Journal of Chromatography, 632 (1993) 177-I 84
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2575

Prediction of the migration behavior of organic acids in micellar electrokinetic chromatography

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ABSTRACT

The migration behavior of an homologous series of phenols in micellar electrokinetic chromatography is quantitatively presented. This model describes mobility in terms of fundamental physical and chemical constants of each solute (acid dissociation constant K_a, micelle binding constant Km), the pH of the buffer, and the micelle concentration ([M]) in the buffer. The model was used to predict the mobility of each solute over a two-dimensional pH/[M] space. Predicted and actual electropherograms show the usefulness of this technique.

INTRODUCTION

In any separation technique it is desirable to have the option of manipulating a variety of parameters in order to affect and subsequently control the quality of a separation. In high-performance capillary electrophoresis (HPCE), the urgency for controlling migration and selectivity has been lessened because of the high efficiency of the technique. There are cases, however, in which manipulation of the separation selectivity parameters is needed [1–9]. Until recently, however, little work has been performed in the areas of prediction of migration behavior and subsequent optimization of the separation.

Recently, models that describe the migration behavior of solutes have been developed [10]. These models describe migration in terms of fundamental physical and chemical parameters of the solutes: pK_a, micelle-water binding constant, and (for acidic or anionic solutes) the mobility of the anionic solute in the absence of micelles. A model was used to correctly predict the migration behavior and optimize the capillary zone electrophoretic (CZE) separation of halogenated phenols in aqueous buffer through manipulation of buffer pH [11]. However, CZE separation of solutes with similar pK_a values was inadequate.

The inclusion of a second chemical equilibrium, namely the solute association equilibrium with micelles, is discussed in this paper. The results of the prediction of migration behavior in micellar electrokinetic capillary chromatography (MECC) using the mathematical models are presented.

EXPERIMENTAL

The experimental apparatus and calculations have been presented in detail previously [11], and follow the work of Jorgenson and Lukacs [12]. The experimental reagent differs only by the inclusion of sodium dodecyl sulfate (SDS; Fisher Scientific, Raleigh, NC, USA) as the surfactant and Sudan III (Aldrich, Milwaukee, WI, USA) as the marker for the elution of the micelles injected with the sample. The procedure differs only by the alteration of the pH range to 8-12.

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0021-9673/93/$06.00

RESULTS AND DISCUSSION

The mobility of an acidic solute in MECC can be described by the following model [10]:

$$\mu = \frac{\mu_{HA} + \mu_{A^-}(K_{a,app}/[H^+])}{1 + (K_{a,app}/[H^+])} \quad (1)$$

where μ is the total mobility of the solute, μ_{HA} is the mobility of the neutral form of the solute, μ_A is the mobility of the anionic form, and $K_{a,app}$ is the apparent acid dissociation constant in micellar media. The mobilities of the neutral and anionic forms of the solute are defined as follows:

$$\mu_{HA} = \frac{K^m_{HA}[M]\,\mu_{mc}}{1 + K^m_{HA}[M]} \quad (2)$$

and

$$\mu_{A^-} = \frac{\mu^{aq}_{A^-} + K^m_{A^-}[M]\,\mu_{mc}}{1 + K^m_{A^-}[M]} \quad (3)$$

where K^m is the binding constant of the solute form to micelles, [M] is the concentration of surfactant present as micelles, μ_{mc} is the mobility of the micelles, and $\mu^{aq}_{A^-}$ is the mobility of the anionic form in the absence of micelles. The concentration of surfactant present as micelles is the total surfactant concentration ([S]) minus the critical micelle concentration (CMC).

Eqn. 1 predicts a sigmoidal behavior for the variation of mobility as a function of pH. At low pH values ($\leqslant pK_{a,app} - 2$) the acid is uncharged and mobility is a result of binding with the mobile micelles as described in eqn. 2. Conversely, at high pH ($\geqslant pK_{a,app} + 2$) the acid is fully charged and the mobility is a combination of two factors: the binding with the mobile micelles and the mobility in the absence of micelles, as described in eqn. 3.

