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Modelling of the retention behaviour of solutes in micellar liquid chromatography with organic modifiers

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ABSTRACT

Most of the reported procedures for the determination of compounds by micellar liquid chromatography make use of micellar mobile phases containing an alcohol. The retention of a solute in a purely micellar eluent has been adequately described by the linear equation $1/k'$ *vs.* micelle concentration. This equation seems also to be valid for mobile phases with the same alcohol concentration and varying micelle concentrations. A model to describe the retention behaviour of solutes in any mobile phase of surfactant and alcohol is proposed, which makes use of the elution data in five mobile phases of surfactant with different amounts of alcohol. A function of the type $1/k' = A\mu + B\varphi + C\mu\varphi + D$, where μ and φ are surfactant and alcohol concentration, respectively, proved to be satisfactory for different solutes (catecholamines, amino acids, phenols and other aromatic compounds).

INTRODUCTION

Armstrong and Henry [1] indicated in 1980 the feasibility of using mobile phases containing a surfactant solution above the critical micelle concentration (cmc) in reversed-phase liquid chromatography (RPLC). The technique was called micellar liquid chromatography (MLC). The complexity of MLC is much greater than that of conventional RPLC with aqueous–organic solvents, owing to the large number of possible solute–micellar mobile phase–stationary phase interactions, which affect the retention of the solutes. Other factors to be considered are micelle concentration, pH and ionic strength. Almost any compound can be determined by MLC [2].

The retention of a solute usually decreases with increasing micelle concentration in the mobile phase, the retention change depending greatly on the nature of the solute. Three models have been proposed to describe the retention of solutes at various micelle concentrations: the three-phase model of Armstrong and Nome [3], the equilibrium approach of Arunyanart and Cline Love [4] and the model of Foley [5], which considers the interactions with the micelles as a secondary equilibrium. These models lead to similar equations, which can be written as

$$\frac{1}{k'} = \frac{K_{AM}}{(V_S/V_M)P_{SW}} \cdot [M] + \frac{1}{(V_S/V_M)P_{SW}} \qquad (1)$$

where k' is the capacity factor, [M] is the total concentration of surfactant in the mobile phase minus the cmc, V_S the volume of the stationary phase, V_M the volume of the mobile phase in the column, P_{SW} the partition coefficient of the solute between the stationary phase and water and K_{AM} the solute–micelle binding constant. This equation has been verified experimentally for a large number of solutes [6–9].

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The most serious problems with MLC are, on the one hand, the weak solvent strength of purely micellar eluents and, on the other, the poor efficiency of the chromatographic peaks compared with aqueous–organic mobile phases, which has been related to a restricted mass transfer of the solute towards the stationary phase [10]. Dorsey *et al.* [11] recommended the addition of an organic solvent, such as a short-chain alcohol, to the micellar eluent, to enhance the chromatographic efficiency. The addition of alcohols also causes an increase in solvent strength, the effect being larger with more hydrophobic solutes. Ternary surfactant–water–organic modifier eluents have been called hybrid micellar eluents [12]. It has been indicated that eqn. 1 is also valid for these eluents [12,13].

When hybrid micellar eluents were first used, they were severely criticized. However, most of the papers published in the last 5 years on MLC reported procedures with these eluents. Our own experience with the determination of different drugs (diuretics, narcotics, stimulants, anabolic steroids and β-blockers) has shown that in most instances, the retention of the solutes with purely micellar eluents is excessive, which forces one to add a modifier to achieve adequate retention times [9,14]. To predict the retention behaviour of a solute in hybrid micellar eluents, it is necessary to find an equation to describe the change in capacity factor with varying concentrations of surfactant and modifier.

Schoenmakers *et al.* [15] proposed in conventional RPLC the following relationship between capacity factor and volume fraction of organic modifier, φ:

$$\log k' = A\varphi^2 + B\varphi + C \quad (2)$$

where A, B and C are constants which depend on the solute. However, in the usual $1 < k' < 10$ range and a small range of concentrations of modifier, this equation may be approximated to

$$\log k' = -S\varphi + \log k'_0 \quad (3)$$

where S is the solvent strength parameter. The intercept $\log k'_0$ does not coincide with the logarithm of the capacity factor of the solute in a purely aqueous mobile phase, being much smaller [16].

