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# Study of amphiphilic behaviour of alkylglycoside surfactants using reversed-phase liquid chromatography

M. Lafosse, P. Marinier, B. Joseph and M. Dreux

*Laboratoire de Chimie Bioorganique et Analytique (LCBA), URA 499, Université d'Orléans, B.P. 6759, 45067 Orléans Cedex 2 (France)*

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ABSTRACT

Alkylglycosides are biological surfactants used to solubilize membrane proteins. A large series of these compounds (commercial or synthesized) were chromatographed using reversed-phase liquid chromatography to study their amphiphilic properties. The latter were found to depend on the length of the alkyl chain, the nature of the polar head and the bonding nature between the sugar and alkyl chain. A comparison with hexaethylene glycol monoalkyl ethers was made. This chromatographic system with evaporative light-scattering detection allows excellent separations of all the compounds.

## INTRODUCTION

Surfactants required for the solubilization and purification of intrinsic membrane proteins showed possess the following properties: high solubilizing power, no denaturation of proteins, optical transparency allowing spectrophotometric detection of purified membrane proteins, chemical purity and easy removal by dialysis necessary for the assay of the protein activity. Among the usual non-ionic surfactants, alkylglycosides are preferred because they have a high critical micelle concentration (CMC) which facilitates their easy removal by dialysis [1,2] and their non-denaturing properties make them very mild surfactants [3,4].

Surfactant properties depend on the nature of the polar head (hydrophilic sugar moiety), the length of the non-polar hydrocarbonaceous chain, the $\alpha$- and $\beta$-anomeric form [3] and the bonding nature between the sugar and alkyl chain. Saito and Tsuchiya [5,6] have shown that octylthioglucoside is superior to octylglucoside and very useful in membrane biochemistry.

These surfactants are often synthesized from the corresponding long-chain alcohols. To produce such compounds on an industrial scale, mixtures of alcohols can be used and purification of the alkylglycosides obtained is difficult because their chromatographic separation in a normal-phase system is very poor, depending on the chain length [4,7]. It was therefore of interest to develop a selective chromatographic method that allows analysis during their synthesis and that resolves a mixture of surfactants according to their non-polar moieties and polar heads. Moreover, a better characterization of the hydrophilic–lipophilic behaviour of these non-ionic surfactants may permit their comparison in order to modify alkyl-bonded silicas and achieve hydrophilic shielding to prevent proteins from being adsorbed while analysing small molecules [8].

In this work we examined these surfactants using reversed-phase liquid chromatography (RPLC). As retention mechanisms of neutral substances in RPLC are principally based on Horváth's solvophobic theory [9], this chromatographic method permits an evaluation of the hydrophobic properties of solutes

*Correspondence to:* Dr. M. Lafosse, Laboratoire de Chimie Bioorganique et Analytique (LCBA), URA 499, Université d'Orléans, B.P. 6759, 45067 Orléans Cedex 2, France.

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[10–12], which is useful because of the high hydrophobicity of the membrane proteins. The retention behaviour in RPLC can thus reflect the hydrophobic associations of the alkyl chains observed in the CMC that characterizes the properties of a surfactant. In the same manner, Dinten *et al.* [13] used reversed-phase thin-layer chromatography for the determination of lipophilicity values of neutral carriers as liquid membrane components.

## EXPERIMENTAL

### *LC system*

Chromatography was carried out using a Spectra-Physics (San Jose, CA, USA) Model 8800 ternary pump, a Rheodyne (Cotati, CA, USA) Model 7125 valve, a Shimadzu CR 6A integrator (Touzart et Matignon, Vitry-sur-Seine, France) and a Model Sedex 45 light-scattering detector (Sedere, Vitry-sur-Seine, France). This detector uses the following principle: the effluent is nebulized by an inert gas and the solvent is vaporized in a warm tube. The non-volatile solutes give a mist of small particles which scatter the light. Scattered light is measured at 120° to the collimated light source. This permits a universal detection method for solid and liquid solutes with or without a chromophore and is compatible with gradient elution [14].

### *Columns*

The columns used were LiChrospher 100 RP-8 (125 × 4 mm I.D.), LiChrospher 100 RP-18 (125 × 4 mm I.D.) and LiChrospher 100 RP-18 end-capped (125 × 4 mm I.D.), purchased from Merck (Nogent-sur-Marne, France), Zorbax octyl (150 × 4.6 mm I.D.) and Zorbax phenyl (150 × 4.6 mm I.D.), purchased from DuPont (Wilmington, DE, USA) and Asahipak ODP-50 (150 × 6 mm I.D.), purchased from Asahi Chemical Industry (Kawasaki, Japan).

### *Reagents*

The mobile phase solvents used were reversed-phase HPLC-grade methanol (Prolabo, Paris, France), HPLC-grade acetonitrile (Merck) and distilled water (Cooperation Pharmaceutique Française, Melun, France). 1-O-Hexyl-β-D-glucopyranoside (C6Glu), 1-O-octyl-β-D-glucopyranoside (C8Glu), 1-O-decyl-β-D-glucopyranoside (C10Glu), 1-O-dodecyl-β-D-glucopyranoside (C12Glu), 1-O-decyl-β-D-maltoside (C10Mal), hexaethylene glycol monodecyl ether (C10E6), hexaethylene glycol monododecyl ether (C12E6), hexaethylene glycol monotetradecyl ether (C14E6) (Fluka, Mulhouse, France), 1-O-dodecyl-β-D-maltoside (C12Mal) and 1-S-octyl-β-D-thioglucopyranoside (C8TGlu) (Aldrich, Mulhouse, France) were all used as received.

