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# APPLICATION OF THE HYDROPHOBIC EFFECT IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TO THE PREDICTION OF THE CRITICAL MICELLE CONCENTRATION

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## SUMMARY

Owing to the occurrence of the hydrophobic effect, a treatment of reversed-phase high-performance liquid chromatographic retention data for the main surfactant families present in α-olefinsulphonates has led to a relationship with the critical micelle concentration (CMC). The CMC values thus estimated are consistent with experimental ones taken from the literature. This method has also been successfully applied to sodium alkanesulphonates.

## INTRODUCTION

The hydrophobic effect is of great importance in biology. For example, this concept has been found useful when determining or predicting the physiological activity of several substances from their structures[1–7]. Hydrophobic interactions have been studied by high-performance liquid chromatography (HPLC), using *n*-alkyl-bonded silica particles as a stationary phase[8,9].

Because the attractive force in micelle formation arises from the hydrophobic effect acting upon the hydrocarbon chain of the amphiphile, the relative prediction of the critical micelle concentration (CMC) from reversed-phase HPLC data seems possible. The aim of the present investigation was to study this possibility.

## EXPERIMENTAL

### *Reagents and materials*

Some pure components and some enriched samples of α-olefinsulphonates (AOS) were prepared according to the described procedures[10]. The straight-chain sodium alkanesulphonates were commercial products (Interchim and Fluka). Water was distilled and filtered; methanol and nitric acid, purchased from Prolabo, were used without further purification.

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TABLE I

EXPERIMENTAL AND CALCULATED CAPACITY FACTORS OF AOS COMPONENTS

$\Phi$ = Volume fraction of water; $C$ = concentration of nitric acid (mmol/l). A = Alkenesulphonate; OH-3 = 3-hydroxyalkanesulphonate; OH-4 = 4-hydroxyalkanesulphonate. Calculated values are given in parentheses.

| *Mobile phase composition* | | $C_{12}$ | | | $C_{14}$ | | | $C_{16}$ | | | $C_{18}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *A* | *OH-3* | *OH-4* | *A* | *OH-3* | *OH-4* | *A* | *OH-3* | *OH-4* | *A* | *OH-3* | *OH-4* |
| $\Phi$ | $C$ | | | | | | | | | | | | |
| 0 | 0 | (0.13) | (0.06) | (0.05) | (0.19) | (0.09) | (0.08) | (0.28) | (0.14) | (0.12) | (0.40) | (0.23) | (0.19) |
| | 1.1 | (0.20) | (0.09) | (0.07) | (0.32) | (0.15) | (0.12) | (0.49) | (0.25) | (0.20) | (0.76) | (0.43) | (0.35) |
| | 1.46 | 0.22 | 0.10 | | 0.35 | 0.20 | | 0.56 | 0.30 | | 0.85 | 0.54 | |
| | | (0.23) | (0.10) | (0.08) | (0.36) | (0.17) | (0.14) | (0.58) | (0.30) | (0.24) | (0.92) | (0.51) | (0.42) |
| 0.125 | 0 | (0.27) | (0.12) | (0.10) | (0.43) | (0.20) | (0.17) | (0.70) | (0.36) | (0.31) | (1.15) | (0.65) | (0.55) |
| | 1.1 | 0.34 | 0.13 | | 0.56 | 0.26 | | 0.92 | 0.54 | | | | |
| | | (0.39) | (0.17) | (0.14) | (0.68) | (0.32) | (0.26) | (1.18) | (0.61) | (0.50) | (2.06) | (1.17) | (0.95) |
| 0.16 | 0 | (0.34) | (0.15) | (0.12) | (0.57) | (0.27) | (0.23) | (0.97) | (0.50) | (0.43) | (1.64) | (0.93) | (0.79) |
| | 1.1 | 0.44 | 0.24 | | 0.71 | 0.41 | | 1.25 | 0.71 | | | | |
| | | (0.49) | (0.21) | (0.17) | (0.89) | (0.42) | (0.34) | (1.59) | (0.83) | (0.67) | (2.87) | (1.64) | (1.33) |
| | 2.2 | | | | 0.84 | 0.44 | | 1.51 | 0.86 | | 3.5 | 2.22 | 1.96 |
| | | (0.65) | (0.28) | (0.23) | (1.28) | (0.58) | (0.47) | (2.31) | (1.20) | (0.98) | (4.36) | (2.49) | (2.02) |
| 0.25 | 0 | (0.54) | (0.23) | (0.20) | (0.99) | (0.47) | (0.40) | (1.8) | (0.94) | (0.80) | (3.3) | (1.89) | (1.61) |
| | 0.73 | 0.62 | 0.27 | | 1.39 | 0.65 | | 2.8 | 1.49 | 1.28 | | 3.13 | 2.65 |
| | | (0.70) | (0.30) | (0.25) | (1.34) | (0.64) | (0.52) | (2.56) | (1.33) | (1.09) | (5.56) | (2.80) | (02.28) |
| | 1.1 | 0.81 | 0.35 | 0.28 | 1.58 | 0.75 | 0.60 | 3.1 | 1.74 | 1.40 | 5.9 | 3.33 | 2.94 |
| | | (0.76) | (0.33) | (0.27) | (1.48) | (0.70) | (0.57) | (2.87) | (1.50) | (1.22) | (5.3) | (3.19) | (2.59) |
| | 1.46 | 0.90 | 0.40 | | 1.76 | 0.80 | 0.60 | 3.7 | 1.96 | 1.7 | | | |
| | | (0.84) | (0.36) | (0.30) | (1.66) | (0.79) | (0.64) | (3.27) | (1.71) | (1.39) | (6.44) | (3.70) | (3.07) |
| | 2.93 | 1.09 | 0.48 | 0.42 | 2.37 | 1.2 | 0.95 | 5.5 | 2.9 | 2.45 | | | |
| | | (1.15) | (0.50) | (0.40) | (2.38) | (1.13) | (0.92) | (4.93) | (2.6) | (2.1) | (10.21) | (5.86) | (4.77) |
| 0.30 | 1.1 | 1.7 | 0.59 | | 2.3 | 1.3 | 0.85 | 5.7 | 2.9 | 2.5 | | | |
| | | (1.3) | (0.43) | (0.35) | (2.01) | (0.96) | (0.78) | (4.1) | (2.14) | (1.74) | (8.27) | (4.76) | (3.88) |

