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Synthesis and use of novel chiral surfactants in micellar electrokinetic capillary chromatography☆

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Abstract

This paper presents the synthesis of some novel chiral surfactants based on (*R*,*R*)-tartaric acid and long-chain aliphatic amines. Evidence for their ability to form micelles based on surface tension measurements will be given. Several chiral separations have been achieved and these will be presented for one of the surfactants. A discussion of the chiral selectivity data will show a preference for resolving certain compounds of a similar structure. Ideas for further work with these surfactants will be outlined.

1. Introduction

In 1981 Jorgenson and Lukacs [1] described the use of small-diameter capillaries in the electrophoretic separations of derivatised amino acids and amines. The small diameters of the capillaries enabled efficient heat dissipation and consequently resulted in highly efficient separations. The technique was named capillary electrophoresis (CE) and proved useful for the simultaneous separation of positively and negatively charged species. For the separation of neutral species this technique was modified by Terabe et al. [2], who added surfactants to the background electrolyte (BGE). The surfactants act as a pseudo-stationary phase and the resulting separation has characteristics of both an electrophoretic and chromatographic nature. Terabe et al. named their modified technique micellar electrokinetic capillary chromatography (MEKC or often abbreviated as MECC). The most common additive used in MECC to date is sodium dodecyl sulfate (SDS), but since it is achiral it cannot differentiate between enantiomers.

To achieve chiral discrimination either a chiral surfactant is required or some chirality must be imparted into the micelle. This second option has been achieved by the formation of mixed micelles, i.e., a chiral compound has been added to an SDS solution and is included into the SDS micelle, thus forming a so-called mixed micelle which will have some chiral selectivity.

Compounds that have been successfully added in this manner include digitonin [3], and sodium N-dodecanoyl-L-valinate [3,4]. Digitonin is a

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non-ionic compound which incorporates into the SDS micelle; this effectively results in a chiral ionic micelle. Sodium N-dodecanoyl-L-valinate has been used in a mixed micelle system containing SDS. These approaches resulted in good separation of phenylthiohydantoin-derivatised amino acids [3].

Relatively few chiral surfactants have so far been used in the absence of SDS in MECC. By far the most widely used chiral surfactants are the bile salts [5,6] which have a steroidal backbone and form helical rather than spherical micelles. Sodium N-dodecanoyl-L-valinate has also been used on its own as a chiral micellar agent. [3] The synthesis of novel chiral surfactants would widen the number and scope of applications for this technique.

The work presented describes the preparation and use of two novel chiral surfactants based on (*R*,*R*)-tartaric acid and long-chain amines. Details of the synthetic methods used will be given, as will evidence of micelle formation. Their use in chiral discrimination will be demonstrated by showing the enantiomeric separation of a number of compounds. Some ideas as to structures likely to be best separated will be proposed. Further work with these and other surfactants will also be outlined.

2. Experimental

2.1. Synthesis of surfactants

Chemicals

(*R*,*R*)-Tartaric acid (99+%), $C_{10}H_{21}NH_2$ (97%) and $C_{12}H_{25}NH_2$ (98%) were purchased from Janssen Chimica (Cheshire, UK), acetic anhydride (99%) was supplied by Aldrich (Milwaukee, WI, USA); dichloromethane was of GLR grade, and ethyl acetate (99%) was obtained from Fisons Laboratory Supplies (Loughborough, UK). Sodium bicarbonate (99.5%) and magnesium sulfate were purchased from Vickers (Watford, UK). Aqueous solutions were prepared using glass-distilled water.

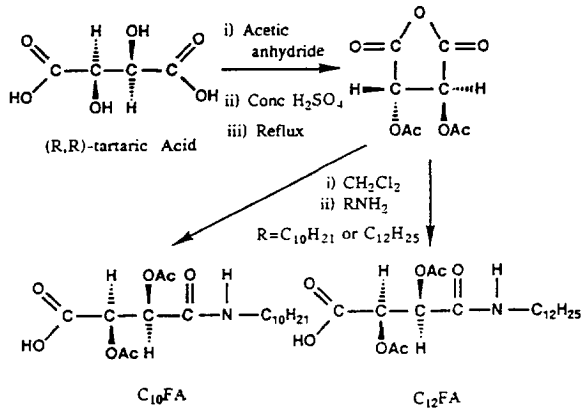

Fig. 1. Reaction scheme 1.