1-O-Octyl-β-D-galactose (C8Gal), 1-O-octyl-β-D-cellobiose (C8Cell), 1-O-octyl-β-D-thiocellobiose (C8TCell), 1-O-octyl-β-D-fucose (C8Fuc) and 1-O-octyl-β-D-xylose (C8Xyl) were synthesized in the laboratory. The main structures of the alkylglycosides are shown in Fig. 1.

### *Void volume ($V_0$) determination*

A knowledge of $V_0$ is required for the determination of capacity factors, $k'$. Linearization of the retention data was obtained using a homologous series [15,16] (*e.g.*, alkylglycosides or hexaethylene glycol monoalkyl ethers) with the following equation [17]:

$$V_0 = \frac{V_3 V_1 - V_2^2}{V_3 + V_1 - 2V_2}$$

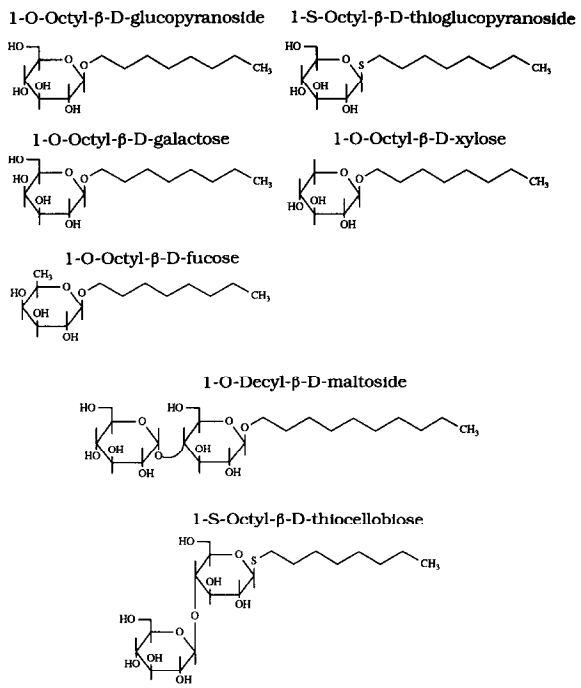


Fig. 1. Main structures of the various alkylglycosides.

where $V_1$, $V_2$ and $V_3$ are the retention volumes of three consecutive homologous surfactants. The value of $V_0$ obtained was slightly higher than that found when sodium nitrate, fructose or glucose was used as the void volume marker.

## RESULTS AND DISCUSSION

In order to elucidate the amphiphilic properties of surfactants, allowing solubilization of highly hydrophobic membrane proteins, the chromatographic behaviour of various alkylglycosides having different alkyl chains and polar heads was investigated. Hexaethylene glycol monoalkyl ethers (RE6) were also tested as these non-ionic surfactants have been widely used for the solubilization of membrane proteins. They are extremely difficult to separate from isolated proteins because of their low CMC and high affinities for membrane proteins [1]. In addition, the number of oxygen atoms in their polar heads is similar to that of alkylglycosides with only one glycosylic group and their hydrophylic–lipophilic balance (HLB) is nearly identical (see Table I).

### *Influence of polar and non-polar moieties of surfactants on retention*

In Fig. 2, the logarithm of the measured capacity factor is plotted against the carbon number of the alkyl chain of alkylglycosides (RGlu), alkylmaltosides (RMal) and RE6 surfactants.

TABLE I

HYDROPHILIC-LIPOPHILIC BALANCE (HLB) AND CRITICAL MICELLE CONCENTRATION (CMC) OF SURFACTANTS

| Solute[a] | HLB[b] | CMC (m$M$) | Ref. |
|---|---|---|---|
| C6Glu | 13.6 | – | |
| C8Glu | 12.3 | 25 | 30 |
| C10Glu | 11.2 | 2.2 | 4 |
| C12Glu | 10.3 | 0.19 | 4 |
| C10Mal | 14.1 | – | |
| C12Mal | 13.4 | 0.16 | 4 |
| C10E6 | 12.9 | 0.92 | 31 |
| C12E6 | 12.0 | 0.082 | 31 |
| C14E6 | 11.3 | 0.0104 | 31 |

[a] For abbreviations, see Experimental.
[b] HLB: weight % polar head / 5.

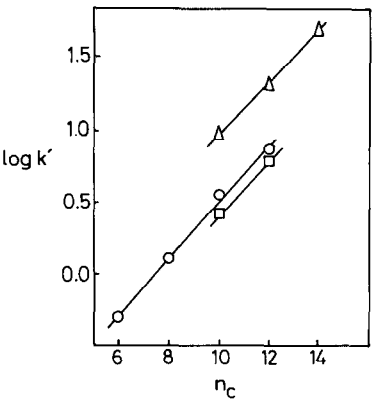


Fig. 2. Experimental plot illustrating the relationship between log $k'$ and carbon number, $n_c$, of non-ionic surfactants in methanol–water (70:30) on a LiChrospher RP-8 column. Solutes: △ = RE6; ○ = RGlu; □ = RMal.

