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Simultaneous optimization of pH and micelle concentration in micellar liquid chromatography☆

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ABSTRACT

A retention model for ionizable compounds in micellar liquid chromatography is derived and verified. The use of the model for the prediction of retention is illustrated and appropriate optimization strategies for the separation of ionizable compounds in Micellar Liquid Chromatography are discussed.

INTRODUCTION

Reversed-phase liquid chromatography (RPLC) is the method of choice for the analysis of ionizable compounds with adequate retention. However, the method is unable to retain hydrophylic, ionizable compounds [1].

Poorly retained ionizable solutes can be retained by the addition of submicellar quantities of ionic surfactants acting as ion-pairing agents. However, this modification suffers from the drawback of extending the time required to equilibrate the stationary phase. This is due to the direct dependency of retention on the charge density of ionic surfactant adsorbed on the stationary phase [2]. Similarly, during gradient elution, the increasing organic content of the mobile phase reduces the charge density of surfactant on the stationary phases. As a consequence, the column must be re-equilibrated with numerous column volumes of the weaker mobile phase to regenerate the same surface coverage of pairing reagent required for consistent retention. This can lead to poor retention reproducibility and makes the prediction of optimum separation conditions difficult [3].

Micellar liquid chromatography (MLC) is also capable of the retention and separation of ionizable and neutral compounds. In MLC, surfactant concentrations in excess of the critical micelle concentration (CMC) are used so that micelles are formed in the mobile phase. The presence of micelles in the mobile phase allows for the direct on-column injection of physiological fluids [4–10] and offers enhanced detection possibilities [11–16].

Ionizable compounds are retained in a manner

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similar to ion-pair chromatography due to surfactant deposition on the stationary phase but here elution strength (in the isocratic and gradient modes) is related to the concentration of micelles in the mobile phase. Since in MLC the surfactant concentration is greater than the CMC, then the variation of surfactant concentration on the stationary phase will be limited. Hence, the composition of the stationary phase in MLC is less variable [17]. Consequently, the regeneration capabilities of MLC are comparatively rapid [18,19] and reproducibility of retention is attained in a shorter period of time and with a greater degree of certainty. The stable and predictable nature of MLC retention facilitates the rapid optimization of retention and prediction of optimum conditions [20].

The intent of pH manipulation in RPLC is to increase the retention (through ion suppression) and selectivity of ionizable solutes [21,22]. In addition, the quality of separation could be improved by optimizing the mobile phase organic modifier content or type. Foley and May [21] demonstrated an approach whereby selectivity enhancement can be achieved by optimizing the mobile phase pH. They verified their theory by predicting the optimum pH for the separation of a group of methylated cresols. The mobile phase pH was targeted to separate a critical peak pair based upon the effective ionization constants and self-selectivities of the two solutes in a mobile phase containing 30% acetonitrile.

One should be cautious, however, in optimizing one parameter at a time as this approach can only be effective when the parameters are not interactive. This is usually not the case for optimizing pH and micelle concentration in micellar mediated techniques such as MLC [23–28] and micellar electrokinetic capillary chromatography (MECC) [29,30]. This is mainly due to the fact that the apparent ionization constant in micellar media is a function of micelle concentration, and more importantly, the magnitude of micellar induced shift of ionization constants is a function of solute type [31]. In order to disclose the full resolving power of the method this paper reports the preliminary results of the optimization of retention in MLC using an appropriate retention model that simultaneously describes retention in terms of pH and micelle concentration.

Zwitterionic amino acids are selected as the test solutes because the thermodynamic ionization constants of these solutes are very similar and it has been shown that certain protonated amino acids in MLC are essentially unresolved at low pH [28]. Consequently, it is unlikely that the independent variation of mobile phase pH will provide the desired separation. Interaction with additional parameters such as micelle concentration will probably be required.

