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Theoretical estimation of capillary zone electrophoresis behaviour of metal complexes using multivariate regression analysis

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Abstract

The migration of lanthanide metal ions complexed with aminopolycarboxylic reagents (ethylenediaminetetraacetic acid and chemically similar analogues containing varying degrees of basicity and size) in capillary zone electrophoresis was investigated. Electrophoretic mobilities were measured for a series of complexes in borate buffer electrolytes of variable pH and buffer concentration (62 different compositions). A migration model was developed to relate the electrophoretic mobility with charge density parameters of a metal complex (the net charge, the stability constant and ligand structural increments) and electrophoretic system variables such as the electrolyte pH. The predictive ability of the derived regression equation was evaluated in terms of its statistical significance. The good agreement observed between the calculated electrophoretic mobilities and experimental values demonstrates the applicability of the multivariate regression approach to the prediction of migration behaviour in capillary electrophoresis on the basis of small-number measurements. The results confirm also that the separation mechanism for metal complexes is based on the differential electrophoretic migration governed by differences in charge-to-size parameters.

1. Introduction

High-performance capillary electrophoresis (CE) is receiving considerable attention as a highly efficient tool for separating and detecting metal ions. Significant advances have been made by a number of workers, as summarized in recent books [1–3] and reviews [4,5]. In CE it is now common to use the complexing agent added

to the capillary electrolyte or to the sample, to complex metal ions, partially or completely, and thereby to facilitate the resolution. This CE methodology holds even greater promise than ion chromatography regarding the achievement of multi-element separations. Nevertheless, as metal ion analysis is a relatively new area of CE application, specific advances are still needed to develop the method as an alternative separation technique.

In this respect, a better understanding of the migration mechanism and the ionic or molecular properties that control the separation of metal

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species during CE would be of great value. Further, optimization of the electrophoretic behaviour requires the use of the quantitative relationships between migration parameters and different charge-to-size characteristics of the analytes. To the best of our knowledge, there has been only one published report, by Shi and Fritz [6], where a correlation was established between the migration times of lanthanide cations in the presence of a weak complexing agent, 2-hydroxyisobutyric acid (HIBA), and a charge-related parameter such as the average number of ligands associated with a metal atom. No-one seems to have addressed the issue of how the separand parameters affect the electrophoretic behaviour of precapillary-formed metal complexes, which are generally distinguished by a more complicated migration mechanism than partially complexed metal ions. This feature is related to a diversity of interactions between the complexes and electrolyte components such as those involved in acid–base equilibria, ion pairing, mixed-ligand complex or adduct formation, dissociation, micellization, etc.

In addition, method development in metal ion CE needs a systematic investigation of the effects of operating variables on the migration behaviour. Few studies have been published which deal with this subject. Jimidar et al. [7] showed that the separation of rare earth metal ions is mainly influenced by the pH and the concentration of HIBA and selected optimized conditions by using computer-assisted modelling. More recently, a mathematical model relating the electrophoretic mobility of a metal cation with the same two experimental factors was reported by Quang and Khaledi [8].

We have previously demonstrated that the iterative regression strategy can be successfully applied to the theoretical estimation of the retention of metal complexes in HPLC, when using well known molecular properties (e.g., the stability constants or the orbital electronegativity of the metal atom) and physico-chemical characteristics of the mobile phase as predictors [9]. Here we describe the extension of this approach to the interpretation of migration properties in CE for a range of metal complexes with the aim

of developing a simple linear model of electrophoretic behaviour. The migration mechanism for lanthanide metal complexes of aminopolycarboxylic acids used as test solutes and the capability of derived multiparametric regression equations to predict migration parameters are discussed.

