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Theoretical Migration Model for Micellar Capillary Electrophoresis and Its Application to the Separation of Anionic Metal Complexes of HEDTC and CDTA

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A mathematical model relating the effective mobility of an analyte in micellar capillary electrophoresis (MCE) to the concentration of surfactant and organic modifier in the background electrolyte (BGE) was derived. Effective mobility is expressed in terms of the electrophoretic mobility of the analyte, the partition coefficient of the analyte into the micelle, and the influence of organic modifier on these two factors. The performance of the model was evaluated using Cd(II), Pb(II), Co(II), Ni(II), Bi(III), Cu(II), and Hg(II) complexes of bis(2-hydroxyethyl)dithiocarbamate, all of which carry a partial negative charge, and Cd(II), Pb(II), Co(II), Ni(II), Bi(III), Cu(II), Hg(II), Fe(III), Ag(I), Tl(I), and Mn(II) complexes of *trans*-1,2-diaminocyclohexane-*N,N,N,N*-tetraacetic acid, all of which are anionic having charges in the range -1 to -3 . These analytes were separated in borate BGEs containing 10–50 mM sodium dodecyl sulfate and 0–20% (v/v) methanol. Nonlinear regression was used to derive parameters for the model from experimental data and these parameters were used to predict effective mobilities of the analytes. Predicted values of effective mobilities agreed with experimental values to within 3.1%. Values of parameters from the model equation are used to explain changes in separation selectivity observed at different BGE compositions and the model equation is shown to be applicable to computer-assisted optimization of the BGE composition, in MCE using a limited number of experiments.

The development of micellar capillary electrophoresis (MCE), named micellar electrokinetic capillary chromatography (MEKC) by Terabe et al.¹ in 1984, extended the application of capillary electrophoresis (CE) to neutral solutes. The separation of analytes is achieved due to differential partitioning of the analyte into the electrophoretically driven micelles of a charged surfactant added to the background electrolyte (BGE). MCE is most frequently applied to the separation of organic and often neutral analytes, but there has been an increasing interest in the separations of

small ions, particularly in regard to different separation selectivity that can be achieved. The separation of metal-containing species by MCE has been recently reviewed by Haddad et al.,² with the most common approach for determining metal ions as the analytes being by complexation with a suitable ligand to form a UV/visible-absorbing complex. Limited attention has been paid to the separation of inorganic species by MCE, with one example demonstrating the ion-exchange capacity of cationic micelles.³

In the separation of analytes using micelles carrying the same charge as the analyte, the exact nature of the micellar interaction and its influence on separation selectivity is unclear. Initial work by Saitoh et al.⁴ using anionic metal complexes of 4-(2-pyridylazo)-resorcinol (PAR) found that sodium dodecyl sulfate (SDS) was crucial to obtain a separation between Fe(III), Co(III), Cr(III), and Ni(II); however, later work by Timerbaev et al.⁵ demonstrated that partitioning into the micelles did not occur. Further work by Timerbaev et al.⁶ using multiply charged anionic complexes of *trans*-1,2-diaminocyclohexane-*N,N,N,N*-tetraacetic acid (CDTA) found that partitioning occurred when less than 15 mM SDS was added, but above this amount, no further partitioning occurred which was attributed to electrostatic interactions (charge repulsion) between the analytes and the micelles. Recently Hilder et al.^{7,8} reported the separation of bis(2-hydroxyethyl)dithiocarbamate (HEDTC) complexes that bear a small partial negative charge and have shown that significant partitioning into the micelle occurred.

Modeling of migration behavior in CE is a useful tool for prediction of optimal separation conditions and for increased understanding of the separation mechanism and has been reviewed as an optimization method in MCE by Corstjens et al.⁹ Terabe et al.^{1,10} established the fundamental equation in MCE

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relating the migration factor, which is a representation of association with the micelle, to surfactant concentration. Khaledi and Quang^{11–13} presented several phenomenological models to describe the migration behavior of ionizable solutes using both mobility and retention approaches. Recently Muijselaar et al.¹⁴ established that a mobility approach is better for modeling migration behavior of hydrophobic solutes due to the interaction with monomer surfactant. Limited attention has been paid to modeling migration behavior of metal complexes in MCE, but Saitoh et al.¹⁵ demonstrated that, for metal acetylacetonate complexes, separation was based solely on partitioning into the micelles. Additionally, little attention has been paid to the effect of organic modifiers on separations of analytes by MCE,^{16,17} in particular with respect to selectivity of the separation.

The aim of this work was to undertake a detailed study of the manipulation of the separation selectivity of anionic metal complexes in MCE using anionic micelles of SDS and methanol as an organic modifier. Emphasis is placed on the development of a theoretical model equation that describes the migration behavior and includes consideration of the influence of changing the concentration of SDS and methanol in the BGE. Slightly charged complexes of HEDTC and multiply charged complexes of CDTA were taken as model systems, representing extreme cases of partition and electrophoretic dominance in the separation mechanism, respectively. Further, charge differences between the two groups of complexes allow the significance of like charge–charge interactions in the separation mechanism to be evaluated.

EXPERIMENTAL SECTION

Instrumentation. Conductivity measurements were performed using a Jenway PWA2 portable water analyzer.

The CE instrument used was a Beckman P/ACE system 2050 (Beckman, Palo Alto, CA) with a Beckman P/ACE station version 1.0 (being used for data acquisition). Separations were carried out using a Polymicro (Phoenix, AZ) fused-silica capillary (75 μ m i.d., length 37.0 cm, 30.5 cm to detector). The capillary was initially conditioned with 0.1 M HCl, followed by 1 M NaOH and then water and BGE (at least 10 capillary volumes each). Injection was performed by applying 0.5 psi pressure for 1 s to the anodic side of the capillary. Direct photometric detection at 254 nm was used for the HEDTC complexes and at 200 nm for the CDTA complexes.