Khaledi *et al.* [17] reported that in hybrid MLC and at a constant micelle concentration, the linear relationship between $\log k'$ and φ is valid. According to them, $\log k'_0$ in eqn. 3 is the logarithm of the capacity factor at a given micelle concentration. However, in the same paper, the plots of retention ($\log k'$) of several amino acids and alkylbenzenes in mobile phases of sodium dodecyl sulphate (SDS) and hexadecyltrimethylammonium bromide (CTAB), respectively, against volume fraction of propanol were not linear, and especially the experimental point for the absence of modifier deviated from linearity. We observed for other solutes that linear $\log k'$ *vs.* φ relationships were only obtained with methanol as modifier [9,18].

Recently, Strasters *et al.* [19] proposed a procedure to describe the change in capacity factor of a solute in hybrid eluents, using the retention data of only five mobile phases. In this approach, linear relationships for $\log k'$ *vs.* total concentration of surfactant, μ, and volume fraction of organic modifier, φ, were assumed. The retention in other mobile phases was calculated by means of a simple linear interpolation. The authors indicated that the agreement between experimental and calculated data for several amino acids and phenols was excellent. However, we found important errors in the prediction of the retention of other solutes when this procedure was applied.

In this paper, a more suitable model is proposed for the description of the retention behaviour in micellar eluents containing an alcohol. In this study, the elution data for five catecholamines in mobile phases of SDS and propanol were used.

EXPERIMENTAL

Reagents

Sodium dodecyl sulphate (99%) was obtained from Merck (Darmstadt, Germany) and propanol (analytical-reagent grade) from Panreac (Barcelona, Spain). The mobile phases were vacuum-filtered through 0.47-μm nylon mem-

branes from Micron-Scharlau (Barcelona, Spain).

Stock standard solutions of the following catecholamines at a $2 \cdot 10^{-3}$ *M* concentration were prepared in 0.1 *M* acetic acid from Probus (Barcelona, Spain): L-adrenaline (biochemical), DL-noradrenaline (pure), dopamine hydrochloride (very pure) and adrenalone hydrochloride (pure) from Fluka (Buchs, Switzerland) and isoprenaline, kindly donated by Boehringer-Ingelheim (Barcelona, Spain). Nanopure deionized water (Barnstead Sybron, Boston, MA, USA) was used throughout.

Apparatus

A Hewlett-Packard (Palo Alto, CA, USA) HP 1050 chromatograph with a UV–visible detector (absorbance was measured at 280 nm) and an HP 3396A integrator were used. Data were acquired by means of a PC and Peak–96 software from Hewlett-Packard (Avondale, PA, USA). The sample was injected through a Rheodyne (Cotati, CA, USA) valve with a 20-μl loop. A Spherisorb octadecylsilane ODS-2 (5 μm), analytical column (12 cm × 4.6 mm I.D.) and a precolumn, placed before the injector, of identical characteristics (3.5 cm × 4.6 mm I.D.) from Scharlau were used. The mobile phase flow-rate was 1 ml min^{-1}. The dead volume was determined by injecting water.

RESULTS AND DISCUSSION

The retention of aminochromes [18] and diuretics [9] in SDS mobile phases containing methanol followed eqn. 3, apparently owing to its weak eluent strength. However, deviations from linearity were observed with other alcohols as modifiers (see Fig. 1). With the data plotted in this figure, excluded the point for $\varphi = 0$, the value of k'_0 was calculated from the intercept of the fitted straight-line according to eqn. 3. This value is compared in Table I with the experimental k' value for mobile phases without modifier. The difference between the experimental and calculated k'_0 values is larger with an alcohol of longer alkyl chain length. Curiously, a linear relationship was found between this difference