THEORY

Fig. 1 shows the equilibria involved in the retention of zwitterionic compounds in MLC. In the figure, anionic micelles are shown but cationic and nonionic micelles could also be considered, although there would be no immediate advantage in using nonionic surfactants for the separation of charged solutes in MLC. K_{a1} and K_{a2} represent the acid dissociation equilibrium constants between the cationic ($HABH^+$), zwitterionic ($^-ABH^+$) and anionic (^-AB) forms

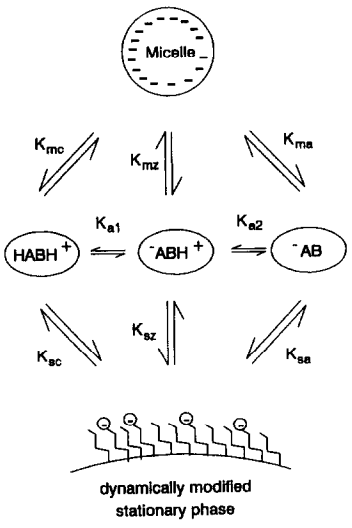

Fig. 1. Equilibria of a zwitterion in MLC with anionic surfactant.

in aqueous solution; K_{mc}, K_{mz} and K_{ma} are the corresponding solute–micelle equilibrium (or binding) constants of the cation, zwitterion and anion. K_{sc}, K_{sz} and K_{sa} are the respective binding constants of the cation, zwitterion and anion to the stationary phase ligands. Note that in MLC, the stationary phase is dynamically modified by the adsorption of surfactant monomers. Following the reasoning given in refs. 23, 25 and 28, the capacity factor is defined as:

$$k' = \{\Phi([HABH^+ - L_s] + [^-ABH^+ - L_s] + [^-AB - L_s])/\{[HABH^+] + [^-ABH^+] + [^-ABL] + [HABH_m^+] + [^-ABH_m^+] + [^-AB_m]\} \quad (1)$$

The following equilibria can be defined:

$$HABH^+ + L_s \rightleftharpoons HABH^+ - L_s \quad (K_{sc}) \quad (2)$$

$$HABH^+ + M \rightleftharpoons HABH^+ - M \quad (K_{mc}) \quad (3)$$

$$^-ABH^+ + L_s \rightleftharpoons {}^-ABH^+ - L_s \quad (K_{sz}) \quad (4)$$

$$^-ABH^+ + M \rightleftharpoons {}^-ABH^+ - M \quad (K_{mz}) \quad (5)$$

$$^-AB + L_s \rightleftharpoons {}^+AB - L_s \quad (K_{sa}) \quad (6)$$

$$^-AB + M \rightleftharpoons {}^+AB - M \quad (K_{ma}) \quad (7)$$

$$^-ABH^+ + H^+ \rightleftharpoons HABH^+ \quad (K_{a1}) \quad (8)$$

$$^-AB + H^+ \rightleftharpoons {}^-ABH^+ \quad (K_{a2}) \quad (9)$$

[M] is the micelle concentration (surfactant concentration, [S], minus the CMC). L_s is the stationary phase ligand and Φ is the phase ratio of the column. Substitution of equilibrium constants of expressions 2–9 into eqn. 1 gives:

$$k' = \{\Phi[L_s](K_{sc} + K_{sz}K_{a1}/[H^+] + K_{sa}K_{a1}K_{a2}/[H^+]^2)\}/\{1 + K_{mc}[M] + (1 + K_{mz}[M])K_{a1}/[H^+] + (1 + K_{ma}[M])K_{a1}K_{a2}/[H^+]^2\} \quad (10)$$

Note that eqn. 10 can also be derived by the phenomenological approach [27,28]. Accurate determination of the constants Φ and $[L_s]$ is difficult and so these terms are incorporated into the constants K_{sc}, K_{sz} and K_{sa} to give the factored constants K'_{sc}, K'_{sz} and K'_{sa} (i.e. $K'_{sc} = K_{sc}\Phi$ $[L_s]$ etc.). This simplification introduces column dependency in the derived values:

$$k' = \{K'_{sc} + K'_{sz}K_{a1}/[H^+] + K'_{sa}K_{a1}K_{a2}/[H^+]^2\}/\{1 + K_{mc}[M] + (1 + K_{mz}[M])K_{a1}/[H^+] + (1 + K_{ma}[M])K_{a1}K_{a2}/[H^+]\}^2 \quad (11)$$

eqn. 11 is shown in Fig. 2. Values for the constants are chosen to reflect those that may be typical for a zwitterionic amino acid with anionic surfactant such as sodium dodecyl sulfate (SDS). The figure shows the predicted retention at variable pH (0–12.5) and variable surfactant concentration ([S] = 0.03–0.074 *M*). Also shown in the figure are the derivatives of eqn. 11 used to determine the apparent ionization constant(s).