For a given alkyl chain ($C_{10}$ or $C_{12}$), the order of retention is RMal < RGlu ≪ RE6, showing the effect of the polar head. For a given mobile phase, the retention of homologues increases with increasing carbon number, $n_c$, as follows [18]:

$$\log k' = \log \beta + n_c \log \alpha \quad (1)$$

where $\log \beta$ is the specific contribution of the polar moiety to the retention (extrapolation to $n_c = 0$) and $\log \alpha$ is the non-specific contribution of the methylene group ($\alpha$ is the ratio of the capacity factors of two consecutive homologous solutes); $\alpha$ is proportional to the free energy of transfer of a hydrophobic group, methylene, between the mobile and stationary phases [19] and characterizes the solvophobic effect [20].

Table II gives the $\log \alpha$ and $\log \beta$ values with a methanol–water (75:25) mobile phase obtained on various hydrocarbonaceous stationary phases. The selectivity $\alpha$ between two homologous compounds of each series (RGlu, RMal, RE6) is similar on a given stationary phase. This value is smaller on Zorbax phenyl (average value of $\log \alpha$ with three families = 0.119) than on octyl phases (average value of $\log \alpha = 0.171$ on Zorbax $C_8$ and 0.163 on LiChrospher RP-8). Hence this non-specific contribution is similar with a multi-layer octyl (LiChrospher RP-8) and monolayer octyl (Zorbax $C_8$) phases. As already noted [20,21], the largest value is obtained on octadecyl phases [average value 0.221 with

a relative standard deviation (R.S.D.) of 4.7% on the seven values] and this value is similar to the means (0.218) reported by Colin *et al.* [22]. These results show that log $\alpha$ does not depend on packing characteristics (specific area, bonding mode, residual silanol groups). This selectivity $\alpha$ depends more on the nature of the hydrocarbonaceous moiety of the stationary phase than on that of the family of solutes for a given mobile phase.

The log $\beta$ term, a measure of the polar moiety contribution, shows a poor contribution to the retention of glucose and maltose ($n_c = 0$). The average log $\beta$ values for glucose and maltose are $-1.83$ (R.S.D. 4%) on the three octadecyl-bonded phases, $-1.52$ (R.S.D. 9%) on the octyl-bonded phases and $-1.3$ on Zorbax phenyl. These values determined from eqn. 1 correspond to a retention volume close to the void volume observed on the non-polar bonded phases in the methanol–water mobile phases.

The log $\beta$ term for the E6 moiety is larger than that for the sugar moiety: the hydrophobic contribution of the polar head is larger with hexaethylene glycol than with sugar.

Table III shows for a given mobile phase (methanol–water, 75:25) the capacity factors of various surfactants on octyl-bonded (LiChrospher RP-8) and octadecyl-bonded phases (LiChrospher RP-18, LiChrospher RP-18 end-capped and Asahipak ODP-50). Octadecyl-bonded phases with end-capped residual silanol groups (LiChrospher) or without silanol groups (Asahipak) involve larger retentions. This effect indicates that the interactions between the sugar moiety of the surfactant and silanol groups are very weak; this is confirmed by the small values of log $\beta$ (Table II).

*Comparison of retention mechanisms*

From the results in Table III, a comparison of the separation on a pair of stationary phases $j$ and $j'$ can be made based on the log $k'_{ij}$ *versus* log $k'_{ij'}$ relationship, where $k'_{ij}$ is the capacity factor of the $i$th solute (alkyl surfactant) in the $j$th chromatographic system. According to Melander *et al.* [23],

$$\log k'_{ij} = a_{jj'} \log k'_{ij'} + C^t \qquad (2)$$

where $r$ (correlation coefficient) and $a_{jj'}$ (proportionality factor) allow the retention mechanism on the stationary phases to be classified as follows: homoenergetic (the same) if $r > 0.95$ and $0.90 < a < 1.10$, homeoenergetic (similar) if $r > 0.95$ and $a < 0.90$ or $a > 1.10$ and heteroenergetic (different) if $r < 0.95$.

For the column pair with $j$ = LiChrosphr RP-18 end-capped and $j'$ = LiChrospher RP-18, the slope $a_{jj'}$ of log $k'_{ij}$ *vs.* log $k'_{ij'}$ is 0.96 ($r = 0.995$), showing a homoenergetic (the same) mechanism, and the $a_{jj'}$ value for the column pair with $j$ = Asahipak and $j'$ = LiChrospher RP-18 end-capped is 0.99 ($r = 0.995$) and also indicates a homoenergetic mechanism. This illustrates a poor interaction of the hydrophilic moiety of the surfactant with silanol groups of the octadecyl phases.

For the column pair with $j$ = RP-18 and $j'$ = RP-8, the value $a_{jj'}$ is 1.19 ($r = 0.988$), showing a homeoenergetic (similar) mechanism with a larger lipo-

TABLE II

VALUES OF LOG $\alpha$ AND LOG $\beta$ OF EQN. 1 IN METHANOL–WATER (75:25)

| Stationary phase | RGlu | | RMal | | RE6 | |
|---|---|---|---|---|---|---|
| | Log $\alpha$ | Log $\beta$ | Log $\alpha$ | Log $\beta$ | Log $\alpha$ | Log $\beta$ |
| Zorbax phenyl | 0.122 | −1.30 | 0.110 | −1.31 | 0.126 | −0.79 |
| Zorbax $C_8$ | 0.163 | −1.49 | 0.178 | −1.72 | 0.171 | −1.07 |
| LiChrospher RP-8 | 0.166 | −1.43 | 0.161 | −1.45 | 0.162 | −0.92 |
| LiChrospher RP-18 | 0.231 | −1.92 | 0.209 | −1.74 | 0.206 | −1.24 |
| LiChrospher RP-18 end-cappcd | 0.228 | −1.82 | 0.218 | −1.77 | – | – |
| Asahipak ODP-50 | 0.231 | −1.82 | 0.227 | −1.89 | – | – |