*Apparatus and procedures*

The liquid chromatograph comprised a Gilson Model 302 high-pressure pump, an Altex Model 210 injection valve, an Altex Ultrasphere 5-$\mu$m, RP-18 (ODS) column (25 cm × 10 mm I.D.), a Pye-Unicam LCM2 moving-wire flame ionization detector and a Shimadzu ICR 1 recorder-data processor. The performance of the detector and the determination of the capacity factors, $k'$, from retention data have already been described[10]. Let us recall only that the reported volume fractions in the methanol–water eluents are always those before mixing.

## RESULTS AND DISCUSSION

The retention behaviour of the main components of AOS (sodium alkenesulphonates, 3- and 4-hydroxyalkanesulphonates) in reversed-phase HPLC was studied as a function of the water content of the eluent at room temperature. Table I shows the capacity factors, $k'$, of these compounds in different solvent mixtures with added nitric acid[10]. Some supplementary data relative to sodium *n*-alkanesulphonates (model compounds not found in AOS) and 2-hydroxyalkanesulphonates (minor components of AOS) are given in Table II.

The retention times were slightly dependent on the sample size, so that highly accurate $k'$ values would have required extrapolation to zero sample size. In practice, a roughly constant sample amount was injected: this allows the determination of $k'$ with an acceptable accuracy while being much simpler. Moreover, it has been stated that the exact meaning of low values of $k'$, *e.g.*, $<0.5$, in reversed-phase HPLC is not obvious[11]. However, it is not possible to study the behaviour of several homologous series (from $C_{12}$ to $C_{18}$) within the most favourable range of $k'$ (roughly $1<k'<10$).