2. Experimental

2.1. Chemicals

Lanthanide metal ion solutions were prepared by dilution of 1000 $\mu\text{g/ml}$ AAS standard concentrates (Aldrich, Milwaukee, WI, USA), except for lanthanum, thulium and lutetium, which were prepared from the nitrates dissolved in 0.01 M HNO_3 . The aminopolycarboxylic acids used were purchased from Aldrich (Steinheim, Germany), Sigma (St. Louis, MO, USA) or Merck (Darmstadt, Germany). They included ethylenediaminetetraacetic, *trans*-1,2-cyclohexanediaminetetraacetic, diethylenetriaminepentaacetic and triethylenetetraaminehexaacetic acid and were used as 0.005 or 0.01 M solutions in 0.01 M sodium tetraborate. Metal complexes were prepared as described elsewhere [10]. All chemicals were of analytical-reagent grade and doubly distilled water was used for all solutions.

Sodium tetraborate was used for the preparation of electrolyte buffers. The pH of the electrolytes, varied as specified below, was adjusted by adding sodium hydroxide solution or hydrochloric acid to the sodium borate solutions. The borate concentration was varied as described under Results and discussion. The carrier electrolytes also contained an appropriate amount of a chelating reagent (usually $1 \cdot 10^{-3} M$).

2.2. Instrumentation

Capillary electrophoresis was performed by using a Waters (Milford, MA, USA) Quanta 4000 capillary electrophoresis system and fused-silica capillaries of 50 cm total length \times 75 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA). A positive power supply of 15 kV was

used for electrophoresis. On-column direct detection was performed at 214 nm. The injection time was 10 s with hydrostatic injections from a height of 10 cm. Electropherograms were recorded and processed with a Hewlett-Packard Model 3359 data acquisition system. To ensure run-to-run reproducibility of migration times, 2-min purges of the capillary with running electrolyte were programmed. The electroosmotic flow velocity was determined from the migration time of acetophenone added to a sample. The electrophoretic mobility, μ_{ep} , was calculated as the difference between the observed mobility and electroosmotic mobility and expressed as negative values because it is opposed to the latter.

2.3. Calculations

Migration values (logarithms of electrophoretic mobilities) were related to analyte and electrolyte parameters by means of multiparametric regression analysis processed with the program SigmaPlot (Jandel Scientific, Corte Madera, CA, USA) on a personal computer. The equations derived were tested according to the requirements of a meaningful correlation analysis, taking into account the correlation coefficient, standard deviation, significance level of the whole equation and number of data points used to derive the equation. Calculated dependences of the electrophoretic mobility on the pH of the electrolyte were obtained by iteration based on a least-squares fit using a standard statistical package.

3. Results and discussion

In free-solution CE with simple electrolytes such as sodium borate buffers, the electrophoretic mobility of ionic species is commonly dependent on the charge and size of the solutes. For the metal complexes under investigation, as for negatively charged species, it can be assumed that adsorption interactions with the capillary wall are negligible. Neither could ion-pairing interactions substantially influence the migration

behaviour. Differences in the mobility of the complexes can thus be ascribed to differential charge densities. Lanthanide metal ions have an equal formal charge (3+) and differ only slightly in the ionic radius. Hence as a central metal atom of the chelate complex, they contribute to the net charge and molecular size to a much lesser extent than an aminopolycarboxylic ligand. Therefore, we shall first discuss the effect of differences in charge-to-size ratio arising from differences in the structure of the organic part of the metal complex.

3.1. Correlations using ligand structural descriptors

As pointed out under Experimental, the four sets of lanthanide metal complexes with different aminopolycarboxylic ligands listed in Table 1 were subjected to CE experiments. Variations in the number of carboxylic groups impart different charges to the lanthanide complex, which should affect the migration behaviour. Indeed, superficial examination of the raw data showed that the migration times of the complexes of any given metal increased in the order CDTA < EDTA < DTPA < TTHA. This order followed the expected trend, i.e., the more charged solutes, having higher electrophoretic mobilities in the direction opposite to the electroosmotic flow,