Reagents and Procedures. Ammonium bis(2-hydroxyethyl)-dithiocarbamate was prepared by the procedure described by King and Fritz²⁷ and was stored at 2 °C to prevent oxidation. A stock

solution of 100 mM HEDTC was prepared freshly each day. CDTA was purchased from Fluka (Buchs, Switzerland) and used without further purification. Metal ion standard solutions of Cd(II), Pb(II), Pt(II), Co(II), Ni(II), Bi(III), Cu(II), Hg(II), Fe(III), Ag(I), Tl(I), and Mn(II) were prepared from the nitrate or chloride salts in 10 mM nitric or hydrochloric acid. Precapillary complex formation of a metal ion with the complexing ligand was performed by addition of the 100 mM stock solution to the metal ion standard to give a concentration of 25–30 mM for the HEDTC complexes (12.5-fold molar excess), and 100 μ M for the CDTA complexes (3-fold molar excess). All other reagents were of analytical grade. Water was purified using a Milli-Q water (Millipore, Bedford, MA) system.

Borate buffer was prepared by dilution of boric acid to 500 mM and adjustment to pH 9.2 with lithium hydroxide. This was used to prepare the final BGEs for the separation of the metal complexes. The base BGE for HEDTC complex separations was 80 mM lithium borate (pH 9.2) and 0.1 mM HEDTC. For the CDTA complex separations, a 20 mM lithium borate (pH 9.2) and 1 mM CDTA BGE was used. Before use, the BGE was degassed by vacuum and filtered through a 0.45- μ m disk filter (Activon, Thornleigh, Australia). The electroosmotic flow (EOF) was determined by injections of acetone and the mobility of SDS micelles by injections of anthracene when detecting at 254 nm or by injections of methanol and Sudan(III) when detecting at 200 nm.

RESULTS AND DISCUSSION

Preliminary Investigations. Organic modifiers, and in particular methanol and acetonitrile, have found frequent application in MCE, most commonly for improving the separation of hydrophobic compounds, and the use of these solvents has been briefly reviewed by Khaledi.²² The addition of an organic modifier to the BGE can influence partitioning into the micelle and the electrophoretic mobility of the analyte.

Methanol was selected as the organic modifier to be used in this study. The influence of methanol on partitioning of the analyte into the micelle has been studied separately by Muijselaar et al.¹⁷ and Chen et al.^{23,24} Muijselaar et al. verified that the solvophobic equation could be applied to MCE, up to the addition of 20% (v/v) methanol. The solvophobic equation for liquid chromatography relates the natural logarithm of the retention factor to the volume ratio of methanol in the eluent as follows:

$$\ln k = \ln k_0 - s\Phi \quad (1)$$

where k_0 is the retention factor with no methanol, s is the slope, and Φ is the volume ratio of organic modifier. Chen et al.^{23,24} observed that the migration factor in MCE decreases linearly with the addition of methanol up to 5 mol/L, according to the equation

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$$\frac{1}{K} = \frac{1}{K_0} + b[L] \quad (2)$$

where b is the slope and $[L]$ is the concentration (mol/L) of organic modifier added.

The influence of methanol on the electrophoretic mobility of the analyte has been studied separately by Yang et al.²⁵ and Stathakis et al.,²⁶ who both reported a near-linear reduction in the electrophoretic mobility with increasing methanol concentration. It should be noted that the addition of methanol reduces the EOF and hence in MCE results in an extension of the separation window,¹⁷ extending analysis times considerably.

Preliminary investigations were conducted to determine which of the equations given above provided an adequate representation of the influence of methanol on the partitioning process and the electrophoretic mobility of the analyte. Also, the influence of methanol concentration on the cmc of SDS in the given BGE was examined. Experiments using HEDTC complexes and adding up to 20% (v/v) methanol in 2% (v/v) increments to the BGE (80 mM lithium borate and 10 mM SDS) showed a linear relationship ($r^2 > 0.99$), between the natural logarithm of the migration factor and the volume of methanol, as observed by Muijselaar et al.¹⁷ The equation proposed by Chen et al.^{23,24} (eq 2) also showed a linear relationship but with a poorer correlation ($r^2 > 0.95$). Thus eq 1 was selected for inclusion in the development of a retention model equation. Experimental results using the highly mobile CDTA complexes indicated that the EOF and mobility of the complexes decreased linearly ($r^2 > 0.99$) up to the addition of 20% (v/v) methanol to the mobile phase (20 mM lithium borate), in agreement with previous work.^{25,26}

The effect of methanol on the cmc was investigated for additions of methanol up to 20% (v/v) in a liquid phase containing 80 mM lithium borate (pH 9.2). The cmc was determined by two methods. The first was by conductivity measurements,¹⁹ which gave a cmc value of 3.1 mM with the addition of no methanol. The second method was that described by Jacquier and Desbène,¹⁶ which uses the dependence of mobility of a lipophilic analyte (naphthalene) versus surfactant concentration to determine the cmc. The cmc determined by this method was 3.0 mM for a BGE with no methanol added. After the addition of 20% (v/v) methanol, the cmc was determined by both methods to be 4.0 mM, which means that the cmc changed by only 1.0 mM after the addition of 20% (v/v) methanol. This result is in agreement with that reported by Jacquier and Desbène,²⁸ who observed a 1 mM change in cmc after the addition of 20% (v/v) methanol. To accommodate this change in cmc in the modeling process, the average value of the cmc before and after the addition of 20% (v/v) methanol was used.