The elution order of the different kinds of sulphonates with a given carbon number (4-, 3- and 2-hydroxyalkanesulphonates, then alkenesulphonates) can be attributed to the extent to which the hydrophobic chains may insert themselves between the *n*-octadecyl chains of the stationary phase. Whether solvated (3- and 4-isomers) or chelated (2-isomer)[12], the hydroxyl group marks the limit to which the solute molecule may penetrate into the alkyl-bonded phase. The rôle played by the ratio of the solute chain length to that of the alkyl-bonded phase has recently been described[13].

Without nitric acid, rather broad, unresolved peaks are observed[10]. All other things being equal, *e.g.*, peak broadening related to the re-dilution of samples after gasification in the detector, the addition of small amounts of nitric acid to the mobile phase results in narrower, symmetrical (instead of leading) peaks, and at the same time increases the retention of AOS components. Since nitric acid and sodium sulphonates are ionic compounds, the latter effect can be viewed as a consequence of electrostatic interactions in solution, which modify the distribution constant, $k$ (ratio of the activities of a solute in both phases, $a_s/a_m$, at infinite dilution). Taking into account the relationship between $k'$ and $K$ ($k' = KV_s/V_m$, where $V_s/V_m$ is the phase volume ratio), one can see that an increase in the ionic strength at moderate values lowers $a_m$ and consequently raises $k'$. However, the influence of nitric acid on the retention of AOS components is much larger than that of a salt like sodium nitrate[14], perhaps because of the different sizes of the hydrated counter ions $H^+$ and $Na^+$. On

TABLE II

CAPACITY FACTORS OF SODIUM SULPHONATES: ADDITIONAL DATA

Mobile phase: methanol–water (3:1, v/v) containing $1.1 \cdot 10^{-3}$ *M* nitric acid.

| *Alkanesulphonates* | *k'* | *2-Hydroxyalkanesulphonates* | *k'* |
|---|---|---|---|
| $C_4$ | 0.047 | $C_{12}$ | 0.71 |
| $C_5$ | 0.052 | $C_{13}$ | 0.93 |
| $C_6$ | 0.080 | $C_{14}$ | 1.30 |
| $C_7$ | 0.147 | $C_{16}$ | 2.50 |
| $C_{10}$ | 0.40 | $C_{18}$ | 4.45 |

the other hand, the suppression of adsorption phenomena should result in a decrease in solute retention. As regards the peak shape, the addition of a mineral acid, especially to acidic samples, usually produces symmetrical peaks; nitric acid can also reduce ion-exchange interactions between solutes and the residual acidic silanol groups on the stationary phase[15].

The Martin rule[16] has been applied to homologous series in reversed-phase HPLC[8,9,17,18]

$$\log k' = A + Bn \tag{1}$$

where $n$ is the number of carbon atoms in the chain, $A$ is characteristic of the functional group (polar head group of amphiphilic compounds) and $B$, the increment per methylene unit, is a consequence of the hydrophobic interactions. This rule can easily be derived from the free energies of transfer, $\Delta G^\circ$, of hydrocarbons (or amphiphilic compounds) from water (or mixtures of water with a very short-chain alcohol like methanol) to a non-polar liquid phase[1]

$$\Delta G^\circ = C_1 + C_2 n \tag{2}^*$$

with the usual hypothesis that phenomena occurring inside the column are well approximated by equilibra; $C_1$ and $C_2$ are constants. Thus the parameters of eqn. 1 can be expressed as:

$$A = -\frac{C_1}{2.3\,RT} - \log \frac{V_m}{V_s} \tag{3}$$

$$B = -\frac{C_2}{2.3\,RT} \tag{4}$$

For a given substance, a quadratic relationship between $\log k'$ and the volume fraction of the organic modifier in the eluent has been found theoretically[18–20], but prac-

* In principle, a different value of $\Delta G^\circ$ should apply for each distinct organic phase, but differences between non-polar solvents, *e.g.*, *n*-hexane, *n*-dodecane, tetrachloromethane, are in fact very small.

tically, and especially with water–methanol mixtures, the second-order term may often be neglected[9,18]; moreover, previous results have shown that the slope of $B$ *vs.* this volume fraction (or that of water) is approximately constant over the whole composition range of the eluent[9]. For instance, Tanaka and Thornton's data for the *n*-alkane, straight-chain saturated carboxylic acid and primary alcohol series[8] lead to

$$B = 0.048 + 0.50\Phi \tag{5}$$

where $\Phi$ is the volume fraction of water.