Table 1
Characteristics of the complexing reagents used

Reagent	Charge of lanthanide complex	Ligand structural descriptors			
		$n_{\text{COOH}} - 3$	n_{COOH}	n_{CH_2}	n_{N}
Ethylenediamine-tetraacetic acid (EDTA)	-1	1	4	6	2
Cyclohexanediaminetetraacetic acid (CDTA)	-1	1	4	10	2
Diethylenetriaminepentaacetic acid (DTPA)	-2	2	5	9	3
Triethylenetetraaminehexaacetic acid (TTHA)	-3	3	6	12	4

migrated longer than less charged species. DTPA and especially TTHA complexes as the compounds carrying charges of 2⁻ and 3⁻, respectively, are strongly attracted to the anode (injection end of the capillary), resulting in a decreased apparent velocity compared with that of monocharged EDTA and CDTA complexes. For the latter, equally charged, complexes, the difference in size must have been substantial. As a result, CDTA complexes, being larger and thus possessing a smaller charge density, always migrated faster than EDTA complexes.

On the basis of the above observation, the net charge of the complex (Z) as the main parameter, determining the electrophoretic mobility of a solute, was taken for the initial analysis of the data. Evidently, the Z value strictly depends on the number of carboxylic groups, n_{COOH} , and can be expressed, in absolute units, as $n_{\text{COOH}} - 3$. While the charge is a very simple function of the ligand structural fragment, the size of the ligand, having an additive character, can be only presented as a sum of contributions (increments) of the various functional groups. In our case these are carboxylic and methylene groups as well as tertiary nitrogen atoms quantified by n_{COOH} , n_{CH_2} and n_{N} , respectively. Combination of all four variables was used for analysing the migration data using stepwise multiple regression. The best regression equation describing $\log \mu_{\text{ep}}$ in the CE system based on a 10 mM borate buffer at pH 9.5 was

$$\begin{aligned} \log \mu_{\text{ep}} = & -2.673 + 0.522(\pm 0.181)(n_{\text{COOH}} - 3) \\ & - 0.262(\pm 0.109)n_{\text{COOH}} \\ & - 0.031(\pm 0.002)n_{\text{CH}_2} \\ & - 0.091(\pm 0.182)n_{\text{N}} \end{aligned} \quad (1)$$

$$R = 0.9851; \text{S.D.} = 0.019; p \leq 9.44 \cdot 10^{-31}; n = 40$$

In this and subsequent equations the values in parentheses are standard deviations of regression coefficients, R is the correlation coefficient, S.D. and p are standard deviation and significance level of the whole equation, respectively, and n is the number of data points used to derive the regression.

Eq. 1 is highly significant statistically, as ex-

pressed by the correlation coefficient, and consistent with the qualitative consideration of migration behaviour given above. This equation shows that a charge-related structural descriptor, $n_{\text{COOH}} - 3$, as expected, provides a positive input to electrophoretic mobility (positive sign of the corresponding coefficient), whereas the size-related parameters, n_{COOH} , n_{CH_2} and n_{N} , account for mobility-decreasing effects in CE. At the same time it suggests that the apparent size of the molecule, as estimated from the number of elementary fragments, is less marked as a factor controlling the migration ability relative to the charge. Although all functional groups are shown to contribute negatively to the mobility, the regression coefficients for the corresponding variables differ significantly. This can be rationalized as the input of $-\text{CH}_2-$ and $=\text{N}-$ fragments appears to be of lesser importance than that of COOH groups. This observation fits well with the relative sizes of different molecular increments. Also, the comparison of the absolute values of the coefficients for $n_{\text{COOH}} - 3$ and n_{COOH} variables would assume that the free carboxylic group reflects the changes in the charge rather than in solute's size.