TABLE III

VALUES OF CAPACITY FACTORS ON ALKYL-BONDED STATIONARY PHASES IN METHANOL–WATER (75:25)

| Solute | $k'$ | | | |
|---|---|---|---|---|
| | LiChrospher RP-18 | LiChrospher RP-18 end-capped | Asahipak ODP-50 | LiChrospher RP-8 |
| C8Glu | 0.833 | 1.075 | 1.077 | 0.803 |
| C8Gal | 0.808 | 1.058 | 0.983 | – |
| C8Xyl | 1.183 | 1.733 | 1.774 | – |
| C8TGlu | 1.033 | 1.400 | 1.500 | – |
| C10Glu | 2.475 | 2.950 | 3.107 | 1.712 |
| C10Mal | 2.233 | 2.567 | 2.360 | 1.492 |
| C10E6 | 6.642 | 9.600 | 8.187 | 4.576 |
| C12Glu | 6.967 | 7.933 | 9.011 | 3.530 |
| C12Mal | 5.850 | 6.992 | 6.702 | 3.136 |
| C12E6 | 17.183 | – | – | 9.652 |

philicity (larger $k'$) on the octadecyl-bonded phase owing to the alkyl chain, which is longer than on the octyl phase.

Based on the average capacity factor ($k'_j = \Sigma k_{ij}/n$) of the values of Table III on the octyl- and octadecyl-bonded phases, the order of the "hydrophilic power" of the polar head is Gal > Glu ≫ ThioGlu > Xyl and Mal > Glu ≫ E6. In fact, the selectivity $\alpha_{RE6,RGlu}$ is 2.74 (average of the six values of the selectivities C12E6–C12Glu and C10E6–C10Glu on the four alkyl phases, R.S.D. = 9.7%; see Table IV) and $\alpha_{RGlu,RMal}$ is 1.19 (average of the eight values of the selectivities C12Glu–C12Mal and C10Glu–C10Mal on the four alkyl phases, R.S.D. = 7.6%).

In the same manner, selectivity is more linked to the number of hydroxylic groups ($\alpha_{C8Xyl,C8Glu}$ = 1.56 with R.S.D. = 7.8%) than to their spatial configurations ($\alpha_{C8Gal,C8Glu}$ = 0.95 with R.S.D. = 4%).

The presence of S-bonding decreases the hydrophilicity of the polar head ($\alpha_{C8TGlu,C8Glu}$ = 1.31 with R.S.D. 5.8%) and this can be taken in combination with the CMC [4] of C8TGlu, which is lower (9 m$M$) than that of the C8Glu (25 m$M$). In a similar

TABLE IV

SELECTIVITY ($\alpha$) ON ALKYL-BONDED STATIONARY PHASES IN METHANOL–WATER (75:25)

For capacity factors, see Table III.

| Pair | $\alpha$ | | | |
|---|---|---|---|---|
| | LiChrospher RP-18 | LiChrospher RP-18 end-capped | Asahipak ODP-50 | LiChrospher RP-8 |
| C8Xyl–C8Glu | 1.420 | 1.612 | 1.647 | – |
| C8Gal–C8Glu | 0.970 | 0.984 | 0.913 | – |
| C8TGlu–C8Glu | 1.240 | 1.302 | 1.393 | – |
| C10E6–C10Glu | 2.684 | 3.254 | 2.635 | 2.673 |
| C12E6–C12Glu | 2.466 | – | – | 2.734 |
| C10Glu–C10Mal | 1.108 | 1.149 | 1.316 | 1.147 |
| C12Glu–C12Mal | 1.191 | 1.135 | 1.344 | 1.126 |

manner, the lipophilicity of S-bonding is larger than that of O-bonding from the Rekker constants [24] in the determination of partition coefficient $P$ in octanol–water.

*Influence of the nature of the mobile phase*

By varying the volume fraction $\varphi$ of the organic modifier, a nearly linear relationship between log $\alpha$ or log $\beta$ and $\varphi$ was usually observed according to [20]

$$\log \alpha = \alpha_0 - \alpha_1 \varphi \quad (3)$$

and

$$\log \beta = \beta_0 - \beta_1 \varphi \quad (4)$$

Fig. 2 shows a comparison of the selectivity, log $\alpha$, between the neighbouring members of the RGlu series for three non-polar supports in the methanol–water and acetonitrile–water mobile phases. The theory [20] predicts very low values of the coefficient $\beta_1$ for a homologous series with strongly polar end groups, so that log $\beta$ may not vary significantly over a certain range of the composition of mobile phases. For example, a decrease in $\beta_1$ with increasing polarity of the end group was found experimentally [18] with almost constant log $\beta$ for *n*-alkanols. Consequently, a nearly constant value of log $\beta$ is not inconsistent with the validity of eqn. 4. Log $\beta$ for the RGlu series remains relatively constant (R.S.D. *ca.* 4–7%) for a given stationary phase whatever be the volume fraction $\varphi$. The average value of log $\beta$ on a given column is underlined on each curve in Fig. 3. The low values of $\beta$ indicate that the specific interactions between the polar head and the stationary phase are very weak.