Using these three equations, the mobility of a solute is described in terms of fundamental physical and chemical constants characteristic of each solute. By knowing the values of these constants, the mobility of the solute can be predicted for any pH and micelle concentration.

By measuring mobility over a pH range that is within $pK_{a,app} \pm 2$, the unknown parameters ($pK_{a,app}$, μ_{A^-} and μ_{HA}) can be determined using weighted non-linear (WNLIN) regression. WNLIN regression is an iterative algorithm that estimates the values of the unknown parameters in order to minimize the error in the fit.

If $pK_{a,app}$ is known, it is possible to determine μ_A and μ_{HA} without WNLIN regression by measuring the mobility at $pH = pK_{a,app} - 2$ (for μ_{HA}) and $pH = pK_{a,app} + 2$ (for μ_A).

Calculation of K^m_A and K^m_{HA}

The migration factor k' of the charged and the neutral solute species can be calculated from μ_A and μ_{HA}:

$$k'_{HA} = \frac{\mu_{HA}}{\mu_{mc} - \mu_{HA}} \quad (4)$$

and

$$k'_{A^-} = \frac{\mu_A - \mu^{aq}_{A^-}}{\mu_{mc} - \mu_{A^-}} \quad (5)$$

Terabe and co-workers [13,14] pointed out the relationship between k' and K^m:

$$k' = (P^m V)([S] - CMC) \quad (6)$$

where P^m is the water-micelle partition coefficient and V is the molar volume of the micelles. (The term $P^m V$ is shown in Appendix 1 of this paper to be equal to K^m.) Therefore, K^m is the slope when k' is graphed as a function of surfactant concentration. In all, there are two ways to calculate $K^m_{A^-}$ and K^m_{HA}: first. by using WNLIN estimates of $pK_{a,app}$, μ_{A^-} and μ_{HA} in eqns. 2 and 3; and second, by plotting k' as a function of [SDS] according to eqn. 6.

Most of the experiments were designed to generate data for use with WNLIN regression. This WNLIN data set was collected at nine pH values (8–12, 0.5 increments) and two surfactant concentrations (20 and 80 mM). (Since the variation of mobility as a function of pH is sigmoidal but the variation with surfactant concentration is linear, more pH data are required for reliable operation of WNLIN regression.) These data were used in the first method described above, and were used in the prediction of migration behavior that will be discussed later. In order to test the second method, a separate set of data more appropriate for the linear form of eqn. 6 was collected at two pH values (8 and 12) and four surfactant concentrations (20, 40, 60 and 80 mM).

The K^m_A and K^m_{HA} values calculated from the two

TABLE I

COMPARISON OF MICELLE BINDING CONSTANTS K^m_{HA} AND $K^m_{A^-}$

		4-Propyl-phenol	4-Methyl-phenol	4-Ethyl-phenol	4-Isopropyl-phenol	Phenol
Set 1[a]	K^m_{HA}	11.1	30.1	74.6	155.4	200.6
	σK^m_{HA}	0.7	0.6	2.9	28.6	27.1
	$K^m_{A^-}$	-0.74	2.8	13.1	26.0	45.8
	$\sigma K^m_{A^-}$	1.10	1.2	6.2	21.2	79.2
Set 2[b]	K^m_{HA}	8.09	21.47	51.03	105.90	137.70
	σK^m_{HA}	0.77	0.95	1.23	1.64	1.87
	$K^m_{A^-}$	2.82	8.60	18.29	30.52	38.12
	$\sigma K^m_{A^-}$	0.95	0.90	1.00	1.31	1.44

[a] Based on WNLIN estimates of μ_{A^-} and μ_{HA} from data taken at nine pH and two [SDS] values, using eqn. 1.
[b] Based on linear regression of data taken at pH 8 and 12 and 20. 40, 60 and 80 m*M* SDS using eqn. 6.

data sets are presented in Table I for phenol and four alkylphenols. Both sets show the same trend of an increase in K^m with hydrophobicity of the phenol substituent. When $\ln K^m$ is plotted as a function of the number of substituent carbons on the phenol, as shown in Fig. 1, all sets show the expected linear behavior.