3.2. Correlations with the stability constant

Regardless of the nature of the ligand, the migration order of lanthanide complexes remained constant with a regular increase in migration times with increasing atomic number across the lanthanide series (Fig. 1). In a recent paper [10], we interpreted such a migration behaviour in terms of the effective charge of the metal ion. Evidently, the lower the electron-acceptor ability of the lanthanide ion, the larger is the negative charge of the complex and therefore the faster will be its electrophoretic mobility. Consequently, the complexes of lanthanide ions with higher atomic number migrate more slowly towards the cathode. However, to the best of our knowledge, no published data on effective charge calculations for the complexes under consideration are available. On the other hand, there are abundant reports on the overall stability constants, β_n (e.g., see Ref. [11] or more recent editions of

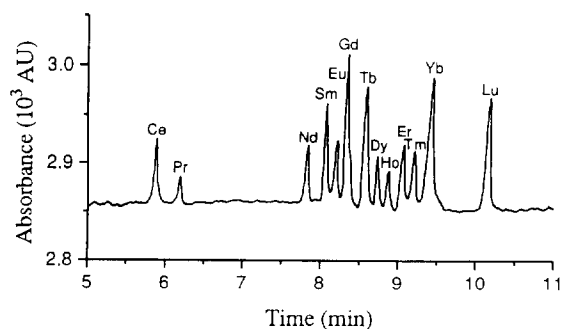


Fig. 1. CE separation of lanthanide metal-CDTA complexes. Capillary, 42/50 cm \times 75 μ m I.D.; electrolyte, 20 mM borate buffer containing $1 \cdot 10^{-3}$ M CDTA (pH 11.1); applied voltage, +15 kV; direct UV detection at 214 nm.

that publication) as a parameter closely connected with the electron-acceptor strength of metal ions. This universal structure-dependent characteristic of metal complexes is commonly accepted as a variable for quantitative structure-chromatographic retention relationships [12,13]. Further, in earlier work [14], the $\log \beta_n$ values were applied successfully in the regression equation describing the observed mobility of transition metal complexes with 8-hydroxyquinoline-5-sulphonic acid in CE.

With that in mind, we subjected the experimental migration data for lanthanide-aminopolycarboxylic acid complexes to linear regression analysis. A simple correlation of $\log \beta_n$ against $\log \mu_{ep}$ values gave the following equation (in this example, for CDTA complexes).

$$\log \mu_{ep} = -2.840 - 0.038(\pm 0.005) \log \beta_n \quad (2)$$

$$R = 0.9308; \text{S.D.} = 0.024; p \leq 3.901 \cdot 10^{-6}; n = 13$$

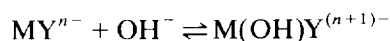
Eq. 2 is of rather good statistical quality that proves the ability of $\log \beta_n$ to approximate mobility differences. It should be also stressed that the coefficient relating $\log \mu_{ep}$ and $\log \beta_n$ is negative. This indicates that more stable complexes with the electron charge density shifted stronger to the central atom are less mobile than less stable complexes and additionally confirms the usefulness of β_n as a reliable measure for the effective charge estimations.

3.3. Dependences of electrophoretic mobility on electrolyte composition

Until now, migration data were analysed separately for each electrophoretic buffer system. For an approximation of the whole data set, knowledge of the electrolyte variables influencing the migration behaviour and selection of the most important ones are required. Included in a single equation with solute descriptors, as carried out below, they can provide a sufficiently complete answer. As borate buffer electrolytes were indicated to be the optimum electrolyte system for metal-aminopolycarboxylic acid complexes [10,15], the parameters considered were the pH and borate concentration.