Fig. 3 shows that the similar behaviour of the octyl-bonded phases, observed in the Table I, with a methanol modifier is maintained with any mobile phase. With an acetonitrile modifier there is a small difference between the octyl phases.

Moreover, a comparison of the curves in Fig. 3 shows that for the same volume fraction $\varphi$ of an organic solvent (*e.g.*, $\varphi = 0.6$), log $\alpha$ values for hydrophobic interactions in an acetonitrile–water mixture are about half those with methanol mixtures. Indeed, the surface tension of the acetonitrile–water mixture is lower than that of the methanol–water mixture [9], decreasing the hydrophobicity of the solute and consequently the retention [10]. Schoenmakers *et al.* [25] has also shown that on a non-polar

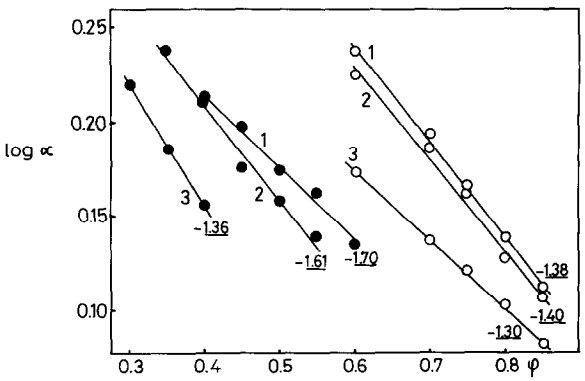


Fig. 3. Log $\alpha$ of alkylglycosides as a function of the volume fraction, $\varphi$, of organic modifier in (○) methanol–water and (●) acetonitrile–water mixtures. Columns: 1 = LiChrospher RP-8; 2 = Zorbax $C_8$; 3 = Zorbax phenyl. The underlined numbers are the average values of log $\beta$.

stationary phase for a given composition, acetonitrile is more sorbed, yielding a decrease in the lipophilic interactions of the solute with a non-polar stationary phase and consequently the retention.

Thus the elution of alkylglycosides on Zorbax phenyl requires a higher content in the mobile phase than on octyl-bonded phases. This is even more so when an acetonitrile–water mobile phase is used. In preparative chromatography, an octyl-bonded (or octadecyl-bonded) phase used with a methanol–water mixture would be better as the mobile phase would be richer in organic modifier, thus avoiding the precipitation of large amounts of the injected solute.

Because the members of these homologous series are not numerous, the values of log $\alpha$ and log $\beta$ are not sufficiently accurate for a detailed study. We therefore investigated the relationship between the capacity factor of each solute and the volume fraction of the organic modifier.

*Characterization of the hydrophobicity of the surfactant*

Fig. 4 shows the plot of log $k'$ *vs.* $\varphi$ for different solutes on LiChrospher RP-8 in various binary mobile phases containing methanol–water. The dependence of the capacity factor of the solute on the volume fraction of organic solvent in the mobile phase can be described by a linear relationship [26]:

$$\log k' = a - m\varphi \quad (5)$$

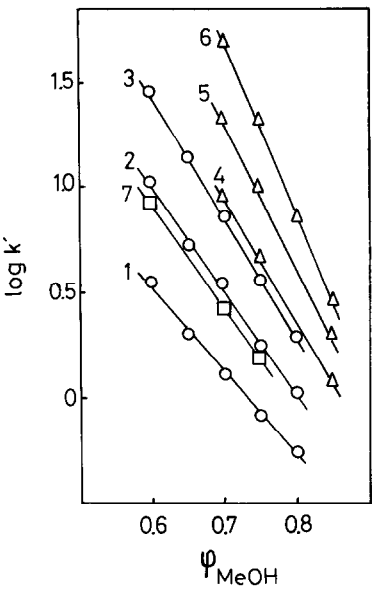


Fig. 4. Experimental plot illustrating the relationship between log $k'$ and the volume fraction, $\varphi$, of methanol (MeOH) in the mobile phase on LiChrospher RP-8. Solutes: 1 = C8Glu; 2 = C10Glu; 3 = C12Glu; 4 = C10E6; 5 = C12E6; 6 = C14E6; 7 = C10Mal.

This equation, a simplified form of a quadratic relationship, is often adequate for a limited range of the volume fraction $\varphi$ [26]. The constants $a$ and $m$ for the three homologous series in each organic modifier and on the three stationary phases are given in Table V. They were derived by linear regression analysis (correlation coefficient $r \geqslant 0.990$).

The intercept value $a$ makes it possible to calculate the extrapolated $k'$ value in pure water and the slope $m$ represents the decrease in retention on increasing the organic modifier content. Jandera [20] has shown that the dependences of the constants $a$ and $m$ on $n_c$ are linear.

The CMC, illustrating the hydrophobicity of a surfactant, is also proportional to $n_c$, following the linear relationship [27]

$$\log \mathrm{CMC} = An_c + B \tag{6}$$

As the value of $m$ represents the increase in retention as the water content in the mobile phase is increased, it permits an evaluation of the solvophobicity of the solutes. Thus a combination of $m$ and log CMC (Table I) yields Fig. 5a, which illustrates the relationship between the solvophobicity measured by HPLC ($m$) and the hydrophobicity evaluated by CMC.

