Chiral Discrimination in Capillary Electrophoresis using Novel Anionic Surfactants related to Cysteine

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Two novel cysteine-based, anionic, chiral surfactants have been designed and prepared from readily available starting materials; these molecules were successfully used as chiral selectors in micellar electrokinetic capillary chromatography (MECC).

Over half of the commonly prescribed drugs contain at least one chiral or optically active centre. This situation is becoming more common with drugs which are currently being developed by several pharmaceutical companies. It is now widely agreed that, in the majority of cases, it is no longer acceptable to commercialise a racemic drug mixture. Thus it is important to know the stereochemical composition of a drug substance and to determine the pharmacologic, toxic and clinical properties of one enantiomer in the absence of its antipode.

Rapid advances in the area of stereoselective synthesis have been equalled by the development of a number of chiral high performance liquid chromatography (HPLC) methodologies, able to resolve a variety of enantiomeric mixtures. More recently, capillary electrophoresis (CE) has proved to be complementary to HPLC even in the area of chiral discrimination.[1]

The majority of chiral separations carried out by CE have involved the addition of commercially available cyclodextrins and their alkylated derivatives to the electrophoresis buffer.[2,3] Examples of other chiral selectors are bile salts,[4] proteins[5] and cyclodextrins combined with sodium dodecyl sulfate (SDS) micelles.[6] As the majority of these chiral complexing agents are naturally occurring, they exist as one stereoisomeric form. This property can restrict their analytical application. The synthesis and use of chiral, functionalised micelles can be of great value for the separation of enantiomers.[7,8] In the first instance molecules can be designed where interaction with chiral analytes can be optimised by altering the nature of the charged chiral 'head' and the length of the hydrophobic 'tail'. Moreover, the availability of both the D- and L-isomeric forms of the parent molecules of these surfactants can be very desirable especially when reversal of the order of migration allows a more accurate estimate of a low level enantiomer impurity.

In this communication we report the stereoselective synthesis and physicochemical characterisation of **1**. We have also shown that stereoisomers of **1** form micelles which are effective chiral discriminators in micellar electrokinetic capillary chromatography (MECC).

The (2*R*,4*R*) and (2*S*,4*S*) diastereoisomers of **1** were prepared in two steps. Dodecylaldehyde was first reacted with either L- or D-cysteine hydrochloride. The resulting 2-undecyl-4-thiazolidine carboxylic acid was stereoselectively *N*-acetylated on reaction with acetic anhydride in aqueous basic media using the procedure of Oya *et al.*[9] Optical rotations for the two diastereoisomers, identified as **1a** and **1b**, were determined in methanol as $[\alpha]_D^{25}$ +21.8 and −21.2, respectively.

The crystal and molecular structures of the (2*R*,4*R*) stereoisomer **1a** were determined from three-dimensional X-ray diffraction data collected at reduced temperature.† The com-

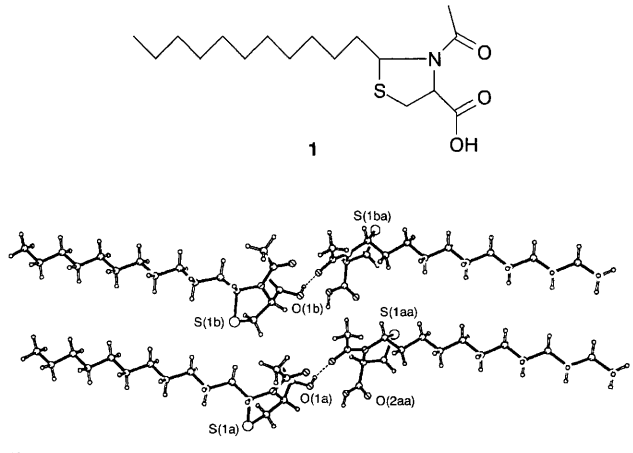

Fig. 1 A view of the two independent molecules of **1a** from the crystal structure. Thermal displacement ellipsoids for non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius. The two unique, crystallographically independent molecules are indicated with the letters a and b and the two symmetry related molecules to which they form hydrogen bonds (dashed lines) through their carboxylic acids are designated by codes aa and ba.