Model Equation. A theoretical equation expressing the migration behavior of an analyte as a function of the methanol content and SDS concentration in the BGE was desired. The relationship between migration factor (K) and surfactant concentration was first established by Terabe et al.:¹⁰

$$K = P\nu([Surf] - cmc) \quad (3)$$

where P is the partition coefficient, ν is the partial molar volume

of surfactant, and cmc is the critical micelle concentration. This equation enables the quantification of analyte association with the micellar phase and has since been shown to be valid for all neutral analytes in MCE.^{11,17–20}

The separation of charged analytes by MCE involves a combination of partitioning into the micelle and the electrophoretic mobility of the analyte.^{11,21} Calculation of the migration factor must therefore include the mobility of the analyte. Equation 4 has been

$$K = \frac{\mu_{ob} - \mu_{ep,eff}}{\mu_{mc} - \mu_{ob}} \quad (4)$$

shown to be valid for charged analytes, where μ_{ob} is the observed mobility of the solute, $\mu_{ep,eff}$ is the apparent electrophoretic mobility of the solute, and μ_{mc} is the mobility of the micelles.

Combining eq 3 relating the migration factor to the concentration of surfactant with eq 1 relating the migration factor to the amount of methanol in the BGE, gives

$$\ln k = \ln(\nu P([Surf] - cmc)) - s\Phi \quad (5)$$

Solving for k , then

$$K = (\nu P([SDS] - cmc))e^{-s\Phi} \quad (6)$$

Substituting K into eq 4 allows the mobility of an analyte to be related to the concentration of surfactant and the amount of methanol in the BGE by the following equation

$$\mu_{ob} = \frac{((\nu P([surf] - cmc))e^{-s\Phi})\mu_{mc} + \mu_{ep,eff}}{1 + ((\nu P([surf]) - cmc)e^{-s\Phi})} \quad (7)$$

The observed linear change in the electrophoretic mobility of an analyte with the addition of methanol to the mobile phase can be represented by

$$\mu_{ep,eff} = \mu_{ep} + K_m\Phi \quad (8)$$

where $\mu_{eff, aq}$ is the effective electrophoretic mobility of the analyte in the aqueous–organic phase, μ_{aq} is the true electrophoretic mobility in the absence of organic modifier, Φ is the volume ratio of methanol, and K_m is the constant of proportionality.

Substituting eq 8 into eq 7 permits the observed mobility of any anionic analyte to be expressed as a function of surfactant concentration and methanol added to the BGE:

$$\mu_{eff,ob} = \frac{((\nu P([Surf] - cmc))e^{-s\Phi})\mu_{mc} + (\mu_{aq} + K_m\Phi)}{1 + ((\nu P([Surf] - cmc))e^{-s\Phi})} \quad (9)$$

There are several assumptions included in this model. The first is that the concentration of methanol has no influence on the cmc of the surfactant. The second assumption is that the concentration of methanol has no effect on the partial molar volume of the surfactant.

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Table 1. Parameters for HEDTC Complexes Determined from Application of Nonlinear Regression to Model Equation without Influence of Methanol on the Electrophoretic Mobility of the Analyte (Eq 7)

metal	P (mol/L)	$(10^{-9} \mu_{ep} / V \cdot s)$	s	r^2
Cd(II) ^a	23.5	5.05	0.13	0.9514
Pt(II) ^a	173.5	2.15	0.09	0.9996
Pb(II)	201.4	2.43	0.10	0.9932
Co(II)	314.2	2.74	0.12	0.9919
Ni(II)	311.5	2.66	0.09	0.9947
Bi(III)	353.1	5.98	0.13	0.9875
Cu(II) ^a	538.0	3.51	0.09	0.9938
Hg(II)	818.8	4.13	0.09	0.9964

^a Model constants determined from fitting to four experimental points. Correlation coefficients determined from 50 points.

Verification of the Equation. The suitability of the model equation with respect to its predictive capabilities and use in optimization was examined for slightly anionic complexes of HEDTC and multiply charged complexes of CDTA.

(a) Partially Charged Anionic Complexes of HEDTC. Due to the working pH range of the BGE being restricted to the neutral to weakly alkaline region,⁸ the methanol content and SDS concentration were selected as the variable parameters and the pH was kept constant by choosing a borate buffer system.^{7,8} Using 80 mM borate buffer (pH 9.2) and 0.1 mM HEDTC as a base system, the concentration of SDS was varied in the range of 10–30 mM in 5 mM increments (five different concentrations). In addition, the methanol content was varied up to 20% in 2% increments (11 different concentrations). This provided data for each solute at 55 different BGE conditions.

Model Application. Hilder et al.⁸ observed that the electrophoretic mobilities of the HEDTC complexes were very low (less than $5 \times 10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$). Therefore, it is likely that the electrophoretic mobility has only a minor influence in the separation mechanism and as such the influence of methanol on the electrophoretic mobility of the analyte should be negligible. Equation 7 should therefore be an acceptable representation of the separation process. Equation 7 was fitted to experimental data acquired for each metal complex at 55 different buffer conditions using nonlinear regression, and the results are shown in Table 1. For this purpose the cmc was constrained to 3.5 mM and v was constrained to 0.2486 mol/L. Agreement between the experimental and predicted mobilities of the solutes was excellent ($r^2 > 0.99$) for all solutes except for Bi(III) ($r^2 > 0.98$) and Cd(II) ($r^2 > 0.95$). This may be attributed to the exclusion of the influence of methanol on the electrophoretic mobility of these solutes in eq 7, in view of the fact the mobilities for these complexes were greater than for the other complexes.