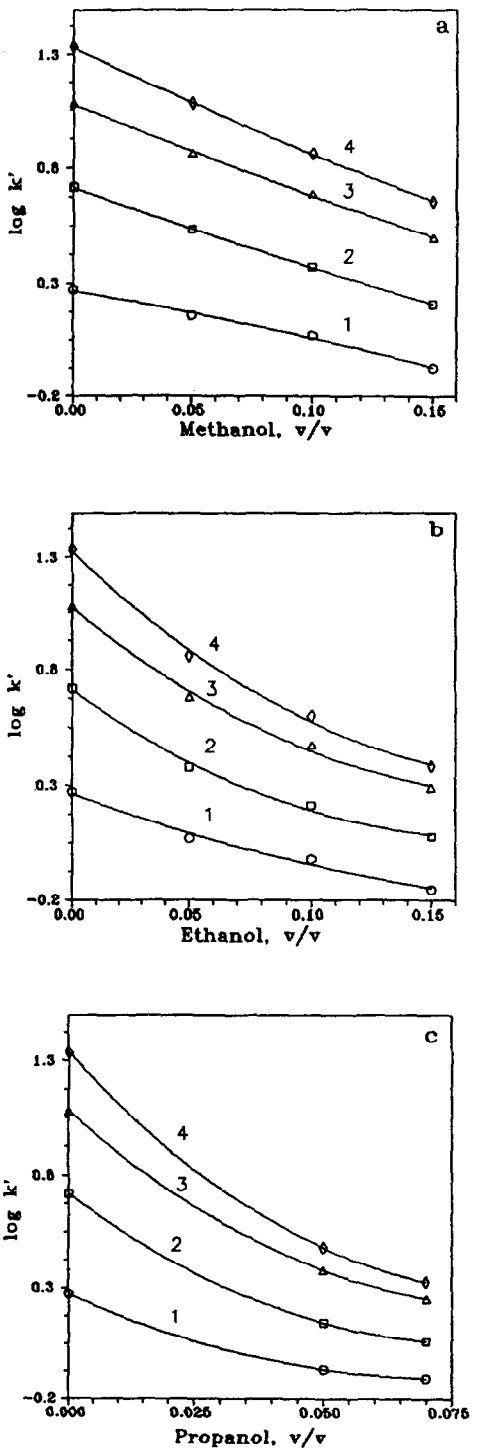

Fig. 1. Log k' *vs.* φ plot for aminochromes: 1 = noradrenochrome; 2 = adrenochrome; 3 = dopaminechrome; 4 = isopropylnoradrenochrome. The mobile phase contained 0.05 *M* SDS.

TABLE I

THEORETICAL AND EXPERIMENTAL k'_0 VALUES FOR 0.05 *M* SDS

Aminochrome	$k'_{0,exp}$	$k'_{0,calc}$		
		Methanol	Ethanol	Propanol
Noradrenochrome	1.87	1.92	1.54	1.10
Adrenochrome	5.21	4.95	3.33	2.29
Dopaminochrome	12.01	11.21	7.42	5.00
Isopropylnoradrenochrome	21.64	19.49	12.33	7.31

and the number of carbon atoms in the alcohol [18].

The search for an equation that will permit the prediction of the capacity factor of a solute in any micellar eluent containing a given concentration of surfactant and of modifier is not easy. The dependence of the capacity factor on the concentration of micelles seems to be different from the dependence of the capacity factor on the concentration of modifier. Eqn. 1 shows a hyperbolic relationship between k' and [M], and in conventional RPLC a quadratic relationship between log k' and φ has been reported [15].