pH

The electrophoretic mobility can reflect the changes in the electrolyte pH provided that the analytes undergo certain charge density alterations. For negatively charged metal complexes, this may concern the influence of pH on acid-base or complexation equilibria. The effect of pH variation was examined experimentally in the range 8.5–11.0 using 10 mM borate buffer. At less basic pH, the mobilities of the complexes were very close or even identical, as for separands with very similar pK_a (or effective charge) values. However, above pH 9.5, a significant increase in μ_{ep} as a function of pH was observed. This increase is thought to be related to the formation of hydroxo forms of the lanthanide complexes, presumably $M(OH)Y^{(n+1)-}$ (M and Y represent a lanthanide ion and the fully deprotonated form of a reagent, respectively). Evidently, an increased negative charge of lanthanide species, following a shift in the equilibrium



in favour of the mixed-ligand form, leads to correspondingly higher mobilities. The observed pH dependence is contrary to the results reported by Motomizu et al. [16], who found that the electrophoretic mobilities of alkaline earth metal-EDTA complexes were identical over the

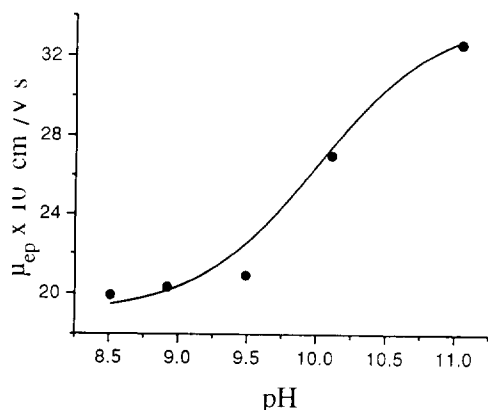


Fig. 2. Electrophoretic mobility of erbium-CDTA complex as a function of pH. ● = experimentally determined values; solid line = theoretical curve calculated according to Eq. 3.

pH range 8–10.5. This contradiction can be explained by a stronger tendency of lanthanide(III) ions to hydrolyse and, consequently, to form hydroxymetal species. Note that the same workers suggested the formation of a mixed-ligand chelate, $\text{Fe}(\text{OH})\text{Y}^{2-}$, for iron(III) with a high tendency for hydrolysis.

To find the relationship between the observed electrophoretic mobility of the complexes and pH of the electrolyte, the above equilibrium was treated in a similar way to an acid–base equilibrium (e.g., Ref. [17]). The resulting theoretical relationship between μ_{ep} and pH is

$$\mu_{\text{ep}} = \frac{\mu_{\text{ep}}^{\text{M}(\text{OH})\text{Y}} - \mu_{\text{ep}}^{\text{M}(\text{OH})}}{1 + 10^{\text{p}K - \text{pH}}} \quad (3)$$

where $\mu_{\text{ep}}^{\text{MY}}$ and $\mu_{\text{ep}}^{\text{M}(\text{OH})\text{Y}}$ are the mobilities of individual complexed forms (the charges are

omitted) and K is the formation constant of a mixed-ligand complex. Curves for μ_{ep} vs. pH dependences were approximated by an iteration procedure using Eq. 3. A typical example of the least-squares fit of the experimental data to the function as given in Eq. 3 is shown in Fig. 2 for the erbium-CDTA complex. The p*K* values resulting from this approximation were used for further evaluation of the electrolyte pH effect within a multivariate migration model.

Borate buffer concentration

Different borate concentrations of the electrolyte were then investigated. In these experiments, the electrolyte pH was kept constant at 9.5. The results are presented in Table 2. As can be seen, the mobility of all the complexes first decreases (up to ca. 10–15 mM) and then increases when the buffer concentration is further increased. This behaviour is probably due to the competing effects of changes in the ionic strength of the electrophoretic medium. At relatively low electrolyte concentrations, the mobilities become lower following a decreased ζ potential, as follows from the double-layer theory. In contrast, when high-ionic-strength buffer solutions are used, an excessive Joule heating, which is likely to occur, leads to an increase in mobilities owing to an influence of temperature on the electrolyte viscosity.

