*M* for (a) and (b), respectively. $L = 72$ cm, $l = 57$ cm. (a) $I = 25$ μA, $V = 23.2$ kV. (b) $I = 50$ μA, $V = 22.7$ kV. Sol = solvent peak; U = unknown peak.

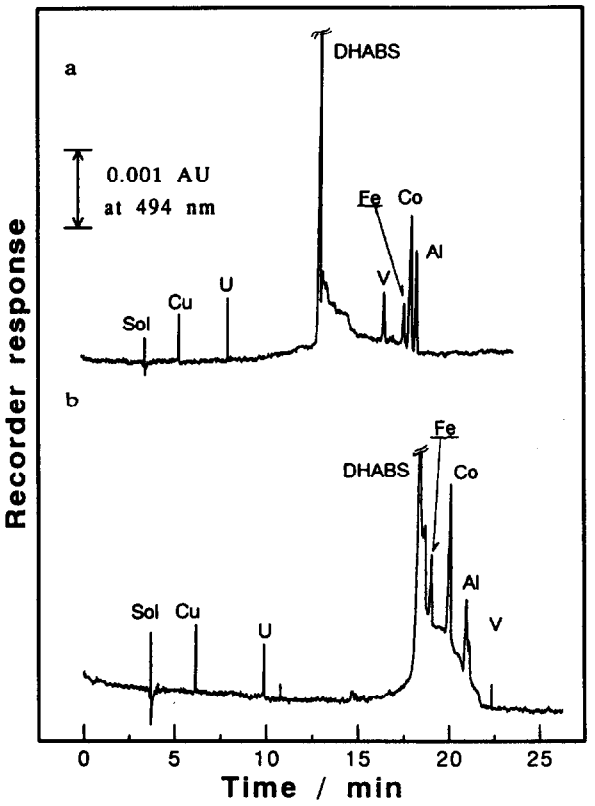

Fig. 4. Electropherograms for DHABS chelates in the TEA system. (a) [TEABr] = 5 m*M*, $I = 25$ μA, $V = 21.2$ kV. (b) [TEABr] = 25 m*M*, $I = 40$ μA, $V = 22.8$ kV. Other conditions are the same as in Fig. 3.

Co(III) chelates or DHABS and Al(III) chelate was observed. The addition of 25 m*M* TBA resulted in excellent electropherograms, as already shown in Fig. 1b. It is noteworthy that only 5 m*M* TAA ion, which is larger than TBA ion, could give quite good electropherograms (Fig. 6a). When the concentration exceeded the optimum TAA concentration at 15 m*M*, the separation of M(III) chelates became poorer (Fig. 6b). The system of 5 m*M* TPP, which is the bulkiest and has π-electron systems, behaved like the 25 m*M* TBA system except for V(V) peak (Fig. 7a). About 5–10 m*M* was optimum in the TPP system, while at 25 m*M*, the electroosmotic flow became extremely slow ($\mu_{eo} = -2.26 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}), and thus a longer migration time was required (Fig. 7b). For instance, the DHABS peak, which was the last one, appeared at 65 min. Peak broadening of

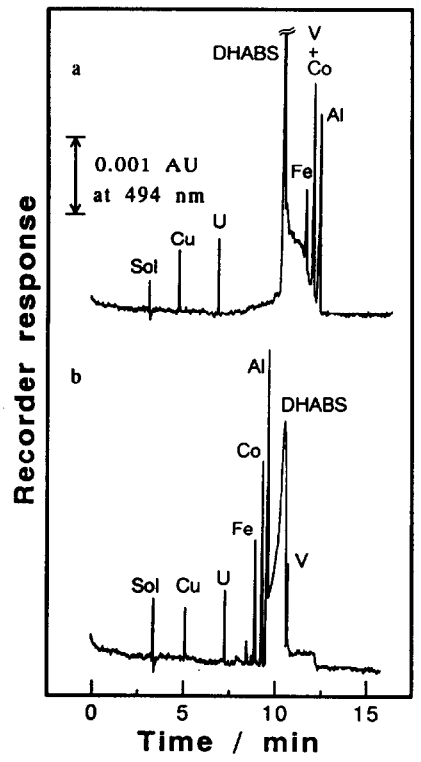

Fig. 5. Electropherograms for DHABS chelates in the TPA system. (a) [TPABr] = 5 m*M*, *I* = 25 μA, *V* = 21.1 kV. (b) [TPABr] = 25 m*M*, *I* = 40 μA, *V* = 22.3 kV. Other conditions are the same as in Fig. 3.

M(III) chelates was also observed, which led to loss of sensitivity.