Procedure

Reaction scheme 1 (Fig. 1). (*R*,*R*)-Tartaric acid was refluxed with acetic anhydride in the presence of sulfuric acid for 40 min. On cooling the acetylated anhydride precipitated out. This was filtered and washed with portions of toluene followed by ether, giving a white crystalline material with a melting point of 129–131°C (Ref. [7] gives a value of 130–132°C). This material was dissolved in dichloromethane and a slight excess of amine was added slowly while stirring. After two hours the dichloromethane was removed under vacuum and the residue was dissolved in ethyl acetate and washed with aliquots of a saturated sodium bicarbonate solution until effervescence ceased; the organic layer was then discarded. The aqueous/bicarbonate layer was then acidified to pH 1 and the precipitated material was re-extracted back into ethyl acetate, dried with magnesium sulfate, filtered and the solvent removed under vacuum, leaving the pure free acid, in most cases as a very sticky/hard gum. Typical elemental analysis results are given below. $C_{18}H_{31}NO_7$ ($C_{10}FA$); calculated: C, 57.9; H, 8.3; N, 3.8%; found: C, 57.8; H, 8.0; N, 3.9%. $C_{20}H_{35}NO_7$ ($C_{12}FA$); calculated: C, 59.9; H, 8.7; N, 3.5%; found: C, 59.9; H, 8.8; N, 3.5%.

Reaction scheme 2 (Fig. 2). The carboxylic acid was dissolved in methanol and a slight molar deficiency of sodium methoxide was added: the mixture was left stirring overnight. The methanol

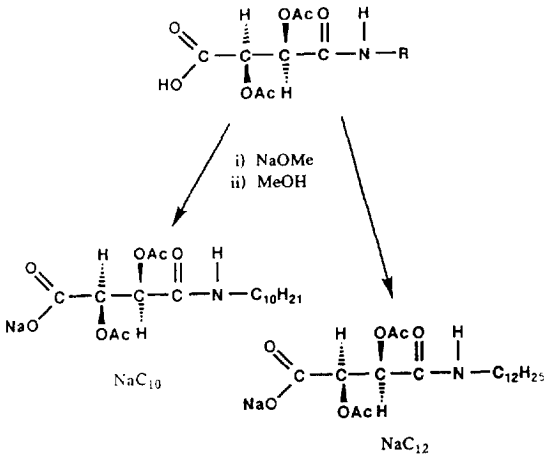

Fig. 2. Reaction scheme 2.

was removed under vacuum, leaving a sticky white residue, which was dissolved in ethyl acetate. After allowing this solution to stand overnight the salt precipitated. It was filtered off and dried under vacuum. Typical elemental analysis results for the salts are given below. $C_{18}H_{30}NO_7Na$ (NaC_{10}); calculated: C, 54.7; H, 7.6; N, 3.5; Na, 5.8%; found: C, 54.4; H, 7.9; N, 3.7; Na, 5.6%. $C_{20}H_{34}NO_7Na$ (NaC_{12}); calculated: C, 56.7; H, 8.0; N, 3.3; Na, 5.4%; found: C, 56.8; H, 8.2; N, 3.3; Na, 5.4%.

2.2. Determination of critical micelle concentration (cmc) by surface tension measurements

Measurements were carried out on a Cruss Model K12 processor tensiometer (Hamburg, Germany) using a Wilhelmy plate. All solutions were thermostatically maintained at 25°C prior to and during analysis. Between each solution the plate was rinsed with deionised water, flame dried and then allowed to cool to room temperature.

A series of solutions of known concentration were prepared in deionised water. A plot of surface tension versus surfactant concentration enabled interpolation of the critical micelle concentration.

2.3. Electrophoresis

A Beckman P/ACE 2050 instrument (Fullerton, CA, USA) was used with UV detection at 254 nm. An uncoated fused-silica capillary with dimensions of 57 cm × 50 μm I.D. was used throughout; the detection window was located 50 cm from the inlet end. Injection was achieved by the use of an overpressure of nitrogen (0.13 MPa) for 1 s in all cases. The electrophoretic medium consisted of 20 m*M* di-sodium hydrogen phosphate at pH 8.2 and 5 m*M* NaC_{12}, and the applied voltage was 20 kV.

3. Results and discussion

3.1. Critical micelle concentrations

Many methods for determining the critical micelle concentration (cmc) of a surfactant are available, such as laser light scattering, conductivity and surface tension [8]. Surface tension is one of the oldest and most widely used techniques and was used in this work, leading to the plots of surface tension versus surfactant concentration shown for NaC_{10} in Fig. 3 and for NaC_{12} in Fig. 4.

By the interpolation shown on the graph in Fig. 3 the cmc of the surfactant NaC_{10} was determined to be 2.6 m*M*, whereas from Fig. 4 the cmc of the surfactant NaC_{12} was determined as 2.4 m*M*.