Individually, the parameters behave as expected, the highest retention is observed at low pH and low micelle concentration and increasing these parameters decreases retention. However, the interactive nature of these parameters is observable through the apparent ionization con-

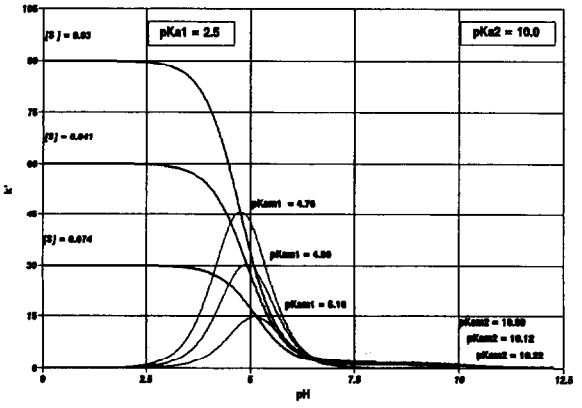

Fig. 2. Predicted retention of a zwitterion as a function of pH and micelle concentration with non-ionic micelles using eqn. 11. Surfactant concentrations: [S] = 0.03 *M*, 0.041 *M* and 0.074 *M*; [CMC] = $8.31 \cdot 10^{-3}$ *M*. Also shown are the derivatives of the double sigmoidal curves at each micelle concentration to give the apparent ionization constants at shown surfactant concentration (pK_{am1} and pK_{am2}). Constants: $K'_{sc} = 19800$, $K'_{sz} = 2$, $K'_{sa} = 0.2$, $pK_{a1} = 2.5$, $pK_{a2} = 10$, $K_{mc} = 10\,000$, $K_{mz} = 10$, $K_{ma} = 0.1$.

stant which is not constant but increases with micelle concentration.

The pH range shown in Fig. 2 (0–12.5) exceeds the pH limitation of the alkyl-bonded silica-based stationary phases (2.5–7.5). However, the pH range 2.5–7.5 is compatible with the retention of zwitterionic solutes in SDS micellar mobile phase as shown in Fig. 2 where, at pH > 7.5, the retention approaches zero and at pH < 2.5 no change in k' with pH is expected. The effect of pK_{a2} on the retention between pH 2.5–7.5 will be negligible and therefore, as this constant tends to zero, eqn. 11 reduces to:

$$k' = \frac{K'_{sc} + K'_{sz}K_{a1}/[H^+]}{1 + K_{mc}[M] + (1 + K_{mz}[M])K_{a1}/[H^+]} \quad (12)$$

Eqn. 12 should be appropriate to test the proposed model for the retention of zwitterionic amino acids with SDS micelles over the limited pH range of a silica based column.

The constants for eqn. 12 can be determined in the following manner. If K_a, K_{mc} and K_{mz} values are available from the literature [27,32,33] or can be determined [32], then K'_{sc} and K'_{sz} can be determined by combining eqn. 12 with the following equations [25,34].

$$K'_{sc} = k'_c(1 + K_{mc}[M]) \quad (13a)$$

$$K'_{sz} = k'_z(1 + K_{mz}[M]) \quad (13b)$$

to give:

$$k' = \frac{k'_c(1 + K_{mc}[M]) + k'_z(1 + K_{mz}[M])K_{a1}/[H^+]}{1 + K_{mc}[M] + (1 + K_{mz}[M])K_{a1}/[H^+]} \quad (14)$$

where k'_c and k'_z are the respective limiting capacity factors for the cationic and zwitterionic forms of the solute. This equation can be linearized:

$$k'\{1 + K_{mc}[M] + (1 + K_{mz}[M])K_{a1}/[H^+]\} = k'_c(1 + K_{mc}[M]) + k'_z(1 + K_{mz}[M])K_{a1}/[H] \quad (15)$$

A plot of the left hand side of the equation *vs.* $K_{a1}/[H^+]$ yields an intercept of $K'_{sc} = k'_c(1 + K_{mc}[M])$ and slope of $K'_{sz} = k'_z(1 + K_{mz}[M])$.