The above results for 5 m*M* and 25 m*M* counter-ions are summarized in terms of the electrophoretic mobility in Figs. 8 and 9. As the added counter-ions become bulkier, the μ_{ep} value of each solute decreased in both cases, except for 5 m*M* TMA. (The reason for the dip in μ_{ep} at 5 m*M* TMA is not clear.) This implies that the hydrophobic interaction between solute anion and counter-cation significantly contributes to the formation of ion associates as well as electrostatic attraction. Also, the fact that each μ_{ep} value was positive and non-zero is a strong evidence of the presence of a negative charge on the ion associate. The resolution between M(III) chelates became better when bulkier counter-cations were used, as shown in Fig. 8. On the other hand (Fig. 9) the optimum resolution was obtained by the TBA ion but the larger counter-cations gave poorer resolution. Counter-ions such as TAA and TPP, which have larger hydrophobic interaction with M(III) chelates, should be used at lower concentrations.

In the area of separation science, ion-association or ion-pairing techniques are employed in various methodologies, such as ion-association

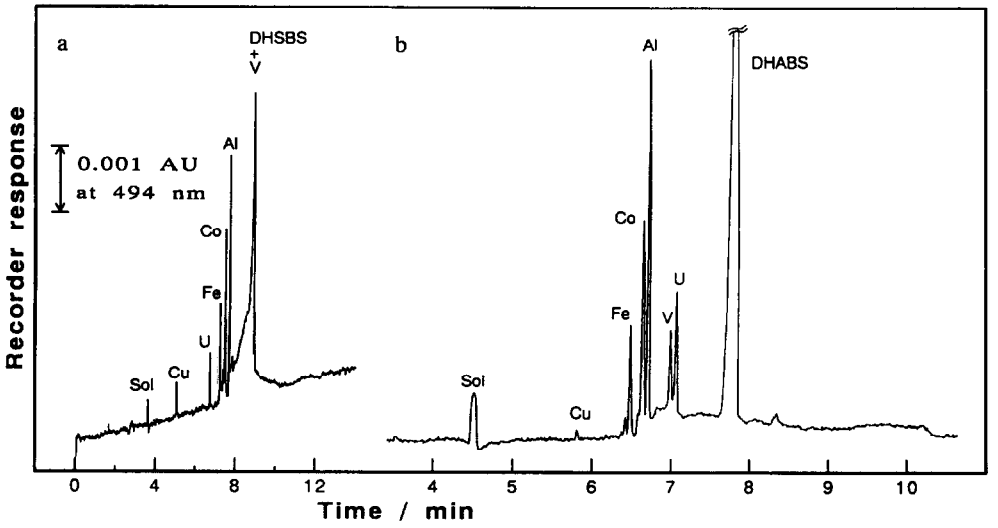

Fig. 6. Electropherograms for DHABS chelates in the TAA system. (a) [TAABr] = 5 m*M*, *I* = 20 μA, *V* = 20.8 kV. (b) [TAABr] = 25 m*M*, *I* = 25 μA, *V* = 20.1 kV. Other conditions are the same as in Fig. 3.

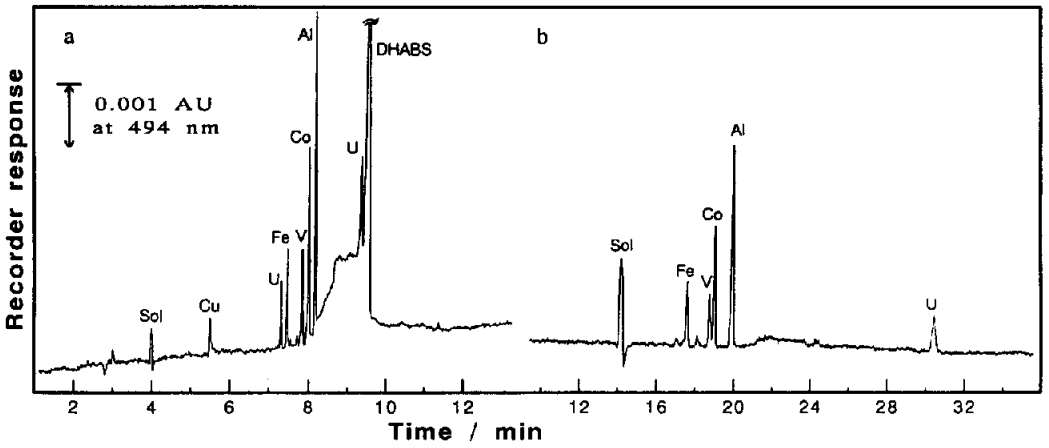

Fig. 7. Electropherograms for DHABS chelates in the TPP system. (a) [TPPCl] = 5 m*M*, *I* = 20 μA, *V* = 20.5 kV. (b) [TPPCl] = 25 m*M*, *I* = 30 μA, *V* = 21.3 kV. Other conditions are the same as in Fig. 3.

extraction [13] and ion-pair RP-HPLC [14]. Importantly in each case, it is essential for the analyte ion to form an ion associate with a suitable counter-ion in the polar phase and to be desolvated before it is partitioned into or adsorbed onto non-polar secondary phase. Also, in the area of HPCE, some attempts to utilize ion association in MEKC [15] and electrochromatography [16] have been made, in which case the ion-association agent serves as a modifier to enhance the phase transfer of analyte onto the pseudo-micellar phase and bonded hydrophobic phase, respectively. From the viewpoint of the separation principle, the CZE system studied in this paper is very peculiar because the separation process depends only on the ion association in the homogeneous phase and is not involved in the secondary phase at all. In conclusion, this separation method is one of a new HPCE mode and should be termed ion association CZE (IA-CZE) or ion association CE (IA-CE). The concept of IA-CE separation can be widely applied to highly charged organic species as well as inorganic molecules.