While testing the validity of the Martin rule for various components of AOS[10], we also studied the behaviour of *n*-alkanesulphonates in order to estimate either the effect of the unsaturation (alkenesulphonates) or that of the hydroxyl group (hydroxysulphonates). Table III presents the values of $A$, $B$ and $\Delta(\Delta G^\circ)$ (free energy of transfer from the mobile to the stationary phase per $CH_2$ unit) for alkanesulphonates and AOS components with methanol–water (3:1, v/v) slightly modified with nitric acid ($1.1 \cdot 10^{-3}$ $M$) as the eluent. The $\Delta(\Delta G^\circ)$ values for aliphatic sodium sulphonates determined under these conditions do not parallel those for transfer of the corresponding hydrocarbons (*n*-alkanes and linear 1-alkenes) from water to liquid hydrocarbon, since, according to Tanford[1], a double bond exerts no noticeable influence on $C_2$, and the effect of one double bond is essentially equivalent to the removal of about one $CH_2$ group from a fully saturated chain. Unexpectedly, in this solvent system, the influence of an OH group in the 3- or 4-position on $\Delta(\Delta G^\circ)$ appears less important than that of a double bond. Besides, the values of $A$ show only small variations with eluent composition: average values, $A_{av}$, determined within the interval $0 \leqslant \Phi \leqslant 0.3$ and relative to the main AOS components are also given in Table III.

TABLE III

CHROMATOGRAPHIC PARAMETERS AND FREE ENERGY OF TRANSFER INCREMENTS, $\Delta(\Delta G^\circ)$, OF SODIUM SULPHONATES

| *Series* | *Range (n)* | $A$ | $A_{av}$ | $B$ | $\Delta(\Delta G^\circ)$ *(cal/mol)* |
|---|---|---|---|---|---|
| Alkanesulphonates | 4–10 | −2.04 | | 0.165 | −225 |
| Alkenesulphonates | 12–18 | −1.81 | −1.84 | 0.144 | −196 |
| 2-Hydroxysulphonates | 12–18 | −1.79 | | 0.136 | −185 |
| 3-Hydroxysulphonates | 11–18 | −2.39 | −2.45 | 0.165 | −225 |
| 4-Hydroxysulphonates | 12–18 | −2.55 | −2.54 | 0.167 | −228 |

After checking that the effects of water and nitric acid, at concentration $C$ (mmol/l), are not independent, a numerical treatment of our results leads to the following expressions:

$$B = 0.080 + 0.015\,[(1 - \Phi) \cdot C]^{0.76} + 0.204\,\Phi \tag{6}$$

(sodium alkenesulphonates)

$$B = 0.100 + 0.015\,[(1 - \Phi) \cdot C]^{0.76} + 0.206\,\Phi \tag{7}$$

(sodium 3- and 4-hydroxyalkanesulphonates)

TABLE IV

PREDICTION OF CMC VALUES (mmol/l) OF SODIUM SULPHONATES

| *Series* | $C_{10}$ | | $C_{12}$ | | $C_{14}$ | | $C_{16}$ | | $C_{18}$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Pred.* | *Exptl.** | *Pred.* | *Exptl.** | *Pred.* | *Exptl.** | *Pred.* | *Exptl.** | *Pred.* | *Exptl.** |
| Alkanesulphonates | 47.8 | 57.3[23]<br>38.5**[24] | 12.0 | 9.18[23]<br>9***[24]<br>10.4[25]<br>11[26] | 3.01 | 2.6***[24]<br>2.7[27]<br>2.4 | 0.76 | 0.74***[24]<br>0.74 | | |
| 2-Alkenesulphonates§ | | | 10 | 13 | 2.7 | 2.7§§ | 0.73 | 0.61 | 0.20 | 0.18 |
| 3-Hydroxyalkanesulphonates | | | 25.8 | 25.0 | 6.3 | 6.3§§ | 1.54 | 1.6 | 0.38 | 0.38 |
| 4-Hydroxyalkanesulphonates | | | 31.7 | – | 7.75 | – | 1.89 | – | 0.46 | – |

* Experimental values obtained at 40°C and taken from refs. 28, 29 unless stated otherwise.
** At 50°C.
*** Extrapolated value.
§ Major positional isomer in AOS.
§§ Standard value used in the calculation of $a_1$.

