Physico-chemical properties of a novel surfactant derived from 6-aminopenicillanic acid and its use in capillary electrophoresis for chiral discrimination

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A novel surfactant based on 6-aminopenicillanic acid is synthesised in good yield, and its physico-chemical properties, such as the critical micellar concentration and the mean aggregation number are determined using fluorescence and capillary electrophoretic methods; the utility of these surfactants for chiral discrimination in micellar electrokinetic capillary electrophoresis is demonstrated.

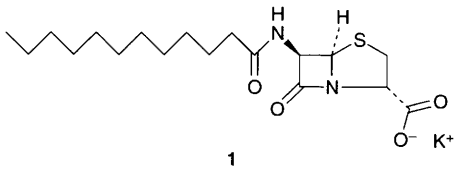
As the commercialisation of racemic mixtures of novel drugs is only acceptable in exceptional circumstances, the discrimination of chiral compounds is one of the most active and challenging areas of chemical analysis. To date, chiral high performance liquid chromatography (HPLC) has been the preferred method for the analysis of racemic mixtures[1] and for the quantification of amounts (<0.1%) of minor enantiomeric impurities.

Compared to HPLC and other commonly used analytical methods [*e.g.* thin layer chromatography (TLC) and supercritical fluid chromatography (SFC)], capillary electrophoresis (CE) is a relatively recent analytical technique.[2] CE has been demonstrated to be a powerful chiral discrimination tool in its free-zone mode and in micellar electrokinetic capillary electrophoresis (MECC).[3]

A variety of chiral discriminating agents have been added to the separation buffer for the resolution of chiral analytes. Native and derivatised cyclodextrins have been the most widely used additives.[4–6] Other naturally occurring neutral[7] and ionic[8] polysaccharides, such as maltodextrin and the sodium salt of chondroitin sulfate C, have been employed as chiral selectors in CE. Recently we have been successful in using anionic glucose-based surfactants for the resolution of a variety of racemic mixtures[9] in MECC. Other synthetic chiral surfactants used in this CE mode have been derived from a number of D- and L-amino acids.[10,11]

Here we report the synthesis, physico-chemical properties and the use of the anionic surfactant **1** in MECC for the resolution of racemic mixtures. The potassium salt of **1** is produced in good yield (unoptimised yield *ca.* 60%) from the reaction of lauroyl chloride with 6-aminopenicillanic acid, an intermediate of widely prescribed antibiotics such as ampicillin and amoxicillin.

The critical micelle concentration (cmc) of **1** was measured by following the variation of the fluorescence intensity of *N*-phenyl-1-naphthylamine with concentration of surfactant. As shown in Fig. 1, the intensity of the fluorescent probe falls off after a concentration of about 3 mmol dm^{-3} surfactant is reached. This behaviour is unusual[12] and indicates that the formation of micelles leads to trapping of the probe followed by quenching of the singlet state. From this study it appears that the cmc of **1** is of the order of 3 mmol dm^{-3}. This value was confirmed by measuring the current in narrow-bore (50 μm) capillaries flushed with buffer solutions containing increasing concentrations of **1** (data not shown).[9] This cmc is about 2–3 times lower than that reported for sodium dodecyl sulfate.[12]



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The mean aggregation number, *N*, of **1** was measured by the method of Turro and Yekta.[13] This method involved monitoring the fall in the fluorescence intensity of $Ru(bipy)_3^{2+}$ [S] with increased amounts of a quencher (9-methylanthracene) [Q] and in the presence of 20 mmol dm^{-3} surfactant (a concentration above the cmc). The excitation and emission wavelengths used in these experiments were 450 and 630 nm, respectively. A plot of the ratio of fluorescence intensity in the absence and presence of quencher against [Q]/[S] gives a straight line (r = 0.993) from the slope of which N = 59 was determined for **1**.

The surfactant properties of **1** and the presence of three stereochemically defined chiral centres make it an ideal candidate as a chiral discriminating agent in MECC. The addition of **1** to the separation electrolyte at concentrations above its cmc has allowed the resolution of a number of racemic mixtures which are electrically neutral under the conditions of analysis. Fig 2. shows electropherograms† of the anti-coagulating agent, warfarin and four of its hydroxy derivatives, the anticonvulsant drug mephenytoin and its major metabolite hydroxymephenytoin, and the antihypertensive drug, cromakalin.