TABLE I

CAPACITY FACTORS FOR THE MLC RETENTION OF IONOGENIC SOLUTES AS A FUNCTION OF SURFACTANT CONCENTRATION AND pH

SDS micellar mobile phase, 50 m*M* sodium phosphate buffer adjusted to pH with concentrated phosphoric acid. Phe = Phenylalanine; Trp = tryptophan; Met = methionine; PPA = phenylpropionic acid.

pH	k'			
	Phe	Trp	Met	PPA
[SDS] = 0.05 M				
7.50	1.23	3.04	0.58	0.30
6.50	1.48	4.31	0.62	1.22
5.60	3.03	6.50	0.75	5.50
3.50	46.7	51.5	17.5	24.8
2.50	56.6	56.7	41.0	25.3
[SDS] = 0.10 M				
7.50	1.60	1.80	0.59	0.30
6.50	1.80	2.57	0.62	1.00
5.60	3.18	5.53	0.72	4.89
3.50	25.6	26.3	13.1	14.1
2.50	28.0	27.1	21.6	14.7
[SDS] = 0.20 M				
7.50	1.00	1.19	0.56	0.31
6.50	1.10	1.71	0.56	0.87
5.60	1.60	2.62	0.65	3.46
3.50	12.9	13.4	7.50	8.33
2.50	13.6	13.6	12.6	8.46

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, MA, USA) liquid chromatographic system was used to collect the chromatographic measurements. The system consisted of a 6000A and an M45 pump, an M680 solvent programmer, a U6K universal liquid chromatograph injector and a Varian UV 50 variable-wavelength detector set at 200 nm. The column was an Ultrasphere, ODS analytical column (Altex, USA, 15 cm × 0.46 cm, d_p 5 μm) protected with a silica precolumn before the injector to saturate the mobile phase with silicates. The silica precolumn and the analytical column were water jacketed and thermostated at 25°C with a Model 1268-02 constant-temperature

recirculating bath (Cole-Palmer). The pH of the mobile phases was measured with Model 231 (Orion) pH meter and 13-639-104 combination electrode (Fisher Scientific).

Reagents

SDS (puriss grade) was obtained from Fluka and used as received. Phosphoric acid (HPLC grade) and the mono- and divalent sodium salts were obtained from Fisher Scientific (NJ, USA). The solutes were purchased from Sigma (St. Louis, MO, USA).

The void volume, V_0, of the system, before exposure to SDS micellar eluents, was measured from the time of injection of $NaNO_3$ to the first deviation of the baseline. A mean value of 0.92 ml ($n = 7$) was used for all subsequent k' calculations. The requisite weight of SDS and 50 m*M* of the sodium salt, were dissolved in doubly distilled, deionized water and filtered through 0.45-μm nylon-66 membrane filters (Rainin). The mobile phase was titrated with concentrated phosphoric acid to pH. The mobile phase was then passed through the system until the column effluent pH equalled the input pH. After the retention volumes were measured the pH of the mobile phase was decreased to the next value and the measurements repeated (Table I).

RESULTS AND DISCUSSION

Regression of eqn. 14 for K'_{sc} and K'_{sz}

The results are shown in Table II where the mean values of K'_{sc} and K'_{sz} are calculated. For each solute the limiting capacity factors are reported with the 95% confidence intervals. For

TABLE II

NON-LINEAR REGRESSION RESULTS OF EQN. 14

Data from Table I; K_m and pK_a values from ref. 29. CMC taken as 0.0081 *M*. ±95% Confidence intervals (CI) reported for limiting capacity factors.