Fig. 2 shows k' as a function of [SDS] for the five solutes at low pH, where the solutes are neutral. According to eqn. 6, the x-intercept is the CMC. In Fig. 2, the value of 4 m*M* appears to be valid. Fig. 3 is the same as Fig. 2 except at high pH, where the solutes are charged. Note that the correlation of the data to the linear regression lit is equally as good in Figs. 2 and 3; however, the x-intercept in Fig. 3 for four of the live solutes is in the range -20 to − 22 m*M*. Of course, negative concentration has no physical meaning, but cannot be dismissed on the basis of misinterpreted data or faulty calculations.

Prediction of mobility

After determining estimates of $pK_{a,app}$, μ_{A^-} and μ_{HA} by WNLIN regression, eqns. 2, 3 and 4 can be used to predict mobility over the entire pH/SDS

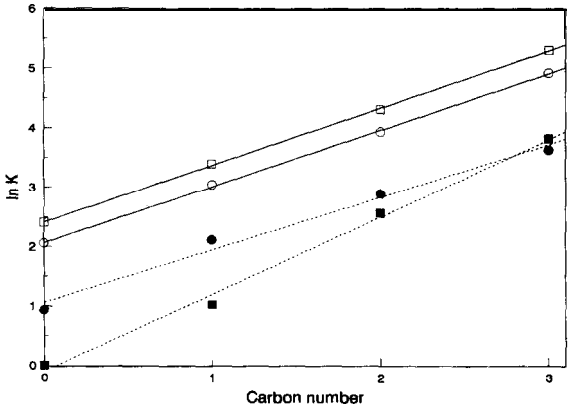

Fig. 1. Relationship between In K^m_{HA} (———) and $\ln K^m_{A^-}$ (- - - -) and the number of carbons in the phenol substituent for set 1 (□ and ■) and set 2 (0 and ●).

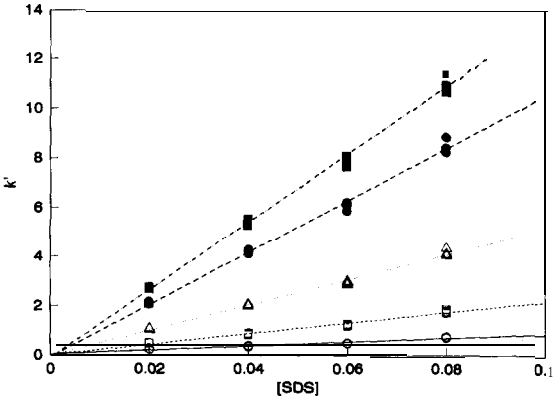

Fig. 2. *k'* as a function of SDS concentration at pH 8 for the neutral forms of phenol (0), 4-methylphenol (□), 4-ethylphenol (△), 4-isopropylphenol (●) and 4-propylphenol (■).

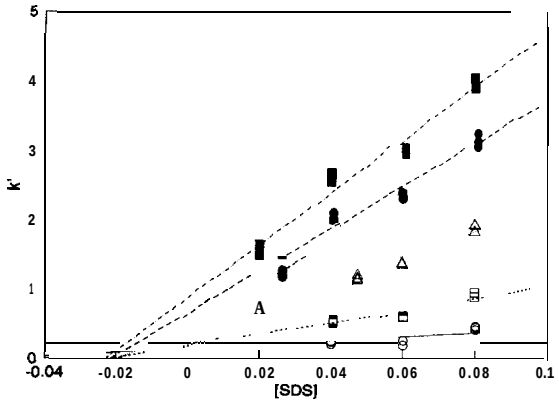

Fig. 3. k' as a function of SDS concentration at pH 12 for the anionic forms of phenol (0), 4-methylphenol (□), 4-ethylphenol (A), 4-isopropylphenol (●) and 4-propylphenol (■).