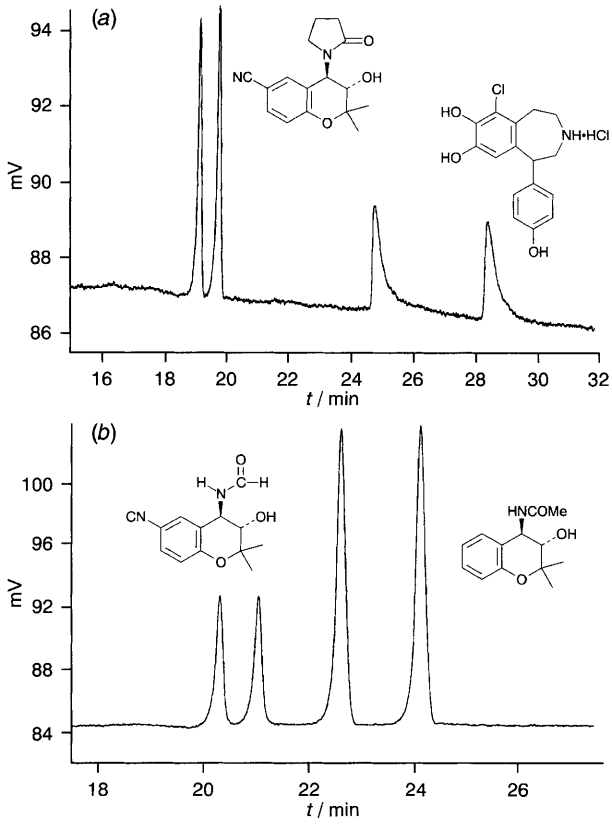

Fig. 2 Electropherograms of racemic mixtures of (*a*) cromakalin and fenoldopam and (*b*) two cromakalin-related molecules using **1a** (25 mmol dm^{-3}) in the separation buffer. *Conditions*: Fused-silica capillary, 670 mm × 50 μm; 50 mmol dm^{-3} borate–25 mmol dm^{-3} phosphate buffer, pH 7.0; 20 °C; voltage, 12.5 kV; sample, 3s pressure injection of a solution *ca.* 1 × 10^{-4} mol dm^{-3} in water; detection wavelength, 254 nm.

pound crystallises (colourless plates from acetone) with two independent molecules in the asymmetric unit as shown in Fig. 1. Each molecule is of identical configuration and virtually identical conformation. The absolute configuration at the cysteine C_α is (*R*), in agreement with the preparation of this compound from the naturally occurring L-amino acid. The configuration at the second chiral centre also is (*R*). Other interesting structural features of these molecules are a fully extended all-*trans* conformation of the hydrocarbon chain, the hydrophobic sheet formed by these chains within the crystal and the intermolecular hydrogen bonds formed by the carboxylic acid groups (Fig. 1). Interestingly, these hydrogen bonds are not of the carboxylic acid dimer type which might have been expected; unusually, both acids hydrogen bond to the acetyl amide carbonyls.

Both **1a** and **1b** show surfactant properties at neutral or alkaline pH. The critical micelle concentration (c.m.c.) of these molecules was determined in phosphate–borate buffer (pH 7) by measuring current at different concentrations of these compounds, using a procedure we outlined recently.[8] For both anionic surfactants a break in the concentration-current profile, related to c.m.c. occurs at a concentration of about 3.5 mmol dm^{-3}. This value is about half that reported for SDS under similar pH and buffer conditions.[10]

The addition of either **1a** or **1b** to the separation buffer, at concentrations above their c.m.c., produces a chiral environment in capillary electrophoresis, suitable for the resolution of the enantiomers of a number of drug molecules. Figs. 2(*a*) and (*b*) show the resolution of racemates related to cromakalim, an anti-hypertensive drug, and fenoldopam, a drug which stimulates renal dopamine D_1 receptors. These chiral separations are baseline and are quite suitable for the measurement of low level enantiomeric impurities.