[SDS]	k'_c + 95% CI	k'_c	k'_c − 95% CI	K'_{sc}	k'_z + 95% CI	k'_z	k'_z − 95% CI	K'_{sc}
Solute: Phe; $K_{mc} = 2100$; $K_{mz} = 1.8$; $pK_a = 2.18$								
0.05	58.2	58.0	57.9	5161	1.36	1.25	1.15	1.34
0.10	28.7	28.6	28.6	5548	1.62	1.59	1.57	1.85
0.20	14.4	13.7	12.9	5535	1.36	0.74	0.12	1.00
Mean				5415				1.40
Solute: Trp; $K_{mc} = 7210$; $K_{mz} = 6.5$; $pK_a = 2.35$								
0.05	59.8	55.9	52.0	16 943	5.45	2.22	−1.00	2.82
0.10	28.3	27.1	25.8	17 984	2.57	1.48	0.40	2.36
0.20	15.5	13.4	11.4	18 554	2.36	0.62	−1.11	1.39
Mean				17 827				2.19
Solute: Met; $K_{mc} = 940$; $K_{mz} = 15$; $pK_a = 2.28$								
0.05	51.9	39.7	27.4	1603	1.67	0.00	−1.61	0.00
0.10	24.6	21.3	17.9	1861	2.60	0.35	−1.91	0.83
0.20	14.6	11.9	9.22	2158	2.27	0.38	−1.51	1.47
Mean				1874				0.77
Solute: PPA; $K_{mc} = 110$; $K_{mz} = 0.3$; $pK_a = 4.63$								
0.05	26.9	25.2	23.5	141	1.53	0.00	−0.52	0.00
0.10	14.9	14.5	14.1	161	0.52	0.16	−0.20	0.16
0.20	8.56	8.46	8.37	187	0.37	0.28	0.19	0.30
Mean				163				0.15

Phe, the values of k'_c are relatively precise, at 0.05 M the value is 58.2 ± 0.2. For Met the value is less precise, at 0.05 M the confidence intervals are k'_c ± 30%. However, the degree of variability tolerable for any one component in the separation of a test mixture, is dependent upon how important that particular component is to the overall quality of the separation. If methionine is not a component of the critical peak pair that determines the minimum resolution of the separation, then a greater latitude in precision is acceptable. For Phe, Trp and PPA, the respective standard errors of the mean (S.E.M.s) for the values of K'_{sc} are 2, 3 and 8% respectively. The mean K'_{sc} values are much greater than the mean K'_{sz} values, for Phe, Trp, Met and PPA, the mean K'_{sz} values are 0.026, 0.012, 0.041 and 0.092% of the respective K'_{sc} values. As these values are so small, then their overall impact on predicting retention at low pH will be small.

All five parameters are available and Fig. 3 shows the fitting of eqn. 14 to the experimental data for Phe. The figure shows that the apparent ionization constant in micellar mobile phase is 1.92–2.60 pH units greater than the aqueous ionization constant. This means that the limiting retention, k'_c, is approached at the low pH limit of a silica-based column (pH 2.5) especially at the higher micelle concentrations. Note that at high pH the solute is barely retained (limiting capacity factors k'_z 0.74–1.59) and therefore it is reasonable to neglect the contribution to retention of the anionic form of the solute and the second ionization constant.

The fit between the experimental and predicted data is not as good at [SDS] = 0.05 M. Inspection of Table II reveals that the calculated K'_{sc} of 5161 at this surfactant concentration is below the mean value of 5415 used in eqn. 12. However, a single mean value is required if eqn. 12 is to be used to predict retention at variable micelle concentration.

Optimization strategy

Fig. 3 shows that a change in the eluent strength of the mobile phase in MLC (changing the micelle concentration) also results in a change in the apparent ionization constant of the