According to Khaledi *et al.* [12], LC with hybrid micellar eluents is similar to that in purely micellar eluents, based on the similar retention characteristics of homologous series. In contrast, Tomasella *et al.* [13], in a study on the role of the

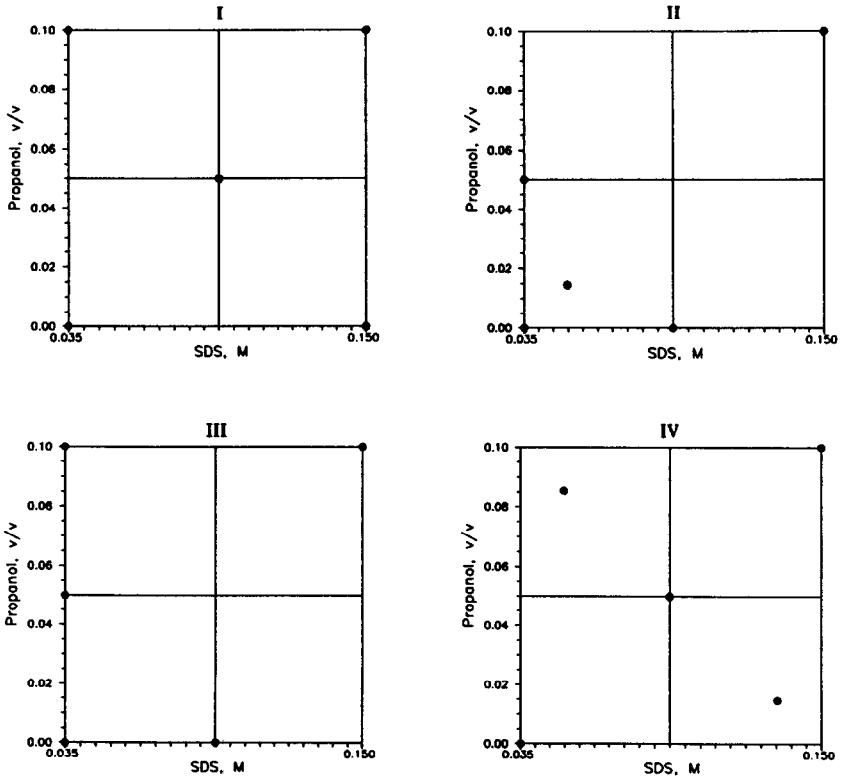

Fig. 2. Experimental designs used to check the retention equations in Table III.

organic modifier in MLC through the use of the free-phase equilibrium model and in correlation with the thermodynamic properties, concluded that, in the presence of a modifier, the retention mechanism is not the same as the mechanism governing aqueous MLC.

The addition of an organic modifier would change certain micellar properties, such as the cmc and the aggregation number of the surfactant, which may influence the retention behaviour of ionic compounds [17]. Also, the equilibrium of the solute is displaced away from the micelle towards the bulk aqueous phase, which becomes more non-polar [13]. On the other hand, the alcohol in the micellar mobile phase solvates the hydrocarbonaceous bonded phase and reduces the amount of sorbed surfactant on the stationary phase. This effect is larger with increasing concentration and hydrophobicity of the modifier [12,17,20]. With hybrid eluents, solute binding constants to micelles and their partitioning into the stationary phase both decrease as a result of the addition of the modifier. However, the K_{AM}/P_{SW} ratio increases and, therefore, the elution power of the mobile phase increases [13].

In the procedure of Strasters *et al.* [19] for predicting retention in hybrid micellar eluents, the retention is determined at five mobile phase compositions (μ,φ), four measurements at the corners of the selected two-dimensional parameter space and one measurement in the centre (see Fig. 2, design I). The extreme values of the parameters are dictated by the practical limitation of the chromatographic system: the lower surfactant concentration must be well above the cmc and must be strong enough to cause elution of all components. The upper surfactant concentration is determined by a combination of the solubility of the surfactant, the viscosity of the resulting mobile phase and the degradation of the efficiency at higher concentrations. The organic modifier concentration is limited to a maximum to ensure the integrity of the micelles. The square parameter space consists of four triangle subspaces. A separate linear model is determined for each of the four subspaces defined by three of the five measurements, *i.e.*, two corner points and the central point. Although it is not explicitly indicated, a different equation of the type

$$\log k' = A\mu + B\varphi + C \quad (4)$$

is fitted in each subspace. The calculation of the k' values is made by interpolation in the subspace where the coordinates belong. The scheme of interpolation followed is not very simple from a practical point of view.