The experimental data were reapplied to eq 9, which includes the influence of methanol on the electrophoretic mobility. Parameters determined from this equation are shown in Table 2. There was considerable improvement in the correlation for Cd(II) (from 0.95 to 0.98); however, there was no significant improvement for all of the other metal ions. Equation 7 was selected for further use as the results were similar to eq 9, and it requires four experiments (three unknowns) rather than five experiments (four unknowns) for solution. The combined correlation plot (using eq

Table 2. Parameters for HEDTC Complexes Determined from Nonlinear Regression to Model Equation with the Influence of Methanol on the Analyte Electrophoretic Mobility Included (Eq 9)

metal	P (mol/L)	$(10^{-9} \mu_{aq} / V \cdot s)$	$10^{-9} \frac{K_m}{m^2/V \cdot s}$	s	r^2
Cd(II) ^a	18	5.77	−0.051	0.13	0.9821
Pb(II)	181	3.74	−0.094	0.09	0.9948
Co(II)	295	3.61	−0.057	0.12	0.9923
Ni(II)	298	3.31	−0.045	0.08	0.9949
Bi(III)	286	8.29	−0.146	0.12	0.9908
Cu(II) ^a	476	5.52	−0.135	0.08	0.9957
Hg(II)	742	6.01	−0.118	0.09	0.9965

^a Model constants determined from fitting to five experimental points. Correlation coefficients determined from 50 points.

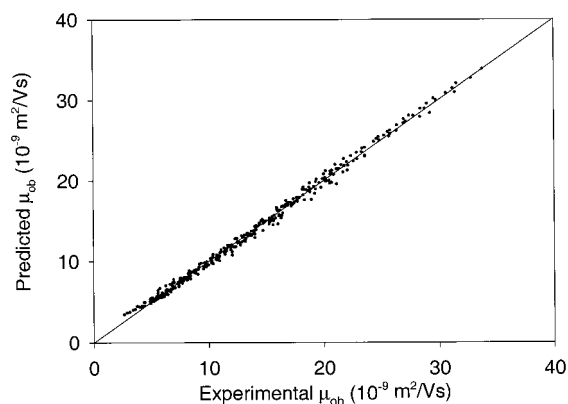


Figure 1. Correlation plot for HEDTC complexes of Cd(II), Pb(II), Co(II), Ni(II), Bi(III), Cu(II), and Hg(II) at 50 different buffer conditions: slope 1.003, intercept −0.0287, $r^2 = 0.9961$, and number of points 358.

7) for the seven metal complexes is shown in Figure 1, and the high correlation indicates the suitability of the model equation for description of the migration behavior of analytes which have a significant influence from partitioning into the micelle in the separation mechanism.

Predictive Capabilities and Accuracy of the Model. To determine the predictive capabilities of eq 7, Cu(II) (which partitions significantly into the micelle) and Cd(II) (which partitions slightly) complexes were selected as test analytes and the observed mobilities for the complexes were determined experimentally at four different buffer conditions (10 and 30 mM SDS with 0 and 20% (v/v) methanol). The model equation (eq 7) was fitted to the migration data using nonlinear regression, and values for the unknown parameters in the equation were obtained (Table 1). To check the validity of the model, the theoretical value of mobility was determined from the model and also determined experimentally at 51 different BGE compositions. Correlation coefficients for the Cd(II) and Cu(II) complexes were 0.981 and 0.994, respectively. The average relative difference between the experimental and theoretical mobilities was less than 3.7% for both complexes, while the maximum error was 6%. These results clearly indicate that the model equation can be used to predict the mobilities of slightly charged species on the basis of only four initial experiments.

Selectivity. The model equation can be used to predict the effective mobilities for the test analytes over a range of BGE

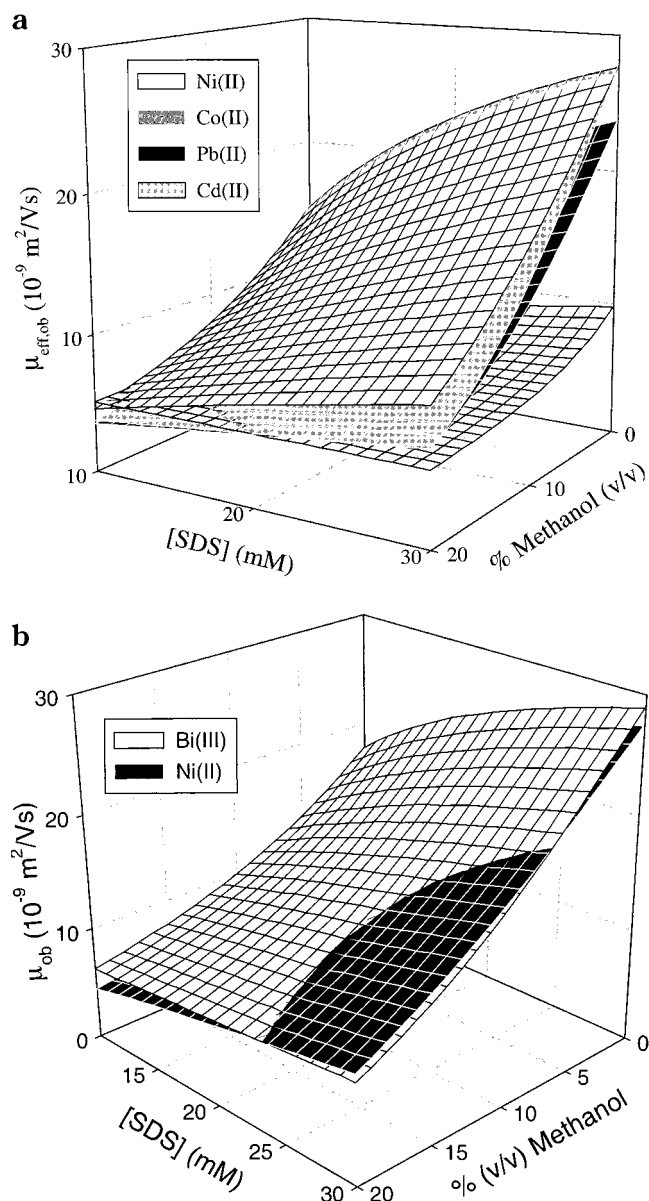


Figure 2. Response surface for effective mobilities of HEDTC metal complexes as a function of methanol content and SDS concentration added to the BGE: (a) Cd(II), Pb(II), Co(II), and Ni(II); (b) Ni(II) and Bi(III).