Nevertheless, these results indicate that the buffer concentration alters the electrophoretic mobility of lanthanide complexes only moderately and thus is a less important controllable factor than the pH; it is therefore not considered further. Hence pH was chosen as a variable that has the greatest impact on migration parameters

Table 2

Effect of borate buffer concentration on the electrophoretic mobilities of lanthanide complexes (pH 9.5)

Sodium tetraborate (mM)	$-\mu_{\text{ep}} \times 10^5 \text{ (cm}^2 \text{ V}^{-1} \text{ s}^{-1}\text{)}$			
	Ce-EDTA	Eu-EDTA	Dy-CDTA	Gd-CDTA
5	26.8	26.6	23.1	23.2
10	25.4	25.3	19.5	19.4
15	22.8	21.8	20.6	20.0
20	23.2	24.0	22.1	21.7

which can be described by a non-empirical function.

3.4. Multiparametric migration model for lanthanide complexes

Obviously, we approached the situation when a migration model relating $\log \mu_{ep}$ values to solute and electrolyte parameters taken simultaneously into consideration as predictors can be derived to evaluate the migration behaviour of lanthanide complexes in different buffer systems. Therefore, an attempt was made to incorporate all of the above variables in one regression equation to be utilized for analysing all of the data combined into one set. The resulting migration model is expressed by the following six-term equation:

$$\begin{aligned} \log \mu_{ep} = & -2.575 + 0.510(\pm 0.169)Z \\ & + 0.0041(\pm 0.0022) \log \beta_n \\ & - 0.285(\pm 0.099)n_{COOH} \\ & - 0.016(\pm 0.002)n_{CH_2} \\ & - 0.141(\pm 0.171)n_N - 0.077(\pm 0.006) \\ & \times \log(1 + 10^{pK - pH}) \end{aligned} \quad (4)$$

$$R = 0.9643; \text{S.D.} = 0.025; p \leq 3.17 \cdot 10^{-50}; n = 86$$

This equation describes the linear change in $\log \mu_{ep}$ as a function of charge and structure characteristics for any pH of borate buffer electrolyte. The last term was included after taking the common logarithm of both sides of Eq. 3 and operated with pK values determined by electrophoretic measurements. As the magnitude of the regression coefficient reflects both the contribution and the individual scale of each parameter, it is not easy to interpret their relevance. Nonetheless, as might be expected, the largest parameter is the net charge of the metal complex. Also, the coefficients for this parameter and ligand structural variables (terms 3–5) are in accord with those for similar terms of Eq. 1.

The statistical goodness of Eq. 4 is high enough to predict the mobility of a metal complex. In order to illustrate this value of the proposed model, the calculated logarithms of

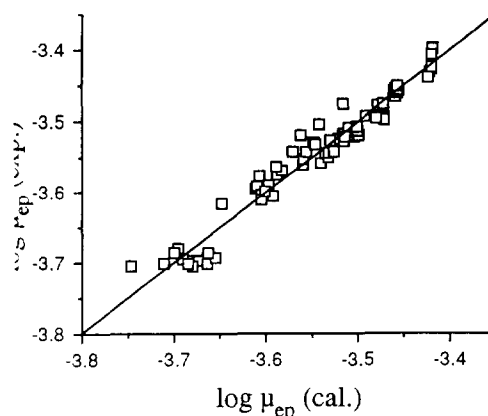


Fig. 3. Relationship between the migration parameters of lanthanide complexes determined experimentally and calculated using Eq. 4 ($\mu_{ep} \times 10^5 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$). The correlation coefficient (R) is 0.9791. The average deviation of the calculated and observed $\log \mu_{ep}$ values is 0.40%.

electrophoretic mobility were plotted against the observed values. Fig. 3 shows the result of applying Eq. 4 to the experimental data set for 43 complexes under 14 different electrolyte conditions (80 data points). A linear relationship with zero intercept and unit slope was obtained. The predictive quality expressed as the percentage deviation of the calculated and observed $\log \mu_{ep}$ values is generally less than 1%.