Eqns. 6 and 7 may be used for extrapolation only to eluents not containing nitric acid ($C = 0$), because in principle the effect of nitric acid on $B$ should not vanish in pure water ($\Phi = 1$). In spite of the rather uncertain meaning of low values of $k'$, the calculated values of $k'$ (using eqns. 1, 6 and 7, and $A_{av}$ values from Table III) and the experimental ones are in satisfactory agreement (Table I).

Micelle formation can be considered as a compromise between the tendency of alkyl chains to avoid any contact with water (hydrophobic interaction) and the strong affinity of polar groups toward water. In homologous series, a linear dependence of the CMC on the alkyl chain length is observed and the following relationship holds approximately[21,22]

$$\log \text{CMC} \approx \text{const.} + \frac{\Delta G^\circ}{2.3\,RT} \approx a_1 - b_1 n \tag{8}$$

where $\Delta G^\circ$ has the same meaning as above (free energy of transfer of an amphiphile from a polar solvent to an hydrocarbon phase). By means of the previous equations, the parameters $a_1$ and $b_1$ can be identified straightforwardly:

$$a_1 = \text{const.} - \log \frac{V_m}{V_s} - A = \text{const}'. - A \tag{9}$$

$$b_1 = B \tag{10}$$

Most chromatographic data were obtained in the presence of nitric acid, which makes reversed-phase HPLC resemble micelle formation, and with methanol-rich eluents. However, the values of $b_1$ for the AOS components in pure water can be obtained by using eqns. 6 and 7 ($C = 0$, so extrapolation is allowed).

The direct calculation of $a_1$ from eqn. 9 is complex and an empirical approach was used: the value of const'. had to be taken from one experimental CMC value in each series; however due to the lack of experimental data in the 4-hydroxyalkane sulphonate series, the same value of const'., was used for both sodium 3- and 4-hydroxyalkanesulphonates. In the alkanesulphonate series, the variation of $B$ as a function of $\Phi$ was not studied: taking into account the similar chromatographic behaviour of these last compounds and hydroxyalkanesulphonates under certain conditions (Table III), the constants pertaining to the hydroxyalkanesulphonates were also employed for alkanesulphonates ($b_1 = 0.30$; const'. $= 0.367$), but the specific value of $A$ was taken from Table III. Table IV presents predicted and experimental CMC values for sodium sulphonates in pure water.

The agreement between the calculated and measured CMC values is quite good, considering the approximations carried out. In particular, the chromatographic data were obtained at room temperature where some longer chain surfactants have no CMC: therefore the comparison is done with CMC values determined at higher temperature, inclusing those of the standard $C_{14}$, so that $a_1$ is corrected; besides, it is well known that $B$, related to the selectivity in homologous series, changes very little with temperature, as does $b_1$. The CMC values of 2-alkenesulphonates are rather close to but somewhat lower than those of alkanesulphonates: this may mean that a double bond near the polar head group has a small influence on the hydrophobic interactions.

It seems this method could be used for the prediction of CMC values of homo-

logous surfactants in water or in mixed aqueous–organic solvents from reversed-phase HPLC data, when the CMC of only one homologue in the proper medium is known, and even when minute, not very pure samples of a few other homologues are available. For this method to be applied to the prediction of CMC values in mixed aqueous–organic solvents or in the presence of an electrolyte without data pertaining to the same medium, the constant $A$ should not be greatly dependent on the mobile phase composition, which has been neglected in the present paper, and the dependences of the constants $b_1$ and $B$ (or $a_1$ and $A$) on eluent composition should have essentially the same form in order to allow extrapolation. Qualitatively, the trends seem consistent with each other: a decrease in $k'$ like that provoked by methanol is associated with an increase in CMC; with nitric acid as with other electrolytes the opposite effect is observed.

However, above all it must be kept in mind that such an indirect method affords a CMC value even when micelle formation does not take place.

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