compositions and together with values for the model parameters (especially P) can be used to explain the observed changes in selectivity. From Table 1 it can be seen that the values of P are generally large and are therefore the dominant influence on the equation, except for Cd(II) which has a value of P that is a factor of 10 lower than for the other complexes. This suggests that the partition process played only a minor role in the separation of the Cd(II) complex. Therefore, the addition of SDS and methanol should have little effect on the observed mobility of the complex, as illustrated in the retention surface diagram shown in Figure 2a. With 20% (v/v) methanol and 10 mM SDS added to the BGE, Pb(II), Co(II), and Ni(II) migrate before Cd(II) but the situation is reversed at 30 mM SDS (Figure 2a). This can be explained in terms of a shift in the relative weighting between the partitioning and the electrophoretic mobility in the separation mechanism. At high concentrations of SDS, which corresponds to a high

concentration of the pseudostationary phase, the separation mechanism was based predominantly on partitioning into the micelle, and the migration order generally correlated closely with the values for P (Table 1). At lower concentrations of SDS, and a high content of methanol, partitioning is at a minimum and the electrophoretic mobility had the greatest influence on the separation mechanism. As the electrophoretic mobility of Cd(II) was much higher than for Pb(II), Co(II), and Ni(II) (Table 1), this resulted in a change in the migration order of the complexes with the concentration of SDS and methanol.

The response surface of the effective electrophoretic mobility for Bi(III) and Ni(II) is shown in Figure 2b. Bi(III) and Ni(II), which have similar partition constants, can be separated without the addition of methanol to the BGE due to Bi(III) having a greater electrophoretic mobility than Ni(II). However, changes in selectivity were observed with the addition of methanol. At 7% (v/v) methanol and 28 mM SDS, the migration order of Bi(III) and Ni(II) was reversed. The concentration of SDS required for this change was dependent on the percentage of methanol present in the BGE, which could be attributed to a shift in the mechanism from being predominantly partitioning (low concentration of methanol) to predominantly electrophoretically based (at high concentrations of methanol).

Optimization of Separation Selectivity. The theoretical model equation presents the opportunity to optimize the separation of the metal complexes. The search area of BGE compositions in which the optimum was sought was selected as 10–30 mM SDS and 0–20% (v/v) methanol, and the HEDTC complexes of Cd(II), Pb(II), Co(II), Ni(II), Bi(II), Cu(II), and Hg(II) were used as analytes. The normalized resolution product (r),²⁹ which takes values between 0 and 1, was selected as the criterion to evaluate the separation and is defined

$$r = \prod_{i=1}^{n-1} \left(R_{s(i,i+1)} / \frac{1}{n-1} \sum_{i=1}^{n-1} R_{s(i,i+1)} \right) \quad (10)$$

where $R_{s(i,i+1)}$ is the resolution between adjacent peaks. Values of r were calculated over the search area using the model equation and the parameters shown in Table 1, and a plot of r over the search area is shown in Figure 3. From this plot, the optimum separation conditions were predicted at 8% (v/v) methanol and 10 mM SDS. As experimental data had already been obtained at this point, a point situated between two previously experimental points was chosen to check the validity of the model. This corresponded to BGE conditions of 9% (v/v) methanol and 12 mM SDS. The optimized experimental and predicted separations are shown in Figure 4, from which it can be seen that the model was able to give a very accurate prediction on the separation with all peaks being predicted to within 3.0% of experimental. Additionally, the complexes are well separated, indicating that the model equation can be used to optimize a desired separation.

(b) Multiply Charged Anionic Complexes of CDTA. Metals selected for complexation with CDTA were Ag(I), Tl(I), Hg(II), Mn(II), Co(II), Ni(II), Pb(II), Cu(II), Bi(III), and Fe(III). These metal ions formed complexes with charges ranging from -1 to

(29) Haddad, P. R.; Jackson, P. E. *Ion Chromatography. Principles and Applications*; Elsevier: Amsterdam, 1990; p 798.

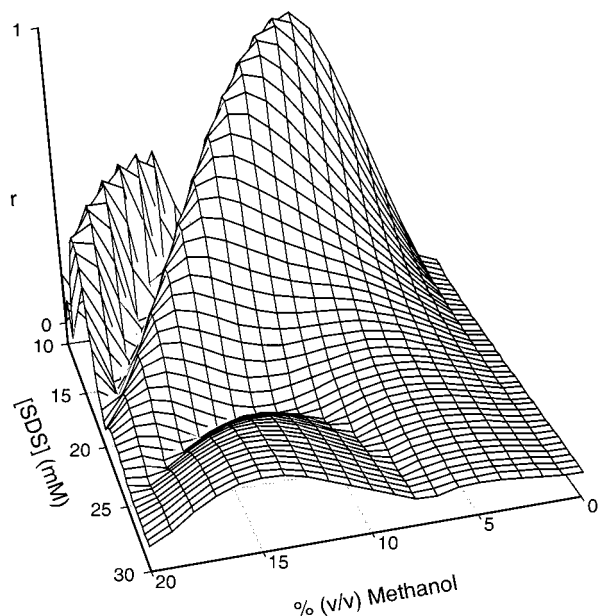


Figure 3. Response surface of the normalized resolution product (r) for HEDTC complexes of Cd(II), Pb(II), Co(II), Ni(II), Bi(III), Cu(II), and Hg(II). The optimum separation conditions are at 8% methanol and 10 mM SDS.