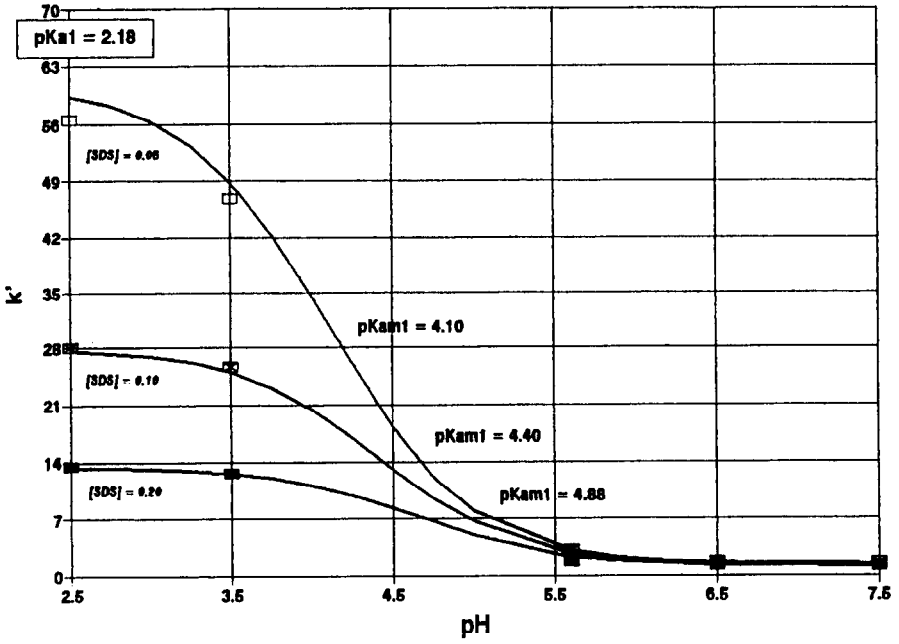

Fig. 3. Retention of Phe as a function of pH and micelle concentration with anionic micelles using eqn. 12 (bold lines). Surfactant concentrations: [SDS] = 0.05 M open squares, 0.10 M square with cross and 0.20 M solid square; [CMC] = 8.1×10^{-3} M. Also shown are apparent ionization constants at each surfactant concentration (pK_{am1} values calculated from the derivatives of the sigmoidal curves; not shown). Constants: $K'_{sc} = 5415$, $K'_{sz} = 1.4$, $pK_{a1} = 2.18$, $K_{mc} = 2100$, $K_{mz} = 1.8$.

solute as the optimum pH and micelle concentration in MLC are directly related. This constraint limits the flexibility of one parameter at a time optimization where the micelle concentration is defined first and then the pH optimized. It will not be possible to optimize by first optimizing pH and then varying the micelle concentration as the ionization constants of the solutes vary with micelle concentration. Therefore a simultaneous, two-parameter optimization of pH and SDS via eqn. 12 should prove an appropriate strategy.

Fig. 4 shows the retention of phenylalanine predicted by eqn. 12. The effect of increasing SDS concentration is shown on the left side of the figure as a parabolic decrease in k' and the sigmoidal effect of pH is shown on the right side of the figure.

Retention surfaces for the other three solutes can be constructed and all four superimposed and a grid search conducted to locate the optimum conditions. The criterion chosen was the maximum minimum resolution, *i.e.* at any location on the grid (in terms of [M] and pH) the resolution is calculated between the four peaks (a total of six computations [35]) and the minimum resolution plotted. The choice of minimum resolution insures adequate separation of the worst peak pair but this criterion does not consider the retention time of the last peak [36] and so the search was limited to [SDS] > 0.05 to ensure a relatively rapid elution of all the components. Fig. 5 shows the calculated minimum resolution for the four solutes as described by eqn. 12 with a maximum minimum resolution of 3.86 at pH 4.5 and [SDS] = 0.05 *M*.

The predicted optimum is investigated further in Fig. 6 which shows the retention as a function of pH at the optimum surfactant concentration (0.05 *M* SDS) in terms of the minimum resolution (line with triangles). The optimum at pH 4.5 and 0.05 *M* SDS is explainable for essentially two reasons. Firstly, at pH less than 3.5 the minimum resolution is less than one due to the similar retention of Trp and Phe but at pH 4.5 these two components will be well separated. It is important to note that the aqueous ionization constants of these solutes are similar (2.35 and 2.18) and below the normal operating pH of silica-based columns (2.5). Therefore the separa-

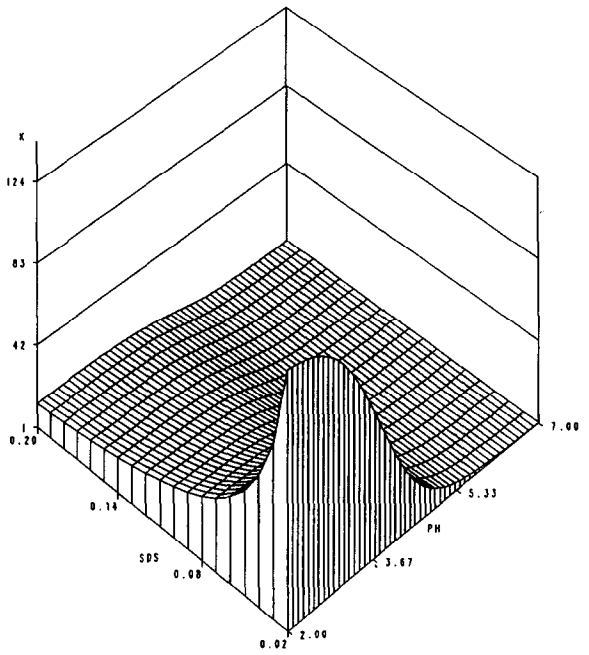

Fig. 4. Retention surface of Phe using eqn. 12, same constants as in Fig. 3.