The 7-hydroxy derivative of warfarin has been included in Fig. 2(*a*) and (*b*) to allow comparison of migration times. Thus migration follows the order: 4′-hydroxy, followed by 6-hydroxy, the parent compound, and 7-hydroxy. Although the introduction of a hydroxy group in warfarin is expected to decrease its hydrophobicity (shorter migration time),[14] migration of these closely related analytes is to a great extent dependent on the position of the hydroxy substituent. This is a good indication that migration is affected not only by differ-

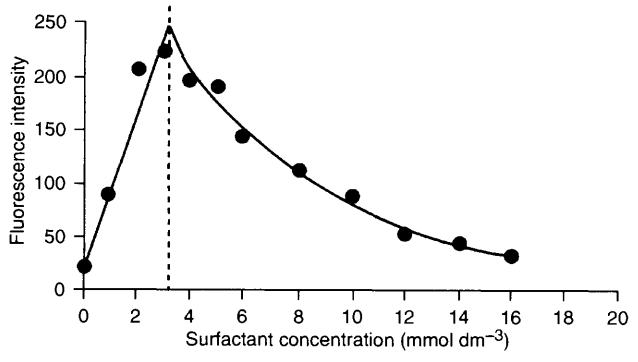

Fig. 1 Variation of the fluroescence of *N*-phenyl-1-naphthylamine with different concentrations of **1**. The dotted line shows the concentration of **1** equivalent to its cmc.

ences in the free energy of partitioning of these analytes across micelles but also by electronic interaction with the hetero atoms

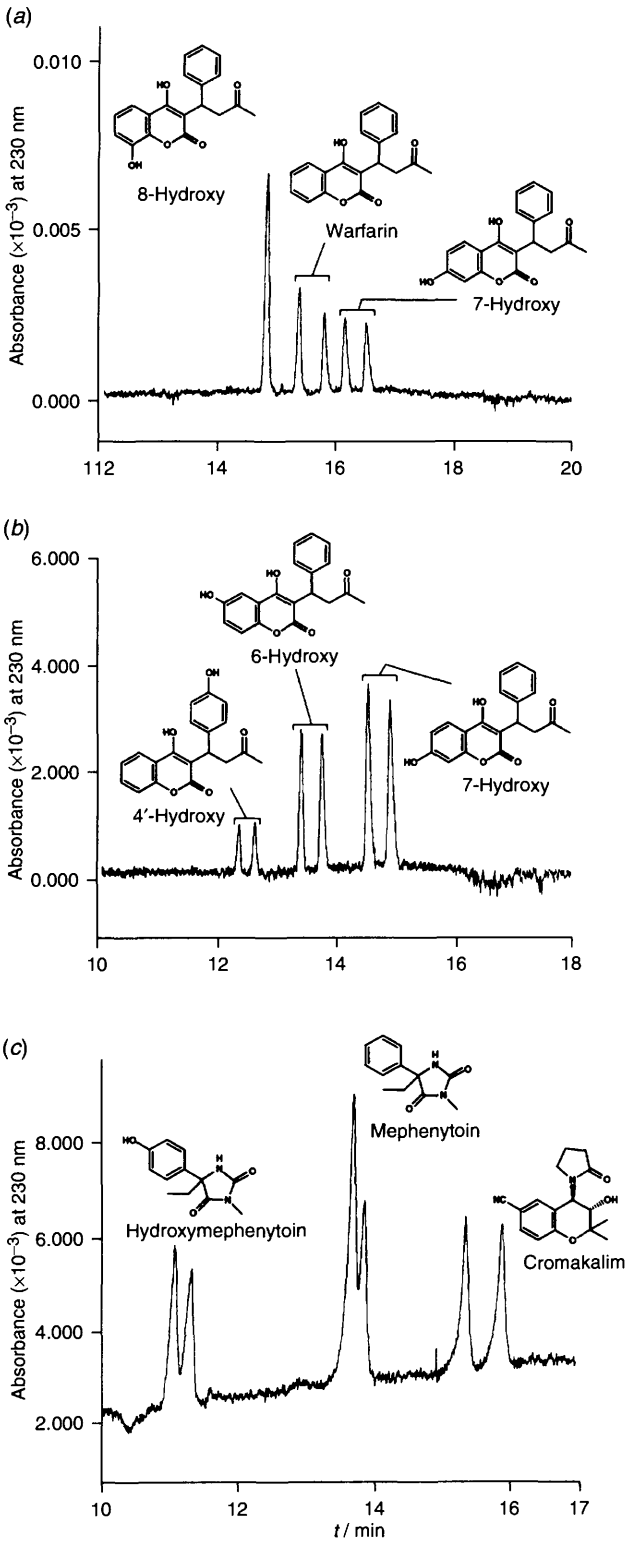

Fig. 2 Electropherograms showing the racemic resolution of (*a*), (*b*) hydroxy derivatives of warfarin; and (*c*) cromakalim, mephenytoin and hydroxymephenytoin