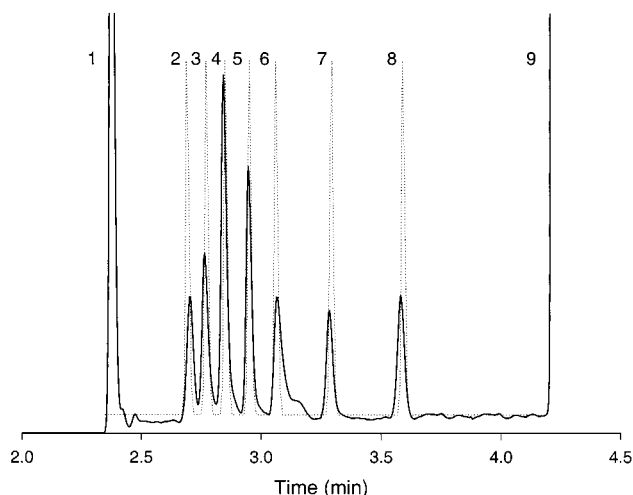


Figure 4. Experimental (solid line) and predicted (broken line) separations of metal HEDTC complexes at 9% methanol and 12 mM SDS. Peaks: 1, EOF; 2, Cd(II); 3, Pb(II); 4, Ni(II); 5, Co(II); 6, Bi(III); 7, Cu(II); 8, Hg(II); 9, HEDTC.

–3. Additionally, several of the divalent metals that were reported by Timerbaev et al.⁶ to comigrate were selected in an attempt to test the applicability of the model to improve the resolution.

A BGE containing 20 mM lithium borate (pH 9.2) and 1 mM CDTA was used as the base system for all experiments. The concentration of SDS was varied between 10 and 50 mM in 5 mM increments (nine different concentrations), while the volume of methanol was varied from 0 to 20% in 4% increments (six different concentrations). This provided data for the 10 complexes at 54 different buffer conditions. Regarding analytical method performance characteristics other than selectivity, it should be noted that the addition of greater than 10% (v/v) methanol resulted in a significant decrease in the baseline noise and reduced detection limits. Furthermore, adding high concentrations of surfactant had a positive effect upon the separation efficiency.

Model Application. The cmc of SDS in a BGE of 20 mM lithium borate (pH 9.2) was determined by a modification of the method of Jacquier and Desbène.¹⁶ This involved determining the mobility of naphthalene with the addition of surfactant well above the cmc, in BGEs used for the collection analyte mobility data. This did not require the use of other buffer solutions with the addition of surfactant near the cmc. Nonlinear regression was then used to fit the data to the equation derived by Jacquier and Desbène. The cmc with 0% (v/v) methanol was 4.5 mM and after the addition of 20% (v/v) methanol it was 5.5 mM. Therefore for the model application, the cmc was defined to be 5.0 mM.

Equation 9 was fitted to the data using nonlinear regression. As the analytes are multiply charged it is expected that the electrophoretic mobility of the analyte will play a significant role in the separation mechanism, and therefore, eq 7 is not suitable. Good agreement between experimental and theoretical results ($r^2 > 0.96$) was obtained for the complexes, except for those of Tl(I) ($r^2 > 0.61$) and Ag(I) ($r^2 > 0.95$). Two reasons may be advanced for this observation. First, Tl(I) and Ag(I) form CDTA complexes having the highest charge (–3) of the group studied and this charge could lead to repulsion effects between the analytes and the anionic micelles. Second, the Tl(I) complex is the least stable of those studied;³⁰ therefore, it is possible that the addition of methanol and SDS might influence the stability of the complex, resulting in the observed poor correlation. While the correlation coefficient for Ag(I) is reasonably high (>0.95), the correlation plot showed horizontal clusters of points (Figure 5a). A closer examination of the data revealed that all of the horizontal points occurred at the same volume of methanol added to the BGE. That is, the model equation predicts the same observed mobility for Ag(I) at all concentrations of SDS.

The results from the nonlinear regression analysis are shown in Table 3. It can be seen that the values for the partition coefficients are small in magnitude and, for most of the complexes, are negative. As the partition constant represents the amount of analyte solubilized inside the micelle, negative values are physically impossible, which suggests an alternative mechanism is responsible. It is interesting to note that the values of P for the monovalent complexes of Bi(III) and Fe(III) are half that of the divalent complexes, which would suggest that the additional interaction may be due to charge–charge interactions with the micelles. However, given that the values for the partition constants are typically at least a factor of 10 less than values for the HEDTC complexes, it is more likely that the resultant negative partition constants are due to errors in approximating the cmc and ignoring the influence of increasing ionic strength on the electrophoretic mobilities of the analytes.

The correlation plot for all of the metal complexes excluding Tl(I) and Ag(I) is shown in Figure 5b. The good correlation between experimental and theoretical observed mobilities indicates that the model equation is suitable to describe the behavior of metal complexes in which the separation mechanism is dominated by the electrophoretic mobility of the analyte.

Predictive Capabilities and Accuracy of the Model. To determine the accuracy of the model equation, five experimental points in a central composite design were used to calculate values of the

(30) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1974; pp 236–240.