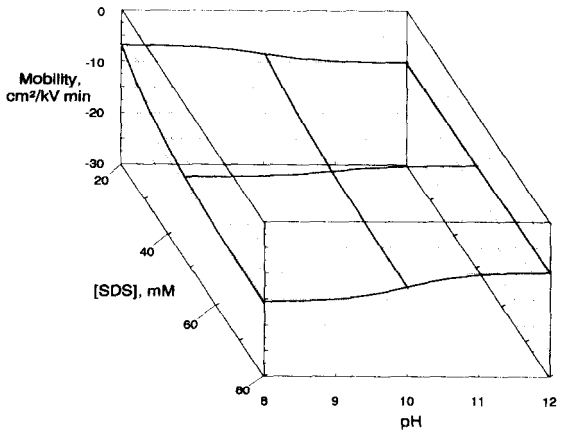

Fig. 5. Variation of mobility as a function of both pH and [SDS] for a hypothetical solute, based on the following values: K^m_{HA} = 14; $K^m_{A^-}$ = 0.002; μ_{A^-} = – 10.25; μ_{HA} = – 12.5; μ_{aq} = –10 and μ_{mc} = -30.

space. Figs. 4 and 5 show the mobility surface for two hypothetical components. The component represented in Fig. 4 strongly interacts with micelles in its neutral form (K^m_{HA} = 220) and moderately in its anionic form ($K^m_{A^-}$ = 1). This component is typical of phenols such as the propylphenols and the di-, tri- and pentachlorophenols. The shape of the sigmoidal curve along the pH axis is generally unchanged as a function of [SDS], and the increase in [SDS] slightly increases mobility. Greatest mobility occurs at pH 8 and [SDS] = 80 mM, which is expected since this point is where the fraction of the neutral form of the solute and the concentration of micelles are the greatest.

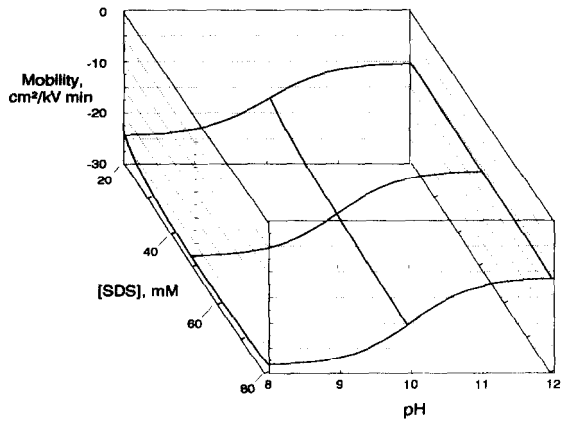

Fig. 4. Variation of mobility as a function of both pH and [SDS] for a hypothetical solute, based on the following values: K^m_{HA} = 220; $K^m_{A^-}$ = 0.8; μ_{A^-} = – 11; μ_{HA} = – 27.5; μ_{aq} = – 10 and μ_{mc} = -30.

The contrast to the strongly interacting component in Fig. 4 is the weakly interacting component in Fig. 5. The degree of interaction with micelles of the neutral (K^m_{HA} = 14) and anionic ($K^m_{A^-}$ = 0.002) forms are representative of phenols such as methyl- and ethylphenol and fluoro-, chloro- and bromophenol. The mobility at high pH is constant over the entire [SDS] range, typical of anionic solutes that interact poorly with micelles.

The shape of the sigmoidal curve along the pH axis changes considerably as a function of [SDS], illustrating the importance of [SDS] on whether the mobility of the neutral form of the solute is greater or less than the anionic form.

The mobilities of the alkylphenols were predicted using the values of $pK_{a,app}$, μ_{A^-} and μ_{HA} estimated by WNLIN. As shown in Table II, $pK_{a,app}$ as estimated by WNLIN did not exhibit consistent behavior as a function of micelle concentration. To account for this variation, an estimate of $pK_{a,app}$ was made by extrapolating between the values measured at 20 and 80 mM.