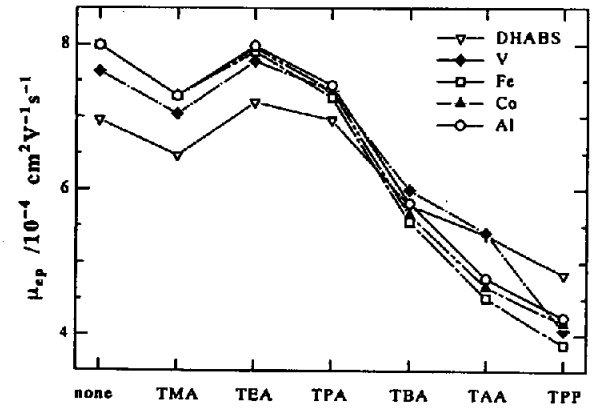

Fig. 8. The effect of counter-cation on the migration of DHABS chelates. Buffer: $[NaH_2PO_4]_T = 0.02$ *M*, pH 7.0, [counter-cation] = 5 m*M* except "none". *L* = 72 cm, *l* = 57 cm.

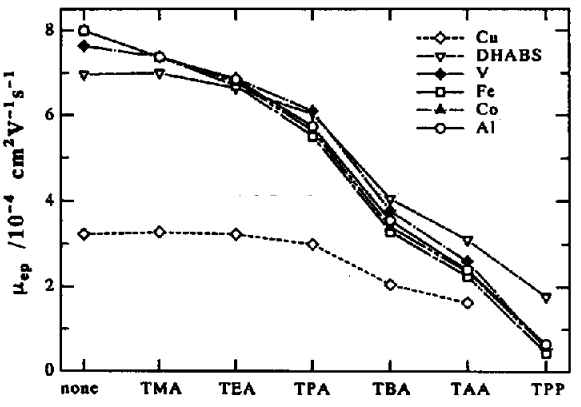

Fig. 9. The effect of counter-cation on the migration of DHABS chelates. Buffer: $[NaH_2PO_4]_T = 0.02$ *M*, pH 7.0, [counter-cation] = 25 m*M* except "none". *L* = 72 cm, *l* = 57 cm.

REFERENCES

1 W.G. Kuhr and C.A. Monnig, *Anal. Chem.*, 64 (1992) 389R.
2 T. Saitoh, H. Hoshino and T. Yotsuyanagi, *J. Chromatogr.*, 469 (1989) 175.
3 T. Saitoh, H. Hoshino and T. Yotsuyanagi, *Anal. Sci.*, 7 (1991) 495.
4 D.F. Swaile and M.J. Sepaniak, *Anal. Chem.*, 63 (1991) 179.
5 S. Motomidzu, M. Oshima, S. Matsuda, Y. Obata and H. Tanaka, *Anal. Sci.*, 8 (1992) 619.
6 S. Motomidzu, S. Nishimura, Y. Obata and H. Tanaka, *Anal. Chem.*, 7 (supplement) (1991) 253.
7 Y. Kiso, I. Yamagata, Y. Yamamoto and M. Shinagawa, *Bull. Chem. Soc. Jpn.*, 38 (1965) 695.
8 Y. Nakabayashi, K. Nagaoka, Y. Masuda and R. Shinke, *Talanta*, 36 (1989) 639.
9 H. Hoshino, K. Nakano and T. Yotsuyanagi, *Analyst (London)*, 115 (1990) 133.
10 N. Iki, H. Hoshino and T. Yotsuyanagi, *Chem. Lett.*, (1993) 701.
11 S. Terabe and T. Isemura, *Anal. Chem.*, 62 (1990) 652.
12 O. Süs, *Liebig's Ann. Chem.*, 556 (1944) 65.
13 K. Tôei, *Anal. Sci.* 3 (1987) 479.
14 P.R. Haddad and P.E. Jackson, *Ion Chromatography (J. Chromatogr. Library*, Vol. 46), Elsevier, Amsterdam, 1990, Ch. 6.
15 H. Nishi, N. Tsumagari and S. Terabe, *Anal. Chem.*, 61 (1989) 2434.
16. W.D. Pfeffer and E.S. Yeung, *J. Chromatogr.*, 557 (1991) 125.