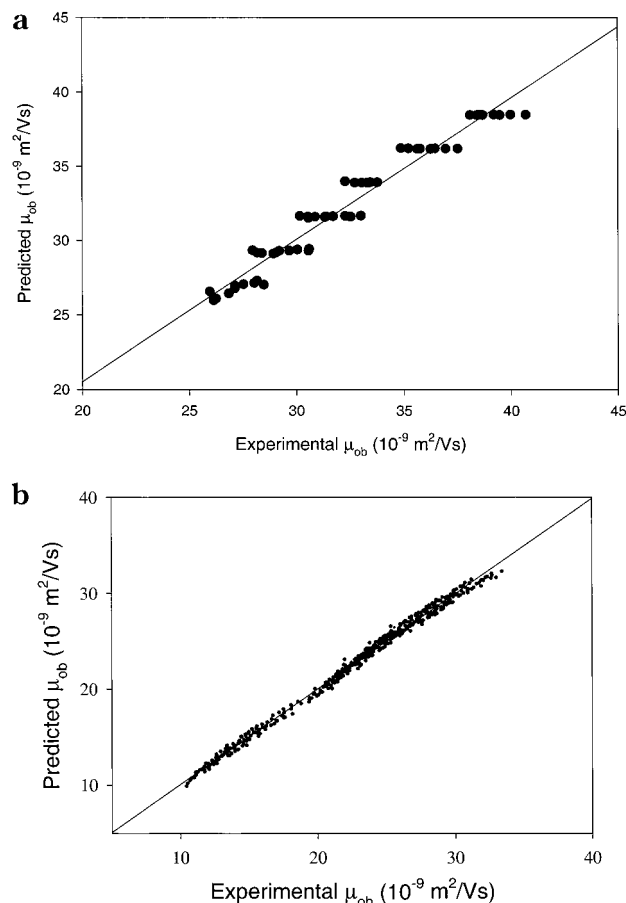


Figure 5. (a) Correlation plot for Ag(I) CDTA complex at 54 different buffer conditions: slope 1.427, intercept 0.954, and r^2 0.9554. (b) Correlation plot for Bi(III), Fe(III), Hg(II), Mn(II), Co(II), Ni(II), Pb(II), and Cu(II) CDTA complexes at 54 different buffer conditions: slope 0.9929, intercept 0.0156, $r^2 = 0.9946$, and number of points 440.

Table 3. Parameters Determined from Nonlinear Regression of Model Eq 9 to Experimental Data for Metal CDTA Complexes

metal	$(10^{-9} \mu_{aq} / \text{V} \cdot \text{s})$	K_m	P (mol/L)	s ($\times 10^{-3}$)	r^2
Bi(III) ^a	17.4	-0.258	-13.2	5.33	0.9615
Fe(III)	19.7	-0.311	-13.8	8.64	0.9785
Hg(II)	30.8	-0.441	-32.5	-10.3	0.9741
Mn(II)	30.9	-0.462	-29.0	-0.265	0.9849
Co(II) ^a	31.6	-0.472	-30.5	0.804	0.9816
Ni(II)	31.9	-0.467	-30.1	-7.61	0.9837
Pb(II)	31.7	-0.460	-30.6	4.37	0.9780
Cu(II)	32.3	-0.466	-32.3	-12.7	0.9780
Tl(I)	15.7	-0.053	22.7	140	0.6115
Ag(I)	38.5	-0.573	225	168	0.9554

^a Model constants determined from fitting to five experimental points. Correlation coefficients determined from 54 points.

constants for the arbitrarily selected complexes of Co(II) and Bi(III). These values were used to predict mobilities of the complexes at 50 different buffer conditions. Correlation coefficients were 0.979 for Co(II) and 0.967 for Bi(III). The average relative error between experimental and predicted mobilities over these conditions was 2.0% for the Co(II) complex and 3.3% for the Bi(III) complex, while maximum error was 4.8 and 8.1% for Co(II) and Bi(III), respectively. These results indicate that the model equation

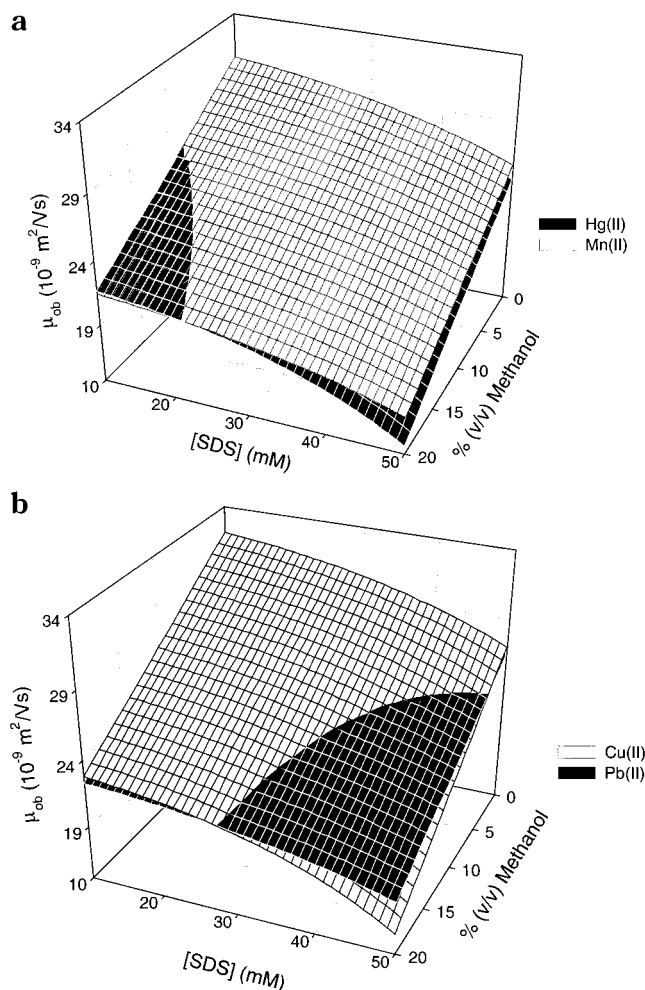


Figure 6. Response surface for effective mobilities of metal CDTA complexes with variation of methanol and SDS concentration to the BGE: (a) Hg(II) and Mn(II); (b) Pb(II) and Co(II).

can be used to predict mobilities of the highly charged complexes on the basis of only five experiments.