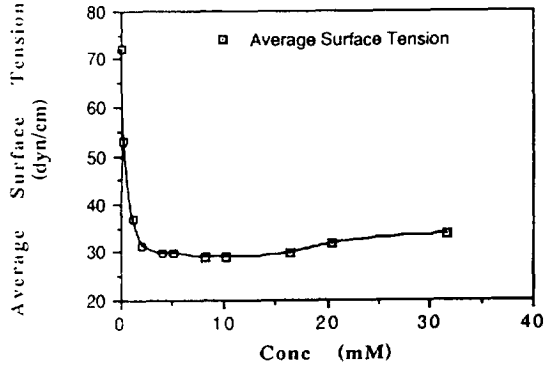

Fig. 3. Surface tension versus NaC_{10} concentration.

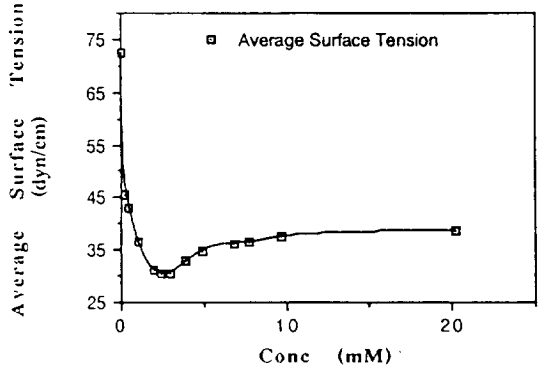

Fig. 4. Surface tension versus NaC_{12} concentration.

In this case the minimum of the plot in Fig. 4 was used due to the fact that interpolation was difficult with this shape of curve. It must be borne in mind that these determinations are only approximate. The shape of the graph in Fig. 4 is indicative of impurities in the surfactant [9], which further complicates the determination. The cmc's of the two salts are very close, which is a little surprising as a change in alkyl chain length usually has an appreciable effect on the cmc. For example, the cmc of SDS is 8.1 m*M* while that of sodium decyl sulfate is 38.8 m*M* [8].

When making up the solutions for analysis the salts were assumed to have 100% purity. Even if this is not the case the value obtained could be used to obtain micelles provided the concentration is maintained above the cmc. Both graphs clearly indicate that the synthesised salts are surfactants that are capable of forming micelles at concentrations of over 3 m*M*.

3.2. Electrophoresis

The percentage separation factor was calculated as $100 \times (p/h)$ (Fig. 5). Baseline separations are obtained as the separation factor approaches 100. (Note: recent guidelines [10] indicate that the term separation factor should be restricted to α, where $\alpha = k'_2/k'_1$, with k' the capacity factor.)

The percentage separation factors quoted in Table 1 were achieved using near-identical conditions (different batches of capillary were used)

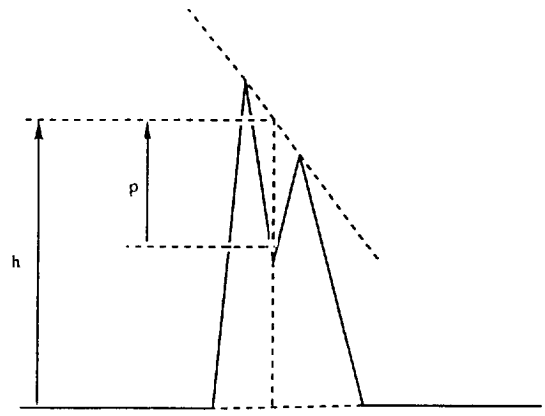

Fig. 5. The calculation of percentage separation factors.

for each of the analytes. The different sample concentrations reflect differences in the UV absorbance characteristics.

It is well established that compounds include into micelles to different extents depending upon their hydrophobicity. In the present work compounds with fused polyaromatic rings appear to separate more easily (Figs. 6–9) than those with only a single aryl group (see compounds 1, 4 and 5 compared with compounds 3 and 6, Figs. 10). A possible reason for this is that separation arises as a result of stronger inclusion within the micelle, promoted by the fused polyaromatic nucleus of the analyte. However, interactions at other points of the molecule are equally important, as demonstrated by compounds 5 and 7 (Fig. 10). Separation is achieved due to very slight differences in stability of the complex

Table 1
Calculated percentage separation factors for compounds 1–8 (Fig. 10)