The synthesis of **1** in two stereoisomeric forms gave us the opportunity to test the possibility of reversing the migration order or two enantiomers in a racemic mixture. This facility is highly desirable when a quantitative estimate is needed of a low level enantiomer impurity, which follows the main antipode. Separation of the enantiomers of cromakalim using the same concentration of either **1a** or **1b** in the separation buffer is shown in Fig. 3. As expected reversal of the order of migration of the (3*R*,4*S*) and the (3*S*,4*R*) enantiomers of the drug occurs depending on which surfactant is included in the electrophoresis buffer system. These data confirm the complementary properties of **1a** and **1b** and the potential advantages of carrying out chiral separations using these two individual surfactants. At present we are carrying out molecular modelling studies to provide insight into the mechanism of stereoselective interaction between these anionic surfactants and chiral analytes. We are also designing other surfactants to provide chiral environments suitable for the resolution of a wide range of racemic mixtures.

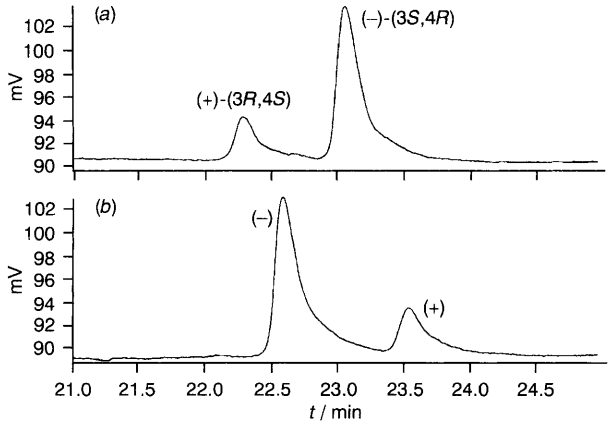

Fig. 3 Electropherograms of a *ca.* 1:4 mixture of the enantiomers of cromakalin using either **1a** or **1b** in the separation buffer. Conditions are the same as in Figure 2, except that a different capillary was used. Small differences in migration time of the individual enantiomers are due to the concentrations of the buffer constituents not being identical for the two experiments.

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Footnote

† The enantiomer was chosen to be consistent with the known (4*R*) configuration of the cysteine C_α and showed **1a** to be (2*R*,4*R*).

Crystal data for **1a**: $C_{17}H_{31}NO_3S$, M = 329.49, colourless plate, 0.60 × 0.40 × 0.04 mm, monoclinic, space group $P2_1$, T = 223 K, a = 9.905(2), b = 8.117(2), c = 24.041(5) Å, β = 92.18(3)°, U = 1931.5(7) $Å^3$, Z = 4, D_c = 1.133g cm^{-3}, Cu-Kα radiation (λ = 1.541 78Å), $F(000)$ = 720, μ = 1.575 cm^{-1}, R_1 (on F^2) = 0.049, wR_2 = 0.129, 3327 observations in the range 3.68 < θ < 62.43°, 409 variables, S = 1.095, final difference map ± 0.43 e $Å^{-3}$. Intensity data were collected on an Enraf-Nonius CAD-4 diffractometer using variable speed ω–2θ scans extended by 25% on each side for background measurements. Cell constants were derived from the setting angles of 25 reflections well distributed in reciprocal space. A quadrant of 3541 data was collected from which symmetry equivalents were averaged (R_{int} = 0.032). Three standard reflections measured every 3 h of X-ray exposure time showed a maximum variation of 5% for which a correction was applied. Data were corrected for Lorentz and polarization effects but not for absorption. The structure was solved by direct methods using the SHELXS program[11] and refined by full-matrix least-squares procedures to convergence (Δ/σ_{max} = −0.01) using the SHELX-93 software.[12] Non-hydrogen atoms were refined with anisotropic displacement parameters. Positional parameters for the hydrogen atoms attached to oxygen were refined. Other hydrogen atom positions were assigned with isotropic thermal factors based on geometrical and crystallographic considerations and held fixed in the last refinement stages. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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