Selectivity. The model parameters listed in Table 3 can be used to explain the observed changes in selectivity in the same manner used earlier for the HEDTC complexes. Here, the value of K_m assumes importance since the partitioning of the complexes into the micelles is very low (P and s are small in magnitude). The response surface for Hg(II) and Mn(II) is shown in Figure 6a. With 10 mM SDS in the BGE, Hg(II) migrates before Mn(II) when less than 10% (v/v) methanol is added. The situation is reversed when more than 10% (v/v) is added. This is due to methanol reducing the electrophoretic mobility of Mn(II) more than for Hg(II) (K_m values of -0.46 and -0.44, respectively). Increasing the concentration of SDS to 25 mM with 20% (v/v) methanol added to the BGE results in a reversal of the migration order. This is due to the interaction between the analyte and the micelle being stronger for Hg(II) than for Mn(II).

The response surface for Cu(II) and Pb(II) is shown in Figure 6b. The migration order changes when the SDS concentration and methanol content added to the BGE are high. This is due to methanol having a more significant influence on the interaction between the analyte and the micelle for Cu(II) than Pb(II) (s values of -12.7×10^{-3} and 4.37×10^{-3} , respectively).

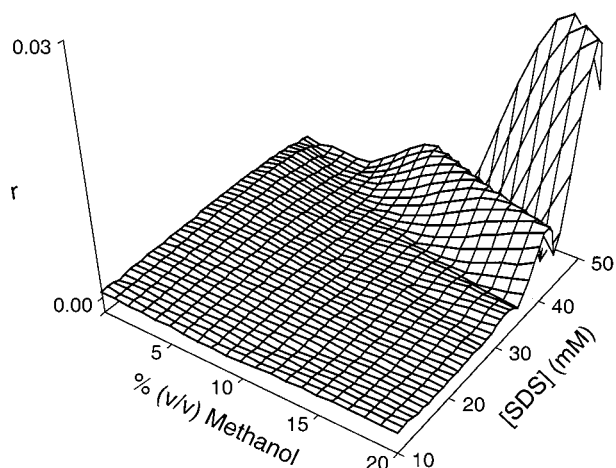


Figure 7. Response surface for the normalized resolution product (r) for Bi(III), Fe(III), Hg(II), Mn(II), Pb(II), Ni(II), Cu(II), and Ag(I) CDTA complexes as a function of methanol content and surfactant concentration. Optimum conditions are at 20% (v/v) methanol and 50 mM SDS, $r = 0.02$.

Optimization of Separation Selectivity. Metal CDTA complexes of Bi(III), Fe(III), Tl(I), Hg(II), Co(II), Ni(II), Cu(II), and Ag(I) were used for evaluating the use of the migration model for the optimization of separation selectivity. Tl(I) was not included due to poor correlation of its effective mobility with the model, and Pb(II) was not used because it comigrated with Co(II) under all conditions. Using the same procedure employed for the HEDTC complexes, the normalized resolution product was calculated over the search area 10–50 mM SDS and 0–20% (v/v) methanol (Figure 7). The optimum separation conditions are at 20% (v/v) methanol and 50 mM SDS. The value for r at this composition was less than 0.03, indicating that there was little or no resolution between the complexes even at the optimum, as shown in Figure 8. Therefore, the addition of methanol and SDS to the BGE has resulted in no significant improvement in the separation of the analytes from that achieved under regular CE conditions. This result is in accordance with the results listed in Table 3, which show that, for the CDTA complexes, interaction with the micelles is minimal and any changes to selectivity arise chiefly from the effects of methanol on mobilities (i.e., K_m values).

CONCLUSIONS

A mathematical model relating analyte mobility in capillary micellar electrochromatography to surfactant concentration and organic modifier content has been presented. Test analytes comprising slightly negative complexes of HEDTC and multiply

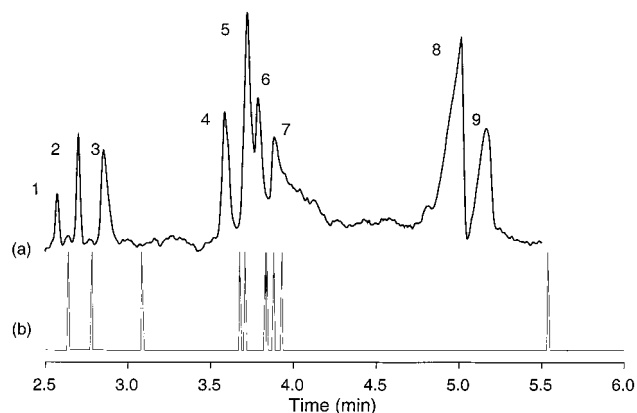


Figure 8. Experimental (a) and theoretical (b) separation of metal CDTA complexes at the optimum separation conditions of 20% methanol and 50 mM SDS. Peaks: 1, Bi(III); 2, Fe(III); 3, Tl(I); 4, Hg(II) and Mn(II); 5, Pb(II) and Co(II); 6, Ni(II); 7, Cu(II); 8, CDTA; 9, Ag(I).

charged anionic complexes of CDTA, separated in electrolytes containing SDS and methanol, gave excellent agreement between experimental and predicted values of effective mobility. Parameters determined from application of the model equation to measured migration data indicated that the separation mechanism for the HEDTC complexes was based predominantly on partitioning into the micelles, whereas that for the CDTA complexes was governed predominantly by electrophoretic migration. Optimum separation conditions could be predicted from four experiments for the HEDTC complexes and five experiments for the CDTA complexes. Predicted values of effective mobility deviated from experimental values by less than 3.1%.

While the theoretical model was verified using stable metal complexes, it should be applicable to other types of analytes. The model may be further improved by including the influence of electrostatic interactions, ionic strength, and the changes in the cmc with the addition of organic modifier. However, given the good results achieved with the present model, the improvements may be unjustified due to the larger number of the initial experiments required. To make the presented model generally applicable to most MCE systems, it may be combined with other models accounting for influences of additional equilibria, such as protonation or complexation.

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