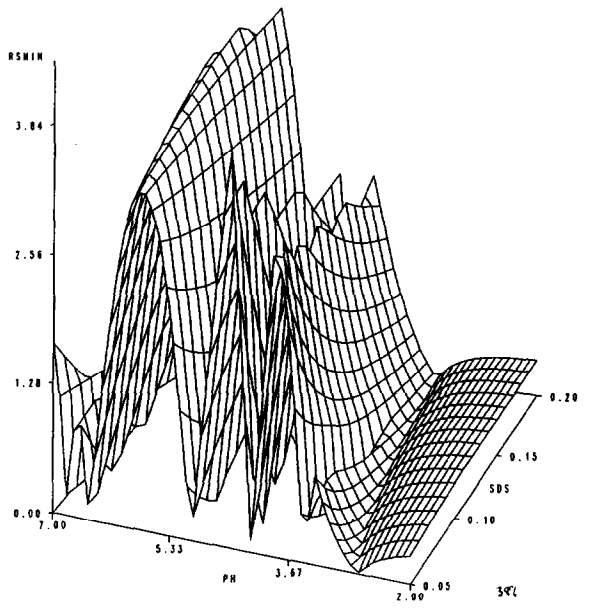

Fig. 5. Response surface in terms of the minimum resolution ($R_{s_{min}}$) for the four solutes as described by eqn. 12 using the constants from Table II.

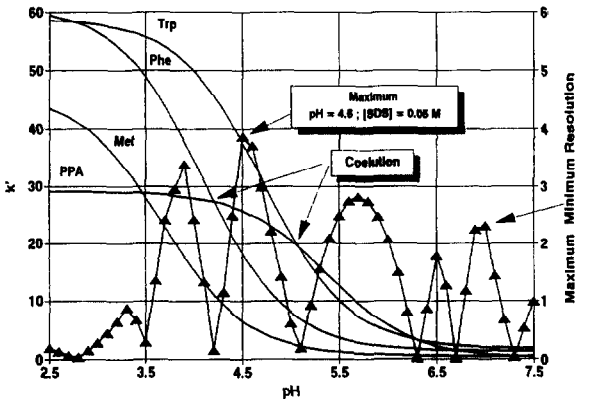

Fig. 6. Cross-section of Fig. 5 at [SDS] = 0.05 *M*. Solid lines, predicted retention of four test solutes. Solid line with triangles, minimum resolution. Also shown are the predicted pH values at which PPA coelutes with Phe and Trp.

tion of these two components at pH 4.5 illustrates the utility of SDS micellar mobile phases which shift the apparent ionization constants of "acidic" solutes to milder pH conditions within the operable limits of silica-based columns.

Secondly, at pH > 3.5 the minimum resolution is dictated by the retention behavior of PPA. Fig. 6 shows that between pH 4.2 and 4.3 the minimum resolution reaches a minimum as Phe and PPA coelute. A similar minimum is found at pH 5.1 where Trp and PPA coelute. Therefore, the model must be sufficiently accurate to predict the behavior of these three solutes between pH 4.3 and 4.8 or PPA may coelute with Phe or Trp.

Fig. 7a shows a simulation of the predicted optimum assuming $N = 2000$. Fig. 7b shows the experimental chromatogram where it is apparent that the first peak (methionine) has a poorer efficiency than the 2000 plates in the simulation. This is probably due to extra-column band broadening which more strongly affects earlier eluting peaks, however for the other three peaks, 2000 plates is a reasonable and typical value for MLC. A comparison of the two figures shows that the predicted and experimental retention are in good agreement with all the peaks well resolved.

Table III shows the predicted retention at the optimum condition compared with the actual retention. The results are consistent in that the

Fig. 7. (a) Predicted optimum using the results from Fig. 5. (b) Experimental verification of (a). 0.05 *M* SDS; 0.05 *M* NaH_2PO_4; pH 4.5. Peaks: 1 = Met; 2 = Phe; 3 = PPA; 4 = Trp.