In the same manner, Fig. 5b illustrates the relationship between the extrapolated $k'$ value in pure water ($a$) and CMC. In each instance, two distinct series, one with alkylglycosides and the other with RE6, can be obtained. As in the plot of log $k'$ *vs.* log $P$ of Sabatka *et al.* [11] for another family of compounds, each curve is related to solutes giving hydrogen bonding or not.

TABLE V

VALUES OF CONSTANTS $a$ AND $m$ IN EQN. 5

| Solute | LiChrospher RP-8 | | | | Zorbax $C_8$ | | | | Zorbax phenyl | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $CH_3OH$–water | | ACN–water | | $CH_3OH$–water | | ACN–water | | $CH_3OH$–water | | ACN–water | |
| | $m$ | $a$ | $m$ | $a$ | $m$ | $a$ | $m$ | $a$ | $m$ | $a$ | $m$ | $a$ |
| C6Glu | 2.793 | 1.689 | – | – | 2.826 | 1.658 | – | – | 2.171 | 1.099 | 3.605 | 1.020 |
| C8Glu | 3.879 | 2.836 | 3.127 | 1.246 | 3.669 | 2.590 | 2.902 | 1.170 | 3.040 | 1.916 | 4.560 | 1.743 |
| C10Glu | 4.925 | 3.953 | 3.952 | 2.046 | 4.632 | 3.625 | 3.803 | 1.943 | 3.691 | 2.646 | 5.863 | 2.564 |
| C12Glu | 5.734 | 4.874 | 4.734 | 2.799 | 5.602 | 4.673 | 4.662 | 2.733 | 4.430 | 3.430 | 7.340 | 3.477 |
| C10Mal | 5.819 | 4.451 | 4.690 | 2.179 | 4.554 | 3.524 | 4.560 | 2.184 | 3.822 | 2.694 | 5.950 | 2.425 |
| C12Mal | 6.558 | 5.290 | 5.255 | 2.829 | 5.325 | 4.422 | 4.921 | 2.692 | 4.517 | 3.440 | 7.383 | 3.296 |
| C10E6 | 5.819 | 5.018 | 4.473 | 3.028 | 5.659 | 4.880 | 3.776 | 2.680 | 4.485 | 3.824 | 2.935 | 2.037 |
| C12E6 | 6.862 | 6.121 | 5.472 | 3.875 | 6.550 | 5.876 | 4.358 | 3.330 | 5.755 | 5.020 | 3.232 | 2.433 |
| C14E6 | 8.382 | 7.581 | 6.384 | 4.687 | 7.852 | 7.198 | 4.935 | 3.990 | 6.803 | 6.079 | 3.724 | 2.965 |

TABLE VI

REGRESSION CONSTANTS AND CORRELATION COEFFICIENTS OF EXPERIMENTAL PLOTS LOG CMC *vs.* *m* AND LOG CMC *vs.* *a* (SEE FIG. 5)

| Mobile phase | Slope | Intercept | *r* | Series | Plot | Relationship |
|---|---|---|---|---|---|---|
| Methanol–water | −1.136 | 2.845 | 9.997 | RGlu | Fig. 5a | *vs.* *m* |
| | −0.737 | 1.111 | 0.987 | RE6 + C12Mal | Fig. 5a | *vs.* *m* |
| | −0.942 | 1.050 | 0.992 | RGlu + C12Mal | Fig. 5b | *vs.* *a* |
| | −0.752 | 0.657 | 0.992 | RE6 | Fig. 5b | *vs.* *a* |
| Acetonitrile–water | −1.318 | 2.531 | 0.999 | RGlu | Fig. 5a | *vs.* *m* |
| | −1.023 | 1.544 | 0.999 | RE6 + C12Mal | Fig. 5a | *vs.* *m* |
| | −1.380 | 0.134 | 0.999 | RGlu + C12Mal | Fig. 5b | *vs.* *a* |
| | −1.174 | 0.500 | 0.999 | RE6 | Fig. 5b | *vs.* *a* |

From the results of the linear regression analysis of these plots (Table VI), C12Mal appears to belong to the RE6 family (Fig. 5a) or the RGlu family (Fig. 5b). This shows that RMal family have a particular behaviour but the lack of CMC data of this series prevents a more precise correlation.

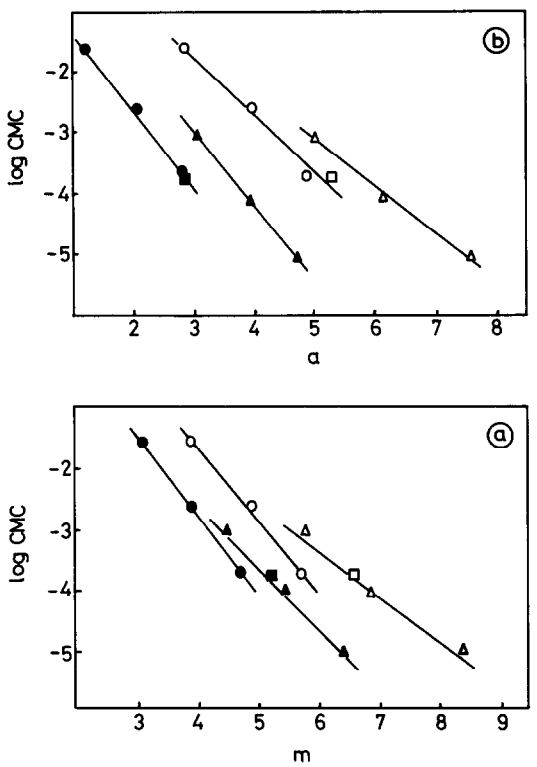


Fig. 5. Correlation between log CMC of surfactant and the slope *m* or the intercept *a* of eqn. 5. Solutes: ▲,△ = RE6; ●,○ = RGlu; ■,□ = RMal. Organic modifier: closed symbols, acetonitrile; open symbols, methanol.