Mobility predictions were made at six pH/SDS locations: 8.0/20, 8.8/20, 9.5/20, 9.9/63, 9.5/77 and 10.1/77. The first three points were selected for their importance in the separation optimization, which

TABLE II

pK_a VALUES OF PHENOL AND FOUR ALKYLPHENOLS, AS ESTIMATED BY WEIGHTED NON-LINEAR REGRESSION, AT 0, 20 AND 80 m*M* SDS

	pK_a			
	Ref. 16	0 m*M* SDS	20 m*M* SDS	80 m*M* SDS
Phenol	9.99	9.98	9.65	9.66
4-Methylphenol	IO.26	10.29	9.94	10.46
4-Ethylphenol		10.22	9.19	10.19
4-Isopropylphenol	10.28	10.37	9.84	10.31
4-Propylphenol		10.33	10.24	10.27

will be discussed later. The last three hold no particular significance. The correlation between actual and predicted mobilities are presented in Fig. 6. From linear regression, the slope of the best fit of the data (1.013) is very close to the slope expected from prefect correlation (1.000); also, the high correlation coefficient (r^2 = 0.998) indicates the usefulness of eqn. 1 as a model of mobility.

The pH range selected in this study was well suited for the alkylphenols since it covered the range of $pK_a \pm 2$. If data are not collected within the $pK_a \pm 2$ range the estimates will become inaccurate. An

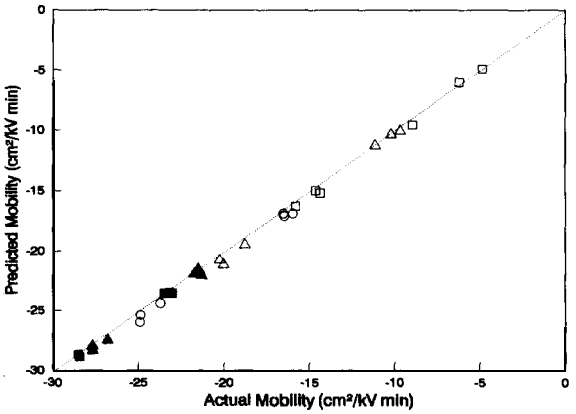

Fig. 6. Correlation between predicted and actual mobilities of phenol(U), 4-methylphenol (A), 4-ethylphenol (○), 4-isopropylphenol (A) and 4-propylphenol (■) at the six pH/SDS locations described in 'the text. The line represents the ideal case of 1: 1 correlation.

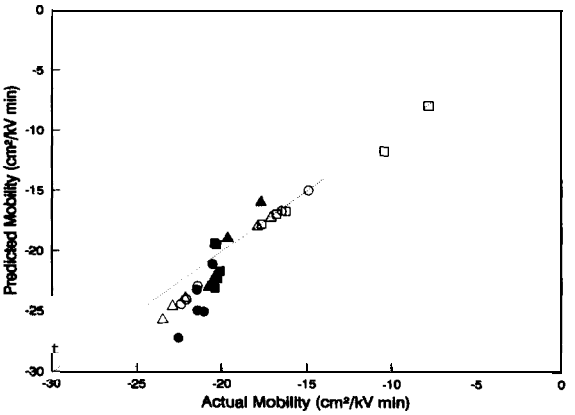

Fig. 7. Correlation between predicted and actual mobilities of 4-fluorophenol (□), 4-bromophenol (A), 4-chlorophenol (0), 2-chlorophenol (A), 2,5-dichlorophenol (■) and 3,5-dichlorophenol (●) at the six pH/SDS locations described in the text. The line represents the ideal case of 1: 1 correlation.

example of this is illustrated in Fig. 7, which is the correlation between predicted and actual mobilities for a set of halogen-substituted phenols. Reasonable correlation exists for 4-fluorophenol, which has a $pK_{a,app}$ of approximately 9.5. As the $pK_{a,app}$ for the solutes shifts away from the center of the pH range studied (pH10), the correlation becomes increasingly worse. The lowest correlations are for 2,5- and 3,5-dichlorophenol, which have $pK_{a,app}$ values of approximately 7.7 and 8.8, respectively.