The capacity factors of five catecholamines in thirteen micellar mobile phases, containing SDS and propanol at pH 6.8, are given in Table II. The concentration ranges studied were μ = 0.035–0.15 *M* and φ = 0–0.10 (v/v). The high k' values in mobile phases without propanol were due to the strong electrostatic attraction between the positively charged amine group and the negatively charged surface of the stationary phase. For the catecholamines, the same as with aminochromes and diuretics, when the experimental data were fitted according to eqn. 3 the value of the intercept $\log k'_0$ was smaller than the experimental value in the absence of propanol.

With the procedure of Strasters *et al.* [19], where five mobile phases are used for the calculation of k', large errors were obtained for catecholamines in the prediction of the k' values for the other eight mobile phases assayed. The errors were in the range 7–31% for noradrenaline, 11–37% for adrenaline, 0.4–39% for adrenalone, 9.8–38% for dopamine and 7.6–44% for isoprenaline.

Table III shows some possible models (equations) to describe the retention of the solutes. In these models the reciprocal of the capacity factor (eqns. a–e) and the logarithm of capacity factor (eqns. f–j) are related to the total concentration of surfactant and the volume fraction of modifier through linear and quadratic expressions. In some of them there is a term that includes both variables. The retention of catecholamines was used to evaluate the quality of the models. Five mobile phases were taken according to different experimental designs. Four of these designs are represented in Fig. 2, as an example of those examined. The data were fitted to each model and the errors in the prediction of k' for the

TABLE II

CAPACITY FACTORS OF CATECHOLAMINES IN SEVERAL MOBILE PHASES OF SDS (μ) AND PROPANOL (φ) AT pH = 6.8

Catecholamine	Mobile phase composition					
	Component	Concentration				
	SDS (M)	0.035	0.035	0.035	0.052	0.052
	Propanol (v/v)	0	0.05	0.10	0.015	0.085
		k'				
Noradrenaline		20.26	11.66	8.50	10.47	6.18
Adrenaline		26.54	11.85	8.24	11.63	6.16
Adrenalone		40.63	22.51	13.00	18.56	10.01
Dopamine		51.38	20.62	12.53	20.86	9.66
Isoprenaline		53.05	18.95	11.42	20.22	8.84
	SDS (M)	0.092	0.092	0.092	0.133	
	Propanol (v/v)	0	0.05	0.10	0.015	
		k'				
Noradrenaline		6.87	4.25	3.12	3.78	
Adrenaline		8.40	4.34	3.14	4.13	
Adrenalone		12.68	7.36	5.86	6.42	
Dopamine		15.86	7.07	4.82	7.14	
Isoprenaline		16.56	6.75	4.40	6.97	
	SDS (M)	0.133	0.150	0.150	0.150	
	Propanol (v/v)	0.085	0	0.05	0.10	
		k'				
Noradrenaline		2.23	3.98	2.56	1.88	
Adrenaline		2.20	5.01	2.55	1.92	
Adrenalone		3.99	7.68	3.91	3.08	
Dopamine		3.41	9.33	4.08	2.92	
Isoprenaline		3.23	9.82	3.75	2.64	

thirteen mobile phases were calculated, comparing the experimental and calculated k' values. The global mean relative errors for the five catecholamines and thirteen mobile phases are indicated in Table III.

The smallest errors were achieved with eqns. b and d with the experimental designs in Fig. 2 and with a large number of other experimental designs checked (more than 100). These equations are similar and contain a term including μ and φ. Equivalent coefficients (A–E) in both equations had almost the same value, and the coefficient of the φ^2 term in eqn. d was negligible compared with respect to the $\mu\varphi$ term. In addition, individual relative mean errors for each catecholamine were in most instances lower with eqn. b. Previously, a linear relationship between the reciprocal of k' and the concentration of modifier at a fixed surfactant concentration was suggested [21].