It should be possible to determine the CMC of a new surfactant by its chromatographic behaviour. Fig. 5 shows linear relationships for both organic modifiers. These slopes illustrate a difference in solvophobicity. For a given CMC value, the solvophobicity derived from *m* is larger in methanol–water, corresponding to higher values of the surface tension of the liquid phase in the range of volume fraction studied [9].

*Chromatographic separation*

As a linear dependence of *a* and *m* on the number of methylene groups $n_c$, of each solute has been observed, the following relationship [20] between *m* and *a* can be obtained after elimination of $n_c$:

$$m = q + pa \quad (7)$$

From the values in Table V, plots of *m* *vs.* *a* are shown in Figs. 6 and 7 for both organic modifiers.

As shown in Fig. 6a, a positive correlation ($r = 0.995$) exists between *m* and *a* in methanol–water mixtures. The resulting values of this correlation are 0.93 for *p* and 1.25 for *q* (see eqn. 7). These values are similar to those found by Jandera [28] on Silasorb $C_8$ with the same mobile phase; they are slightly higher than those obtained by Schoenmakers *et al.* [29] on an RP-18 stationary phase for various solutes without a large alkyl chain.

The correlation observed for all the data points in Fig. 7 is the consequence of small differences between the coefficients *q* in methanol–water. On the other hand, Fig. 6 shows the absence of a

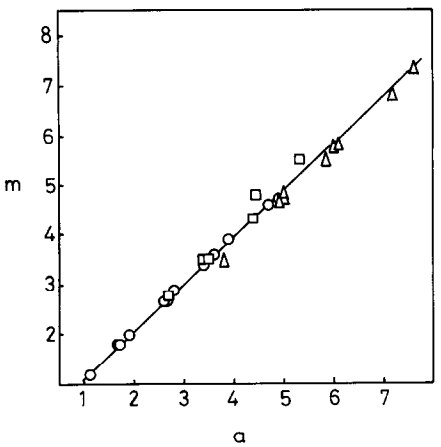


Fig. 6. Correlation between the slope $m$ and the intercept $a$ of eqn. 5 in methanol modifier system. Symbols as in Fig. 5.

TABLE VII

COMPARISON OF THE CONSTANTS $q$ AND $p$ IN EQN. 7 IN ACETONITRILE–WATER MOBILE PHASE FOR VARIOUS HOMOLOGOUS SERIES AND STATIONARY PHASES

| Stationary phase | Homologous series | $q$ | $p$ |
|---|---|---|---|
| Zorbax phenyl | RE6 | 1.180 | 0.854 |
| Zorbax $C_8$ | RE6 | 1.407 | 0.885 |
| LiChrospher RP-8 | RE6 | 0.992 | 1.152 |
| LiChrospher RP-8 | RGlu | 1.837 | 1.035 |
| Zorbax $C_8$ | RGlu | 1.595 | 1.126 |
| Zorbax $C_8$ | RMal | 3.010 | 0.711 |
| LiChrospher RP-8 | RMal | 2.796 | 0.869 |
| Zorbax phenyl | RGlu | 1.974 | 1.530 |
| Zorbax phenyl | RMal | 1.960 | 1.645 |

correlation ($r = 0.65$) with acetonitrile–water. A similar scattered plot was also observed by Schoenmakers *et al.* [29]. According to both plots (Figs. 6 and 7), the nature of the organic modifier significantly improves the difference in the behaviour between these non-ionic surfactants. In fact, the absence of a correlation in Fig. 7 is only apparent; eqn. 7 is theoretically valid only for a given type of homologous surfactant and stationary phase.

The values of $q$ and $p$ were calculated for homologous series and various stationary phases and are listed in Table VII. Except for RGlu and RMal on Zorbax phenyl, the average value of $p$ is 0.95 (R.S.D. = 17%) as in Fig. 6, showing the small difference in the system. In contrast, the constants $q$ differ much more significantly from one another for different homologous series and stationary phases (R.S.D. *ca.* 40%). The difference in $q$ explains the apparent scattering of the plot in Fig. 7.

Fig. 8 illustrates (for alkylglycosides) similar selectivities to those already seen for both the octyl-bonded silicas. In contrast, Fig. 9 shows the influence of the organic modifier on changing the selectivity. In fact, we can observe a better separa-

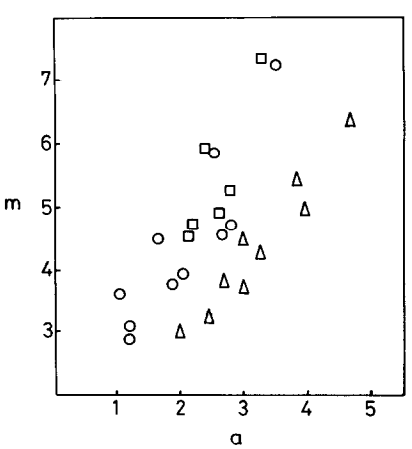


Fig. 7. Correlation between the slope $m$ and the intercept $a$ of eqn. 5 in acetonitrile modifier system. Symbols as in Fig. 5.