Optimization of separations: a preliminary study

In the optimization of separations in CZE, the resolution between the worst resolved peak pair was used as the criterion [11]. Resolution, in its simplest form, is described by refs. 12 and 15:

$$R = \frac{N^{1/2}}{4}\left(\frac{\mu_2 - \mu_1}{\mu_{avg} + \mu_{eo}}\right) \quad (7)$$

where N is the separation efficiency, μ_{avg} is the average mobility of the solutes, and μ_{eo} is the electroosmotic mobility. Resolution is directly proportional to the difference in the mobilities of two solutes ($\mu_2 - \mu_1$). For this preliminary study, this term was used as the optimization criterion.

The minimum $\mu_2 - \mu_1$ over the pH/SDS space is presented in Fig. 8 for the alkylphenol mixture. The optimum separation is at pH 9.4 and 21 m*M* SDS. Experiments were performed at pH 9.5 and 20 m*M*

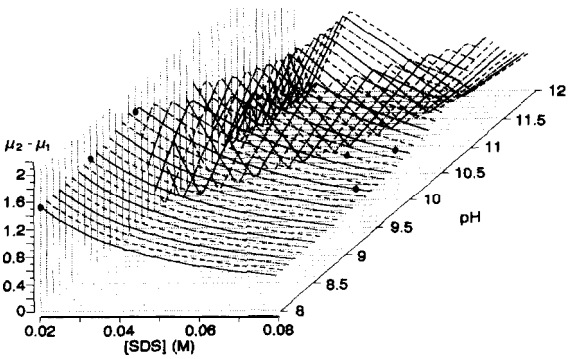

Fig. 8. Smallest difference in mobility between peak pairs as a function of both pH and [SDS]. The circles indicate the pH/SDS locations where confirmatory experiments were performed.

SDS, as **mentioned previously,** in addition to two other points at 20 mM SDS and three in a featureless region at higher SDS concentration. These points are highlighted in Fig. 8.

The actual and predicted electropherograms at each of the six pH/SDS locations are reconstructed from the elution times in Fig. 9a–f. There is excellent agreement at the predicted optimum and at a location near the optimum (Fig. 9b and c). Some of the difference between predicted and actual elution times is most likely due to the extreme sensitivity of the predicted elution time on the value selected as t_0. For example, if the t_0 in Fig. 9a were 1.56 rather than 1.58, the elution times would be 1.77, 2.06, 2.64, 3.35 and 3.63 min, respectively.

Propagation of error in predicting mobility

In all, seven terms are used in the prediction of mobility: pH, [SDS], $pK_{a,app}$, μ_{HA}, μ_{A^-}, μ_{mc} and μ_{aq}. The mobility terms are used to first estimate $K_{A^-}^{m}$ and K_{HA}^{m}, which are then used in the prediction of