Compound number	Concentration (mg ml^{-1})	Percentage separation factor
1	0.20	86
2	0.06	34
3	0.50	12
4	0.25	90
5	0.06	72
6	all concentrations	0
7	all concentrations	0
8	all concentrations	0

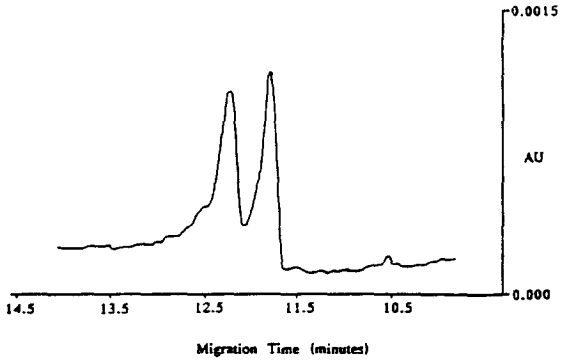

Fig. 6. Enantiomeric separation achieved for compound 1.

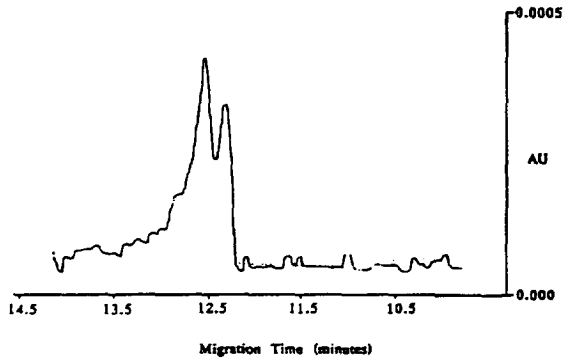

Fig. 8. Enantiomeric separation achieved for compound 4.

formed between each enantiomer and the chiral medium.

4. Conclusions

Some novel chiral surfactants have been shown to be applicable to enantiomeric separations in MECC. As yet the mechanisms involved in the separation process are unclear and further work will be carried out to elucidate them. Measurements of change in surface tension with surfactant concentration have proved to be a convenient method for the determination of critical micelle concentrations. The position of the chiral centre within the micelle may have an important effect on separations, and to investi-

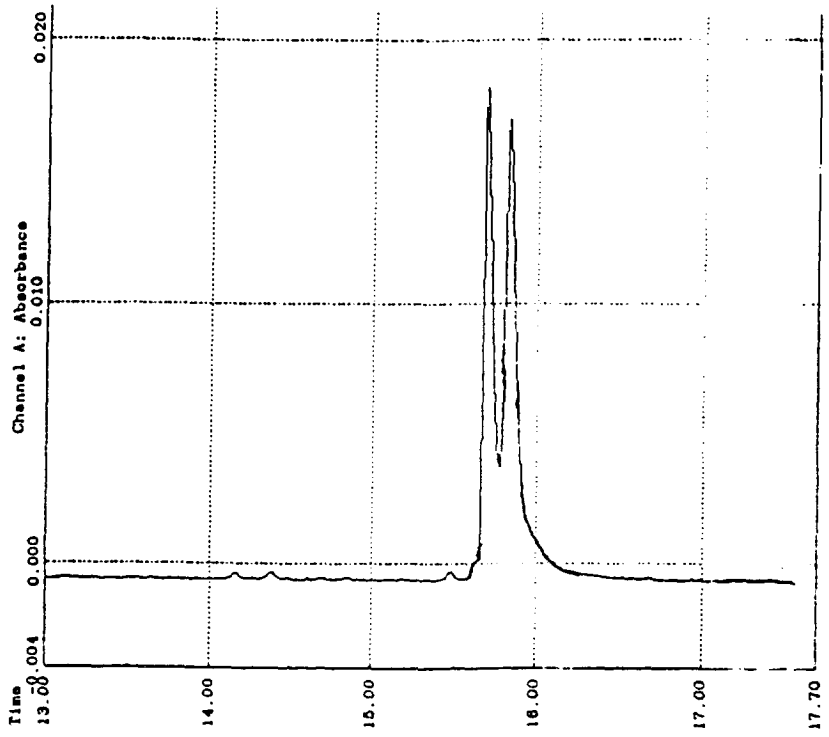

Fig. 7. Enantiomeric separation achieved for compound 2.