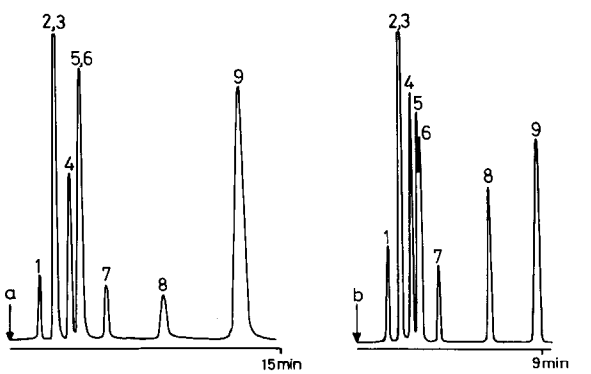


Fig. 8. Chromatograms of alkylglycosides. (a) Column, LiChrospher RP-8; mobile phase, acetonitrile–water (40:60); flow-rate, 1 ml $min^{-1}$. (b) Column, Zorbax $C_8$; mobile phase, acetonitrile–water (40:60); flow-rate, 1.5 ml $min^{-1}$; detector vaporization tube temperature, 45°C. Peaks: 1 = C6Glu; 2 = C8Gal; 3 = C8Glu; 4 = C8TGlu; 5 = C8Xyl; 6 = C10Mal; 7 = C10Glu; 8 = C12Mal; 9 = C12Glu.

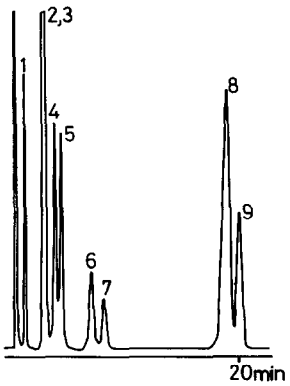


Fig. 9. Chromatogram of alkylglycosides. Column, LiChrospher RP-8; mobile phase, methanol–water (60:40); flow-rate, 1.5 ml $min^{-1}$. Detector conditions and peaks as in Fig. 8.

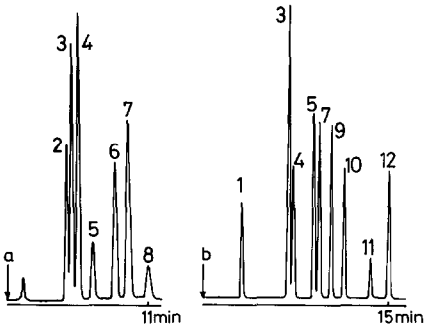


Fig. 10. Chromatograms of alkylglycosides. Column, LiChrospher RP-8. (a) Mobile phase, acetonitrile–water (30:70); flow-rate, 1 ml $min^{-1}$. (b) Linear gradient elution: acetonitrile–water from 25:75 to 55:45 in 15 min; flow-rate, 1 ml $min^{-1}$. Detector conditions as in Fig. 8. Peaks: 1 = C6Glu; 2 = C8Cell; 3 = C8Gal; 4 = C8Glu; 5 = C8TGlu; 6 = C8TCell; 7 = C8Xyl; 8 = C8Fuc; 9 = C10Mal; 10 = C10Glu; 11 = C12Mal; 12 = C12Glu.

tion between the C8Xyl–C10Mal pair with methanol–water (60:40) (Fig. 9), and the selectivity between C12Glu and C12Mal is higher with acetonitrile–water on LiChrospher RP-8 (Fig. 8).

On increasing the water content in the mobile phase, the C8Glu–C8Gal pair is separated. In fact, in acetonitrile–water (40:60) these compounds were not separated ($t_R$ = 2.84 and 2.72 min, respectively; Fig. 8a), whereas in acetonitrile–water (30:70) $t_R$ = 6.65 and 5.79, respectively (Fig. 10a). The same influence of increasing the water content on the resolution is illustrated by comparing Figs. 8a and 10b for C10Mal and C8Xyl.

Following Schoenmakers *et al.* [29], the value of $p$ determines the shape of the optimum gradient programme such that all solutes elute with the same peak width. When $0 < p < 1$, *i.e.*, with a methanol modifier, a convex gradient curve will be optimum. On the other hand, acetonitrile–water affords no correlation and therefore optimum gradients are linear. As our apparatus does not permit a convex gradient, an acetonitrile–water mobile phase with a linear gradient was chosen, resulting in good chromatograms with constant peak widths and a good distribution of peaks (Fig. 10). No baseline drift was observed during the gradient elution due to light-scattering detection. On comparing Fig. 10a and b, a higher retention of alkylthioglycosides is noted (see C8Cell and C8TCell and C8Glu and C8TGlu) and the retention increases as the number of hydroxyl group of the head polar decreases (see C8Cell, C8Glu, C8Xyl and C8Fuc).

## CONCLUSIONS

Reversed-phase liquid chromatography appears to be a good means of comparing the amphiphilic behaviour of non-ionic surfactants. The behaviour of alkylglycosides depends not only on the length of the alkyl chain, but also the nature of the glycoside and S- or O-bonding. A study by molecular modelling of the spatial configuration of these surfactants in water is in progress to elucidate this behaviour.

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