The Synthesis and Micellar Properties of a Novel Anionic Surfactant

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The synthesis is described of dodecyl β-D-glucopyranoside-6-phosphate, a novel surfactant possessing a long-chain hydrocarbon tail and a hydrophilic head consisting of a phosphoryl group covalently linked to a (homochiral) glucose moiety; this compound, which forms micelles at concentrations above 0.25 mmol dm^{-3}, has been successfully used in micellar electrokinetic capillary chromatography for the separation of a variety of analytes, including enantiomers.

Sodium dodecyl sulfate (SDS) is one of the best known anionic surfactants. It is characterised by a saturated hydrocarbon ($C_{12}H_{25}$) tail and a negatively charged sulfate head group. SDS has been extensively used in the analytical separation of a variety of molecules. For example the interaction of this surfactant with proteins leads to the formation of complexes with constant mass to charge ratios.[1,2] This property allows the resolution of a mixture of proteins by polyacrylamide gel electrophoresis (PAGE) and separations are conveniently related to the molecular mass of the native proteins.[3] At concentrations above 8 mmol dm^{-3}, the critical micelle concentration (cmc) of SDS, aggregation of a number of the amphiphilic surfactant molecules occurs. The micelles are usually spherical in nature with the hydrocarbon (hydrophobic) moiety of each constituent SDS molecule pointing inward and the ionic sulfate groups pointing outward into the aqueous solution. As both ionic and neutral molecules can interact with these micelles, SDS has been used in a number of separation techniques, including micellar liquid chromatography (MLC)[4,5] and micellar electrokinetic capillary chromatography (MECC).[6–8] The latter technique is one of the most popular modes of operation of capillary electrophoresis (CE).

Here we report the synthesis of a novel surfactant[9] **5** (Scheme 1), which contains a phosphate group covalently linked to a hydrocarbon tail *via* a chiral glucopyranoside residue. This surfactant aggregates to form micelles with lower cmc values than those measured for SDS under similar conditions. The solubility of **5** in aqueous solution is also far superior to that of lauryl phosphate, a closely related molecule. We show that chromatographic selectivity, including enantiomeric resolution, can be obtained using **5** in MECC.

The synthesis of **5** is summarized in Scheme 1. Dodecyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **2** and dodecyl-β-D-glucopyranoside **3** were prepared using methods outlined in the literature.[10] Selective *mono* phosphorylation of **3** was obtained by an initial reaction with dibenzyl disopropylamino phosphoramidite.

The yield of **4** is low (*ca.* 22%) but deprotection of **4** by catalytic hydrogenation under medium pressure for 24 h to yield the desired final product **5** in quantitative yield proceeded smoothly.

The cmc of **5** was determined in buffer (pH 7.0) solution by studying changes in the fluorescence properties (λ_{ex} 310 nm and λ_{em} 364 nm) of the phenanthrene derivative, halofantrine, with surfactant concentration.[12] As in the case of SDS the emission intensity of halofantrine is more intense in the presence of **5** than in buffer alone. The similarity in the behaviour of halofantrine in solutions of either **5** or SDS

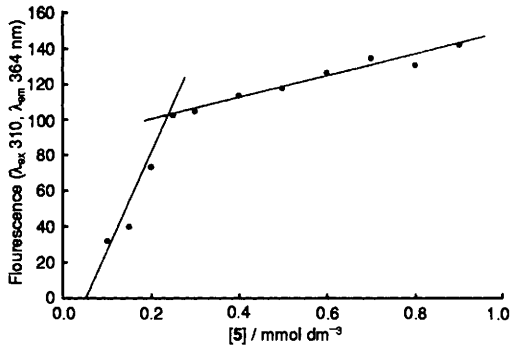


Fig. 1 Variation of the fluorescence intensity of halofantrine with concentration of **5**

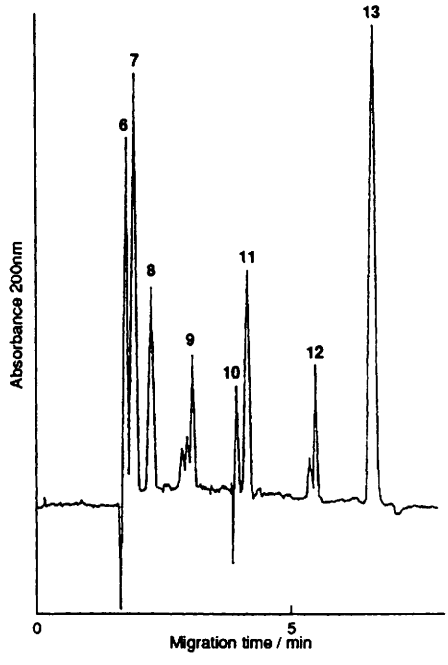
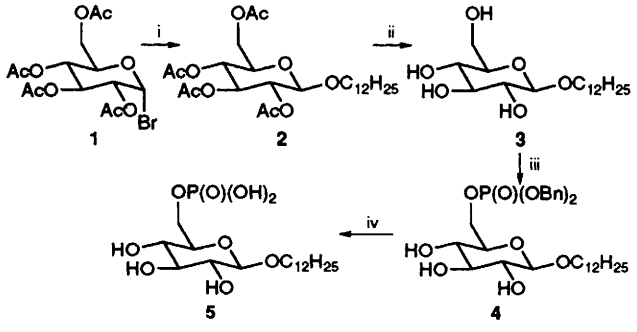


Fig. 2 Separation of a mixture of analytes by MECC. Conditions: 10 mmol dm^{-3} of **5** in 30 mmol dm^{-3} phosphate buffer (pH 7.0); applied voltage, 10 kV; capillary, 270 mm in length and 50 μm ID; temperature of capillary, 25 °C: **6**, mesityl oxide; **7**, nitrobenzene; **8**, toluene; **9**, *o*-, *m*-, and *p*-xylene; **10**, naphthalene; **11**, 1-nitronaphthalene; **12**, butylbenzene; **13**, halofantrine.