Fig. 3 shows the response surface k' *vs.* (μ,φ) according to eqn. b for noradrenaline. This surface is a slightly asymmetric hyperbolic section, the maximum of the function being located

TABLE III

DESCRIPTION OF THE RETENTION BEHAVIOUR AND GLOBAL MEAN ERRORS OBTAINED WITH THE FIVE CATECHOLAMINES AND THIRTEEN MOBILE PHASES

Relationship	Equation	Relative error (%) ($n = 65$)[a]			
		I	II	III	IV
$1/k' = f(\mu,\varphi)$	(a) $A\mu + B\varphi + C$	50.6	65.3	56.2	74.3
	(b) $A\mu + B\varphi + C\mu\varphi + D$	3.7	4.1	3.7	4.2
	(c) $A\mu + B\varphi^2 + C\varphi + D$	52.2	25.7	55.2	34.2
	(d) $A\mu + B\varphi^2 + C\varphi + D\mu\varphi + E$	3.1	8.1	3.2	4.3
	(e) $A\mu^2 + B\mu + C\varphi^2 + D\varphi + E$	1362	51.6	27.8	131.2
$\log k' = f(\mu,\varphi)$	(f) $A\mu + B\varphi + C$	17.3	16.8	17.5	13.1
	(g) $A\mu + B\varphi + C\mu\varphi + D$	17.3	14.2	15.3	13.0
	(h) $A\mu + B\varphi^2 + C\varphi + D$	12.6	39.7	14.1	13.9
	(i) $A\mu + B\varphi^2 + C\varphi + D\mu\varphi + E$	10.6	101.5	13.2	10.2
	(j) $A\mu^2 + B\mu + C\varphi^2 + D\varphi + E$	70.1	–[b]	8.6	–[b]

[a] Roman numbers correspond to the experimental designs in Figure 2.
[b] No results could be obtained.

at the lower surfactant and propanol concentrations.

Fig. 4 shows plots of the reciprocal of the capacity factor of noradrenaline *vs.* (a) SDS concentration for a constant propanol concentration and (b) propanol concentration for a constant SDS concentration. In Fig. 4, the lines correspond to the calculated data (eqn. b) and the points are experimental data. Good agreement between experimental and calculated data was observed. The capacity factor of noradrenaline decreased at increasing propanol volume fraction for each SDS concentration studied (Fig. 4b). However, this effect was attenuated as the surfactant concentration was increased, that is, the eluent strength of propanol decreased at increasing surfactant concentration. A similar observation was made previously for 2-ethylanthraquinone in SDS and several alcohols [21]. The same behaviour was observed with the surfactant (Fig. 4a), *i.e.*, the eluent strength of the surfactant decreased at increasing modifier concentration.

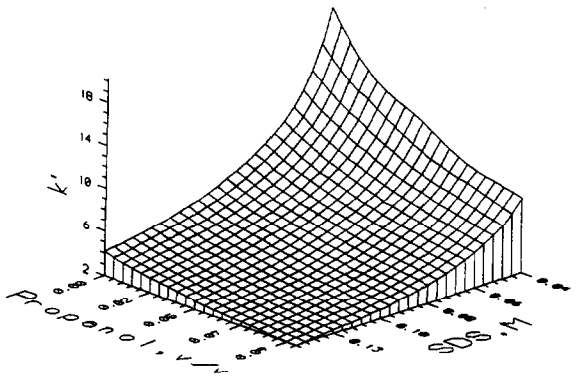

Fig. 3. Response surface k' *vs.* (μ,φ) for noradrenaline (according to equation b in Table III).

For the five catecholamines, the addition of 10% propanol to a 0.035 *M* SDS mobile phase led to relative diminution of the capacity factors by 58–78%. On the other hand, for purely micellar mobile phases, an increase in SDS concentration from 0.035 to 0.15 *M* led to a relative diminution of 81% for the five catecholamines studied. Consequently, it seems that in contrast to the usual behaviour described in the literature [13], for these compounds the eluent strength of the surfactant is the same as or even larger than that of the alcohol. This was due to the high affinity of the positively charged solute towards the negatively charged micelles at the working pH.

Fig. 5 represents the k' *vs.* (μ,φ) contour map for noradrenaline, following the procedure of Strasters *et al.* [19] and the proposed eqn. b.