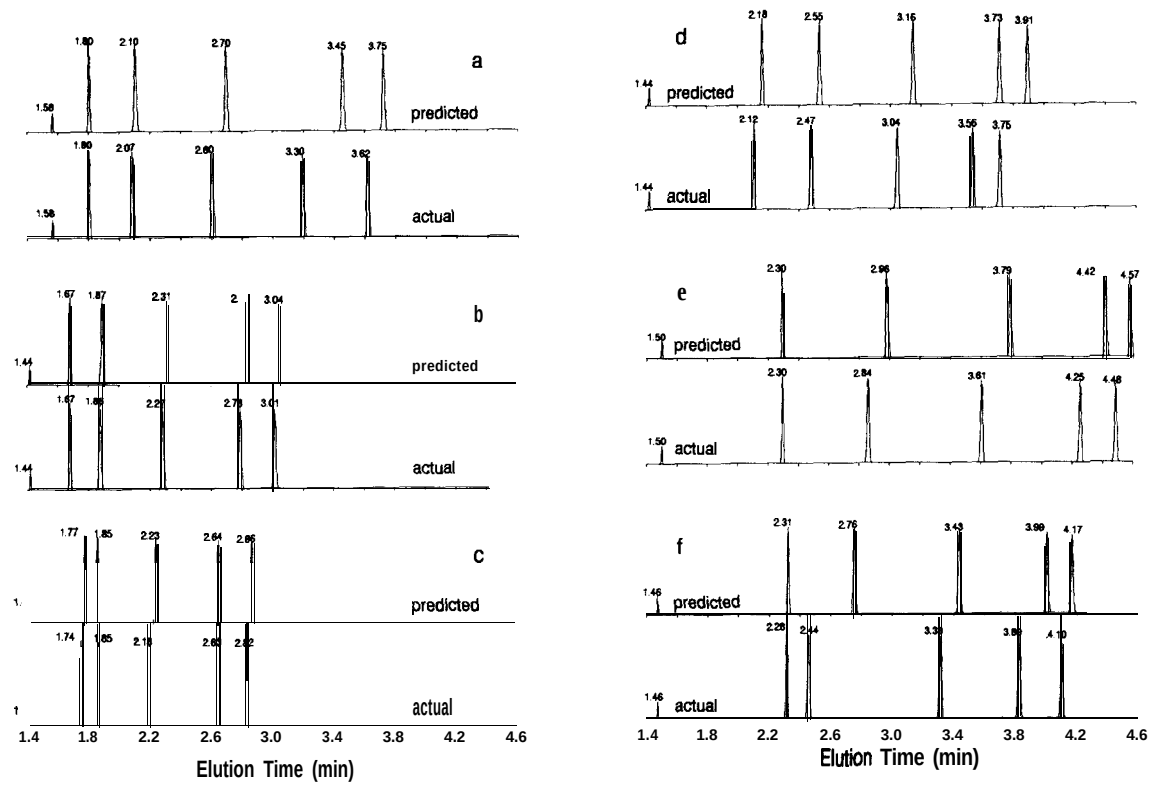

Fig. 9. Predicted and actual elution times at the six pH/SDS locations: (a) 8.0/20; (b) 8.8/20; (c) 9.5/20; (d) 9.9/63; (e) 9.5/77 and (f) 10.1/77. Electropherograms were reconstructed from elution time data.

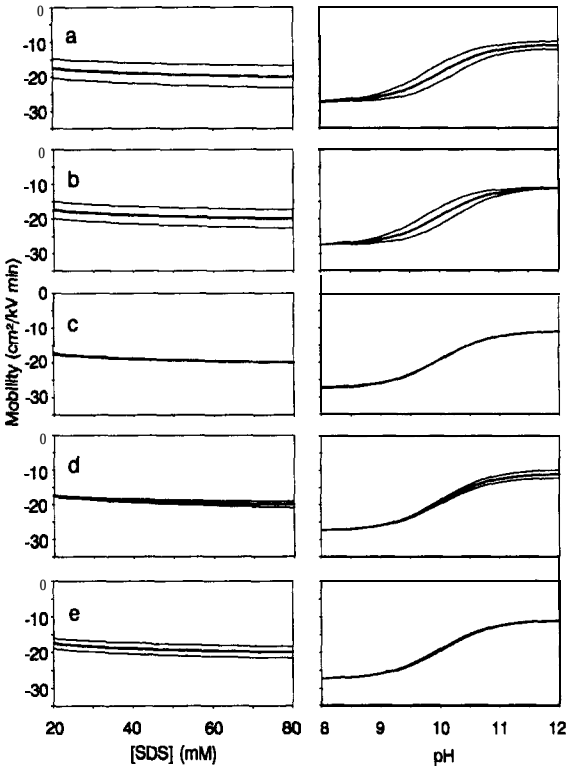

Fig. 10. Total propagated error and individual contributions of five terms to the total error for the hypothetical solute presented in Fig. 4. Each graph presents the mobility (thick line) and the error envelope (thin lines) as a function of either [SDS] at pH10 (left column) or as a function of pH at 50 m*M* SDS. For this solute, the error associated with each contributing term are as follows: (a) total propagated error; (b) $\sigma pK_a = 0.4$; (c) $\sigma K^m_{HA} = 13$; (d) $\sigma K^m_{A^-} = 1.28$ and (e) $\sigma pH = 0.02$.