in the penicillanic acid moiety of **1**. Such interactions are also very selective so that the racemic mixture of the 8-hydroxy derivative of warfarin is not resolved; it is possible that in this case intramolecular hydrogen bonding between the hydroxy group and the lactone moiety disrupts intermolecular interactions with the chiral head of the surfactant.

The order of migration of cromakalim and meophenytoin is directly related to the hydrophobicity of these molecules. The measured values of the logarithms of the octanol/water partition coefficients (*P*) of these drug substances are 2.32 and 1.74, respectively.[14] For comparison, log *P* for warfarin is 2.52, so that it is not surprising that the migration of this drug is close to that of cromakalim. The introduction of a hydroxy group into mephenytoin makes the resulting molecule more hydrophilic, again in agreement with the shorter migration order of this derivative. However, the improved resolution of the enantiomers of hydroxymephenytoin compared to the parent molecule may be due to favourable interactions of the hydroxy group with the chiral surfactant, similar to the case of the hydroxy derivatives of warfarin.

Chiral surfactants can be designed which can be successfully used as buffer additives in capillary electrophoresis for the chiral resolution of drug-related recemic mixtures. The successful outcome of this preliminary work has encouraged us to embark on a program aimed at the synthesis of a variety of other β-lactam surfactants useful for chiral discrimination.

Footnote

† Electrophoresis was carried out using a Beckman P/ACE 2100 system. The lengths of the fused-silica capillary (i.d. 50 μm) from anode to detector and from detector to cathode were 50 and 7 cm, respectively. Samples, dissolved in 50% isopropyl alcohol–water, were introduced into the anodic end of the capillary by applying a pressure of 20 psi for 1–2 s. The analysis was carried out at 25 °C. A voltage of 15 kV was used. The separation electrolyte consisted of 40 mmol dm^{-3} surfactant in a solution made up of a mixture of buffer (10 mmol dm^{-3} boric acid adjusted to pH 7 with 0.1 mol dm^{-3} KOH) and isopropyl alcohol in the ratio of 97:3. Data were analysed using Beckman Gold Software.

References

1 S. G. Allenmark, *Chromatographic Separation: Methods and Applications*, Ellis Horwood, Chichester, 1988.
2 W. G. Kuhr, *Anal. Chem.*, 1990, **62**, 403.
3 P. Camilleri and G. N. Okafo, in *Capillary Electrophoresis: Theory and Practice*, ed. P. Camilleri, CRC Press, Boca Raton, FL, 1993, ch. 5.
4 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 1988, **438**, 211.
5 A. Nardi, L. Ossicini and S. E. Fanali, *Chirality*, 1992, **4**, 56.
6 K. H. Gahm and A. M. Stalcup, *Anal. Chem.*, 1995, **67**, 19.
7 D. Hulst and N. Verbeke, *J. Chromatogr.*, 1992, **608**, 275.
8 H. Nishi, K. Nakamura, H. Nakai and T. Sato, *Anal. Chem.*, 1995, **67**, 2334.
9 D. C. Tickle, G. N. Okafo, P. Camilleri, R. F. D. Jones and A. J. Kirby, *Anal. Chem.*, 1994, **66**, 4121.
10 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 1985, **57**, 834.
11 V. de Biasi, J. Senior, J. A. Zukowshi, R. Curtis Haltwanger, D. S. Eggleston and P. Camilleri, *J. Chem. Soc., Chem. Commun.*, 1995, 1575.
12 P. Camilleri and G. N. Okafo, *J. Chem. Soc., Chem. Commun.*, 1992, 530.
13 N. J. Turro and A. Yekta, *J. Am. Chem. Soc.*, 1978, **100**, 5921.
14 M. Adlard, G. Okafo, E. Meenan and P. Camilleri, *J. Chem. Soc., Chem. Commun.*, 1995, 2241.

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