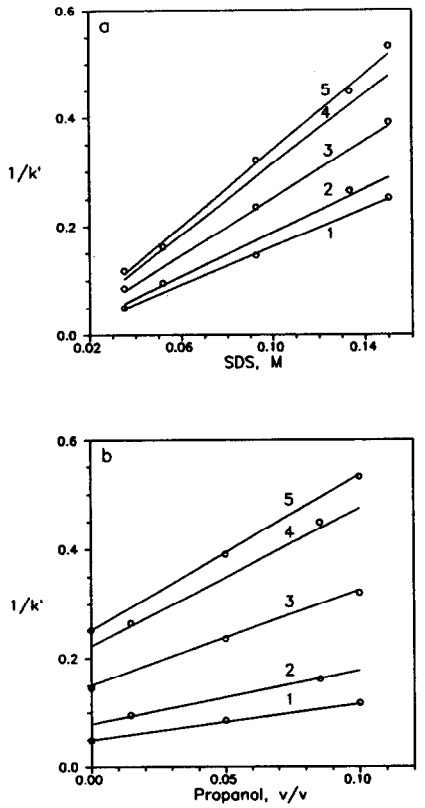

Fig. 4. Retention behaviour for noradrenaline: (a) $1/k'$ *vs.* μ plot for (1) 0, (2) 0.015, (3) 0.05, (4) 0.085 and (5) 0.10 (v/v) propanol; (b) $1/k'$ *vs.* φ plot for (1) 0.035, (2) 0.052, (3) 0.092, (4) 0.133 and (5) 0.150 *M* SDS. Solid lines represent theoretical curves obtained from eqn. b; circles correspond to experimental k' values.

Obvious differences between the contour lines for both models are observed for each k' value.

The calculated k' values according to eqn. b and design I (Fig. 2) are plotted in Fig. 6 against the experimental values for (a) the five catecholamines and thirteen mobile phases, (b) fifteen phenols and five mobile phases [19], (c) thirteen amino acids and five mobile phases [19], and (d) six aromatic compounds and fifteen mobile phases [13]. The equations of the fitted straight lines (linear least squares) were $k'_{calc} = 0.22 + 0.98\ k'_{exp}$ ($r = 0.998$) for catecholamines, $k'_{calc} = 0.05 + 1.00\ k'_{exp}$, ($r = 0.998$) for phenols, $k'_{calc} = -0.41 + 1.05\ k'_{exp}$ ($r = 0.9996$) for amino acids and $k'_{calc} = 0.24 + 0.99\ k'_{exp}$ ($r = 0.996$) for the diverse aromatic compounds. The proximity of the slope to unity and the low intercept revealed the absence of systematic errors. The z value test

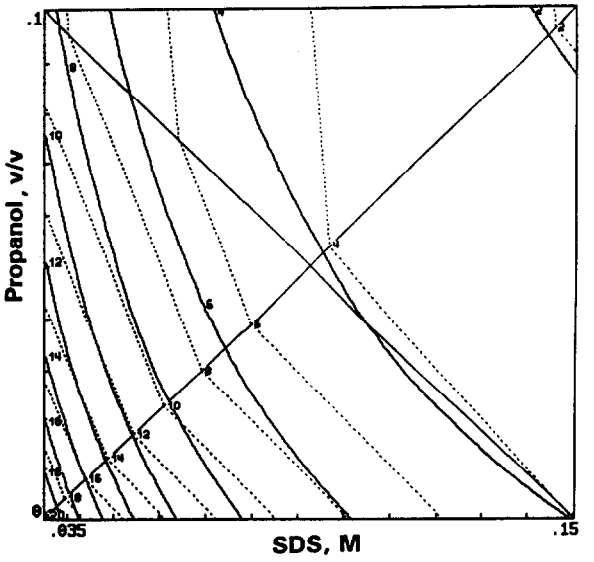

Fig. 5. Contour map k' *vs.* (μ,φ) for noradrenaline, (solid lines) according to eqn. b and (dashed lines) according to Strasters *et al.* [19]. The k' values are indicated on the lines.

for comparing individual differences was also applied [22]. No significant differences existed between the calculated and experimental values.