4. Conclusions

The results of this study demonstrate that the multivariate regression strategy for analysing mobility dependences provides an adequate description and rational interpretation of CE data. The statistically significant relationships derived here can be widely employed to approximate differences in the migration properties of metal ion complexes arising from differences in structure and compositional electrolyte changes. At the same time, they support the validity of the generally observed mechanism of free-solution CE in the case of precapillary-formed metal complexes. The migration behaviour has been revealed to be a pure electrophoretic effect affected solely by charge density parameters of solute molecules without any observable influ-

ence of auxiliary solute–electrolyte or solute–capillary wall interactions. The fact that it is possible to correlate the mobility data directly with simple, readily determinable parameters, possessing a definite physical meaning, confirms also a straightforward theoretical foundation of CE.

As demonstrated above, accurate predictions of migration can be achieved from multiple linear regression when operating meaningful migration models consisting of certain sets of structural and electrolyte variables. It is worth noting that owing to its simplicity, the multivariate statistical approach can be easily adapted to other kinds of metal complexes and further developed to be applicable to partially complexed metal ions. Likely advances with this approach may also concern the migration behaviour of metal complexes in more sophisticated CE modes (e.g., micellar-mediated [18] or ion-association [19] CE), taking into account the inputs to mobility due to the corresponding solute and electrolyte parameters.

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References

- [1] P. Jandik and G. Bonn, *Capillary Electrophoresis of Small Molecules and Ions*, VCH, New York, 1993.
- [2] R. Kuhn and S. Hoffstetter-Kuhn, *Capillary Electrophoresis: Principles and Practice*, Springer, Berlin, 1993.
- [3] F. Foret, L. Krivankova and P. Bocek, *Capillary Zone Electrophoresis*, VCH, Weinheim, 1993.
- [4] P.E. Jackson and P.R. Haddad, *Trends Anal. Chem.*, 12 (1993) 231.
- [5] A.R. Timerbaev, *J. Capillary Electrophoresis*, in press.
- [6] Y. Shi and J.S. Fritz, *J. Chromatogr.*, 640 (1993) 473.
- [7] M. Jimidar, T. Hamoir, W. Degezelle, D.L. Massart, S. Soykenç and P. Van de Winkel, *Anal. Chim. Acta*, 284 (1993) 217.
- [8] C. Quang and M.G. Khaledi, *J. Chromatogr. A*, 659 (1994) 459.
- [9] A.R. Timerbaev, O.P. Semenova, I.G. Tsoi and O.M. Petrukhin, *J. Chromatogr.*, 648 (1993) 307.
- [10] A.R. Timerbaev, O.P. Semenova and G.K. Bonn, *Analyst*, in press.
- [11] A.E. Martell and R.M. Smith, *Critical Stability Constants*, Plenum Press, New York, 1974.
- [12] A.R. Timerbaev, I.G. Tsoi and O.M. Petrukhin, *J. Chromatogr.*, 498 (1990) 337.
- [13] A.R. Timerbaev and G.K. Bonn, *J. Chromatogr.*, 640 (1993) 195.
- [14] A.R. Timerbaev, W. Buchberger, O.P. Semenova and G.K. Bonn, *J. Chromatogr.*, 630 (1993) 379.
- [15] S. Motomizu, S. Nishimura, Y. Obata and H. Tanaka, *Anal. Sci.*, 7 (1991) 253.
- [16] S. Motomizu, M. Oshima, S. Matsuda, Y. Obata and H. Tanaka, *Anal. Sci.*, 8 (1992) 619.
- [17] J. Vindevogel and P. Sandra, *Introduction to Micellar Electrokinetic Chromatography*, Hüthig, Heidelberg, 1992.
- [18] A.R. Timerbaev, O.P. Semenova, P. Jandik and G.K. Bonn, *J. Chromatogr. A*, 671 (1994) 419.
- [19] N. Iki, H. Hoshino and T. Yotsuyanagi, *J. Chromatogr. A*, 652 (1993) 539.