Scheme 1 *Reagents and conditions*: i, HgO_2/HgBr/*n*-dodecanol; ii, NaOMe/methanol; iii, 1*H*-tetrazole–dibenzyl diisopropyl phosphoramidate; iv, Pd/C, THF, NaOH

indicates that the hydrophobic moiety of this probe interacts with a similar environment in the interior of both types of micelle. Increased organisation of the halofantrine molecules within the micelle and decreased interaction with quenching species such as oxygen results in a higher intensity in the presence of surfactant.[13]

A plot of the variation of fluorescence intensity of halofantrine intensity with concentration of **5** is shown in Fig. 1. The point of intersection of the two straight line plots gives an estimate of the cmc of **5** as 0.25 mmol dm^{-3}. This value is of the same order of magnitude as that (0.20 mmol dm^{-3}) for lauryl phosphate and 10 to 15 times lower than values reported[12,14] for SDS under similar buffer conditions.

SDS at concentrations above its cmc is a widely used surfactant in MECC. Under the influence of an electric field the electrophoretic migration of the negatively charged micelle is in the direction of the anode. At neutral and alkaline pH electroosmotic flow, towards the cathode, is strong. In fact under these conditions the electroosmotic mobility of the bulk solution is higher than the electrophoretic mobility of the micelles, leading to an overall migration of the micelles in the direction of the cathode. The presence of the micelles allows resolution of neutral analytes due to differences in distribution behaviour between the aqueous and micellar phases. In the absence of micelles all neutral molecules migrate with the same mobility, under the influence of the electroosmotic flow only.

Fig. 2 shows the resolution of a mixture of eight neutral molecules by MECC, using a 10 mmol dm^{-3} solution of **5** in a pH 7 buffer. The analysis time is less than 7 min. The order of elution of molecules **6** to **12** is closely related to their octanol–water partition coefficient,[15] strongly indicating that the hydrophobic interaction of these molecules with the micelles is the principal mechanism leading to resolution. Halofantrine **13** is last to elute. The positive charge on this molecule allows it to interact by both hydrophobic and ionic mechanisms. These properties, together with its intense UV absorbance, make halofantrine a useful marker for the migration of the micelles.[12]

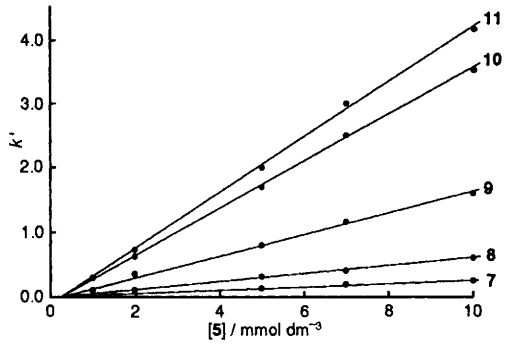


Fig. 3 Plots of k' *vs* the concentration of **5** for the analytes in Fig. 2

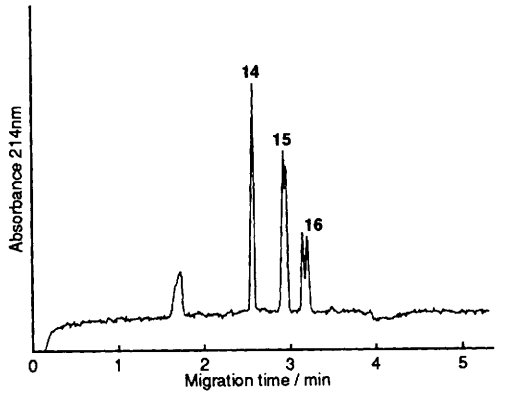


Fig. 4 Separation of a mixture of three dansyl (Dns) amino acids. Electrophoretic conditions are the same as in Fig. 2: **14**, D,L-Dns-Val; **15**, D,L-Dns-Phe; **16**, D,L-Dns-Trp.

We also attempted MECC studies using lauryl phosphate (Lancaster Chemicals) as the surfactant. These experiments were not successful as it was not possible to solubilise this compound in aqueous buffer solutions at concentrations above about 5 mmol dm^{-3}. In contrast we were able to dissolve **5** at concentrations above 100 mmol dm^{-3}.

The relationship between the retention factor k' in MECC and the total surfactant concentration [S] is given by eqn. (1),

$$k' = P_{mw} V \{[S] - \text{cmc}\} \quad (1)$$

where P_{mw} is the partition coefficient of the neutral solutes between the aqueous medium and the micelles and V is the molar volume of the surfactant. Fig. 3 shows the linear relationship obtained from a plot of k' *versus* the total concentration of **5** for a number of neutral compounds. All lines intersect the x-axis at very nearly the same point and the slopes of the lines increases with the hydrophobicity of the compounds. At a value of k' equal to zero the concentration of **5** is equal to its cmc. As shown in Fig. 3 this value is of the same order of magnitude (about 0.25 mmol dm^{-3}) as that obtained from the fluorescence studies (Fig. 1).

As **5** contains the chiral glucopyranosyl moiety, we also attempted to resolve pairs of enantiomers. Fig. 4 shows the separation of three racemic dansyl derivatives of valine **9**, phenylalanine **10** and tryptophan **1**. As in the case of the analytes in Fig. 2 the order of elution of these dansyl derivatives is related to the hydrophobicity of the molecules. Separation is fast and chiral resolution is obtained for **10** and **11**. This result is of much interest as it is one of very few examples where cyclodextrins have not been included in the buffer solution to achieve the resolution of enantiomers.[6,17]

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