mobility. The total propagated error for the strongly interacting hypothetical solute illustrated in Fig. 4 and the contribution to the total error from live each of these terms is presented in Fig. 10a–e. (The contributions of $\sigma[SDS], \sigma\mu_{mc}$ and $\sigma\mu_{aq}$ are negligible.) The graphs on the left were calculated at pH 10; those on the right were calculated at 50 m*M* SDS.

In general, the total error is very small at low pH but rapidly increases to a near-constant level at the pK_a of the hypothetical compound. In Fig. 9, the agreement between predicted and actual elution times became worse with increasing surfactant concentration. This suggests that the main contribution to the error is from $K^m_{A^-}$, since it increases with both pH and [SDS] and since it, along with $pK_{a,app}$, are the largest contributors to the total error.

The total error in the prediction of mobility will also impact on the level of confidence in the prediction of elution order. This problem was addressed in the work with optimization in CZE [1 1]. The level of confidence in the ability to predict elution order is proportional to the difference in the predicted mobilities of the solutes and inversely proportional to the error associated with the predicted mobilities, as shown in eqn. 8:

$$t = \frac{n^{1/2}(\mu_1 - \mu_2)}{(\sigma_1^2 + \sigma_2^2)^{1/2}} \quad (8)$$

where t is the t-distribution value, μ_1 and μ_2 are the average mobilities of solutes 1 and 2, σ_1 and σ_2 are the standard deviations of the mobilities of solutes 1 and 2, and n_1 and n_2 are the number of measurements. This relationship assumes $n_1 = n_2$.

In conclusion, two chemical equilibria characteristic of each solute, pK_a and K^m, can be easily exploited by adjusting the pH and [SDS] of the buffer for the purpose of migration prediction and optimization of the separation. The migration behavior of solutes can be predicted on the basis of a few experiments.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge a research grant from the National Institutes of Health (FIRST Award, GM 38738). The authors also gratefully acknowledge the use of the loan of the SP-4200 integrator from Burroughs Wellcome Company. The authors also acknowledge Joost K. Strasters for assistance in the preparation of some of the figures.

APPENDIX

Relationship between binding constant and partition coefficient

It has been reported [17,18] that the relationship between the binding constant K^m and the partition coefficient P^m is:

$$K = (P - 1)\ I' \quad (A1)$$

where V is the volume occupied by one mole of surfactant present as micelles. Use of this equation has resulted in conflicting results in early derivation

of the models used for this and previous studies. The conflicts were resolved upon re-derivation of eqn. Al, as follows.

Given:

$$P = \frac{\text{concentration of solute in micellar phase}}{\text{concentration of solute in aqueous phase}} = \frac{[S]_m}{[S]_w} = \frac{n_{s,m}/v_m}{n_{s,w}/v_w} = \frac{n_{s,m}v_w}{n_{s,w}v_m}$$

and

$$K = \frac{\text{concentration of solute-micelle complex}}{(\text{concentration of solute in aqueous phase})(\text{concentration of micelles in aqueous phase})}$$

$$= \frac{[S\text{–}M]_w}{[S]_w[M]_w} = \frac{n_{s\text{–}m}/v_w}{(n_{s,w}/v_w)(n_m/v_w)} = \frac{n_{s\text{–}m}}{n_{s,w}n_m/v_w} = \frac{n_{s\text{–}m}v_w}{n_{s,w}n_m}$$

Assuming one solute molecule binds with or partitions with one micelle, the number of solute molecules in micelles must also be the number of solute-micelle complexes and $n_{s,m} = n_{s\text{–}m}$. Combining the above equations yields

$$K = P\frac{v_m}{n_m}$$

Given that V = molar volume of surfactant = v_m/n_m,

$$K = PV \qquad \text{(A2)}$$

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