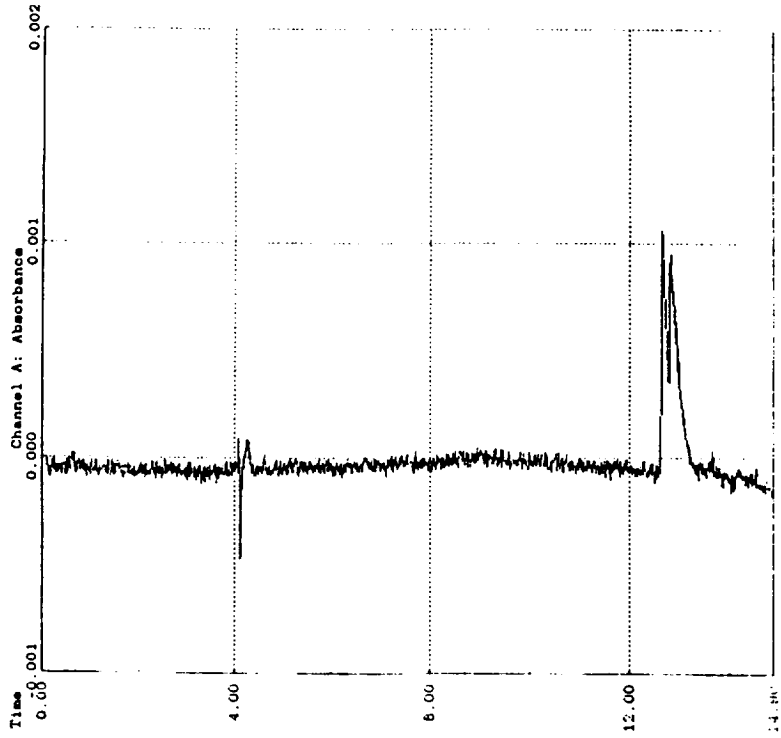

Fig. 9. Enantiomeric separation achieved for compound 5.

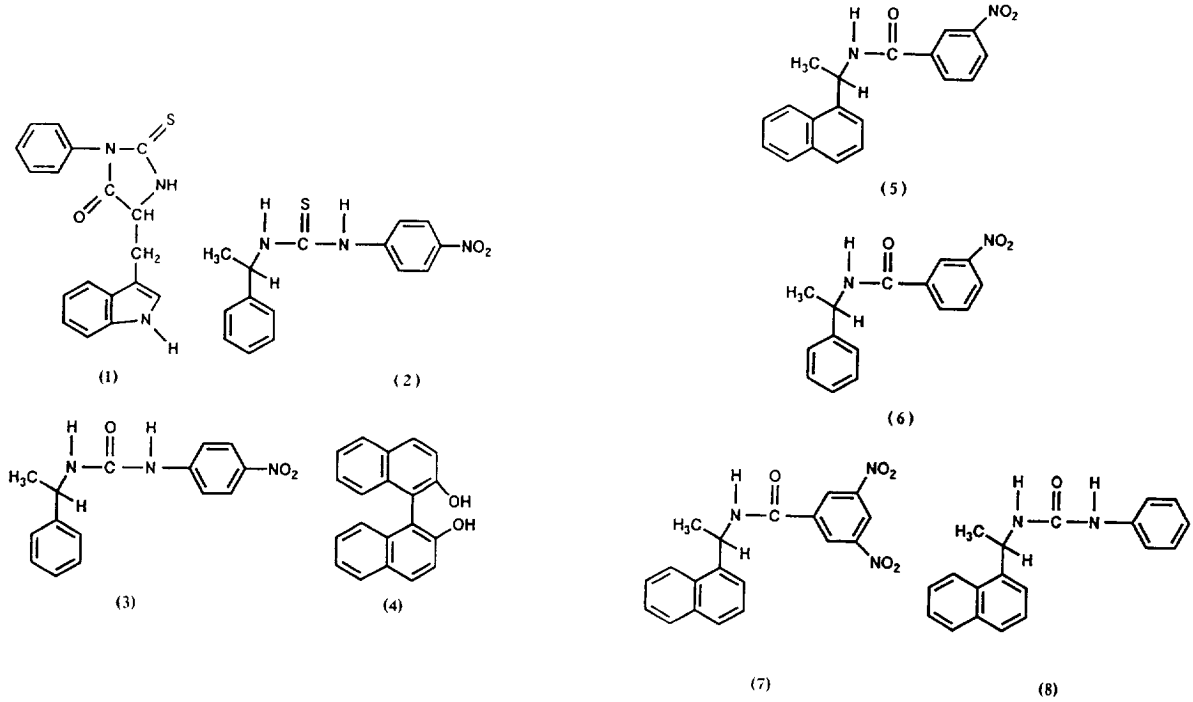

Fig. 10. Some analyte structures examined.

gate this some more novel chiral surfactants are currently being designed and synthesised.

Acknowledgements

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