TABLE III

PREDICTED AND EXPERIMENTAL RETENTION AT 0.05 *M* SDS, pH 4.5

Solute	Predicted		Experimental		% Error	
	k'	α	k'	α	k'	α
Met	6.43		5.95		8.1	
		2.83		2.88		−1.8
Phe	18.2		17.1		6.1	
		1.41		1.35		4.1
PPA	25.6		23.2		10.5	
		1.46		1.55		−5.8
Trp	37.5		36.0		4.1	

predicted retention in all cases exceeds the experimental retention. The errors range from 4.1 to 10.5%. If the results shown in Fig. 7b did not yield the desired separation, then the data from this run would be reentered into the model

(*i.e.* refit eqn. 14 with this additional data point to calculate revised values for K'_{sc} and K'_{sz}) in an iterative procedure until the precision reached an acceptable level [37]. This option may be necessary as normally a precision of 1% or better is required to optimize retention [38]. However, this stringent condition is broadened when systematic as opposed to random error is involved. The results in Table III show that the error results in $k'_{experimental}$ being less than $k'_{predicted}$. What is important is not the absolute error but the relative differences in the errors as all the observed retention are less than the predicted. This is shown in the tabulated % error in α values where a negative value of % error α indicates an increase in peak selectivity while a positive value is indicative of a decrease. Using this criterion, the absolute error of 10.5% in k' for PPA replaced by the more meaningful % error in α of 4.1%.

When interpreting the % error in k' between predicted and experimental values it is important to assess the degree of parameter variability that will produce an equivalent error. This is shown in Table IV where the predicted retention at pH 4.54, [SDS] = 0.05 *M* is compared to the measured retention at pH 4.50, [SDS] = 0.05 *M*. A variation of pH of 0.04 pH units reduces the % error for three of the solutes (Met, Phe and Trp) to less than 1%. This means that when optimizing with pH, a large degree of error in predicting retention is to be expected as a result of small errors in pH measurement. However, if this error is systematic then the selectivity is less likely to be unduly compromised.

TABLE IV

PREDICTED AND EXPERIMENTAL RETENTION AT 0.05 *M* SDS, pH 4.5

Solute	Predicted		Experimental		% Error	
	k'	α	k'	α	k'	α
Met	5.98		5.95		0.5	
		2.86		2.88		−0.7
Phe	17.1		17.1		−0.2	
		1.48		1.35		9.4
PPA	25.4		23.2		9.3	
		1.43		1.55		−7.7
Trp	36.3		36.0		0.8	

TABLE V

PREDICTED AND EXPEIRMENTAL RETENTION AT 0.0566 *M* SDS, pH 4.5

Solute	Predicted		Experimental		% Error	
	k'	α	k'	α	k'	α
Met	6.01		5.95		1.0	
		2.88		2.88		−0.0
Phe	17.3		17.1		0.9	
		1.33		1.35		−1.8
PPA	23.0		23.2		−0.9	
		1.47		1.55		−5.4
Trp	33.8		36.0		−6.2	

Table V shows a similar variation where the influence of errors in surfactant concentration are assessed at the predicted optimum condition. By increasing the surfactant concentration to 0.0566 *M* the % error in retention for three of the solutes are reduced to 1% or less.

CONCLUSIONS

Eqn. 12 predicts that the apparent ionization constants of solutes in micellar solution are displaced from the values measured in purely aqueous media. The pK_{a1} of the carboxylic acid group are shown to increase with anionic SDS micelles.

Due to the dependence of the apparent ionization constants on micelle concentration in MLC, it is demonstrated that a simultaneous optimization of surfactant concentration and pH is the appropriate strategy for the prediction of the optimum condition with a limited number of experiments. In order to demonstrate the validity of the model it is necessary to optimize retention with respect to pH. However, it is difficult to predict and reproduce retention to a high degree of precision with this parameter as retention is strongly dependent on this variable when the pH is within (±) 1 pH unit of the solutes apparent ionization constants. However this does not detract from the utility of the presented approach; the above separation shows

that if the errors are systematic then the separation is not significantly compromised. Also, the multi-parameter approach enables the selection of the optimum condition and allows for the evaluation of the robustness of the optimum. If necessary, an educated decision can be made where selectivity (separation) can be sacrificed for a more robust analysis.

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