CONCLUSIONS

The procedure developed by Strasters *et al.* [19] for predicting retention in hybrid micellar eluents requires four different eqns., one for each established subspace. The retention behaviour of a solute in any micellar mobile phase (at any concentration of surfactant and modifier) should preferably be described by a single equation.

The studies performed with the elution data for catecholamines, obtained by us and the elution data for several aromatic compounds, obtained by Tomasella *et al.* [13], indicated that at least for these compounds the retention behaviour in a micellar mobile phase containing an alcohol did not follow a linear log k' *vs.* (μ,φ) model. It was not possible to check this behaviour with phenols and amino acids, as only the elution data in five mobile phases were available.

Several equations and different experimental designs showed that the best results were obtained with an equation of the type $1/k' = A\mu +$

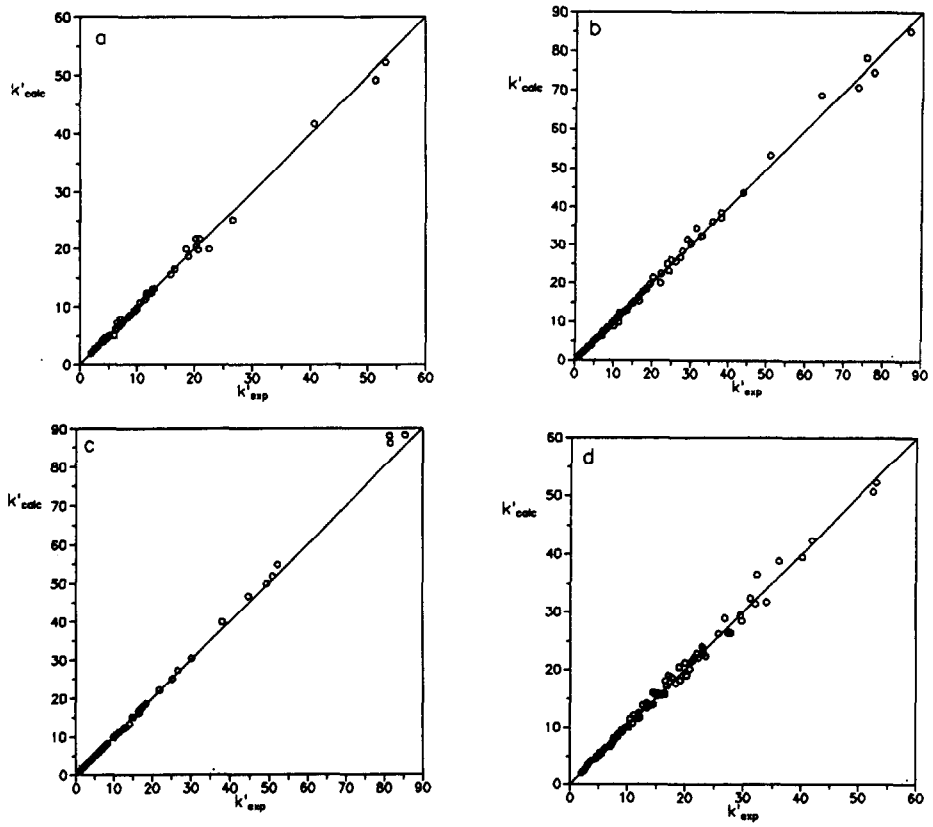

Fig. 6. Calculated k' *vs.* experimental k' values according to eqn. b and design I (Fig. 2) for (a) five catecholamines and thirteen mobile phases, (b) fifteen phenols and five mobile phases [19] (c) thirteen amino acids and five mobile phases [19] and (d) six aromatic compounds and fifteen mobile phases [13].

$B\varphi + C\mu\varphi + D$. This equation was valid for different solutes (catecholamines, amino acids, phenols and other aromatic compounds). The modelling of the retention behaviour is useful for the optimization of resolution in the separation of several compounds, using the elution data for a reduced number of mobile phases. However, more work is necessary on the optimum experimental design.

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