Micellar Electrokinetic Capillary Chromatography in a Mixture of Taurodeoxycholic Acid and β-Cyclodextrin

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A mixture of taurodeoxycholic acid and β-cyclodextrin is found to form a 'pseudo-stationary' phase, which is very effective for the separation of the enantiomers of a variety of chiral compounds using micellar electrokinetic capillary chromatography (MECC); resolution is considerably superior to that obtained by using either taurodeoxycholic acid or β-cyclodextrin alone.

Bile acid salts, such as taurodeoxycholate, aggregate in water to form micelles. However, the presence of both a hydrophobic and a hydrophilic face in these molecules allows them to aggregate in a different manner to that observed with long-chain alkyl surfactants, such as sodium dodecyl sulfate (SDS).[1,2] A helical model has been found to describe the behaviour of deoxycholate and taurodeoxycholate micelle aggregates in aqueous solution.[3] It is also thought[4] that both the hydroxy groups on the cholic acid moiety as well as the terminal hydrophilic group (–CO–NH–$[CH_2]_2SO_3$, in the case of taurodeoxycholate) play an important part in their formation of micelles.

Deoxycholate and taurodeoxycholate micelles have been used[5–7] in micellar electrokinetic chromatography (MECC) where the overall negatively charged micellar aggregate moves towards the positive electrode mostly under the influence of the electroosmotic flow of the bulk electrolyte (pH around 7). Differential partitioning of molecules across the micelle leads to separation of analytes. Moreover, as bile salts are chiral they have been used to separate enantiomers in racemic mixtures.[5,6] A difference in the free energy of formation of the transient complexes between the bile salts and the separate enantiomers leads to resolution.

α-, β- and γ-Cyclodextrins (CDs) and their derivatives have also been widely used[8,9] for the separation of enantiomers in high performance capillary electrophoresis (HPCE). The addition of CDs to SDS micelles has been applied recently to the separation of the DL forms of several amino acids which had been derivatised with naphthalene-2,3-dicarbaldehyde (NDA).[9] The high resolving power achieved in the latter experiments was said to be due to additional distribution mechanisms of analytes between the SDS micelles and the cyclodextrin cavity.

In this communication we report the use of a mixture of taurodeoxycholic acid and β-cyclodextrin acid for enantiomeric separation in MECC. We show that the distribution

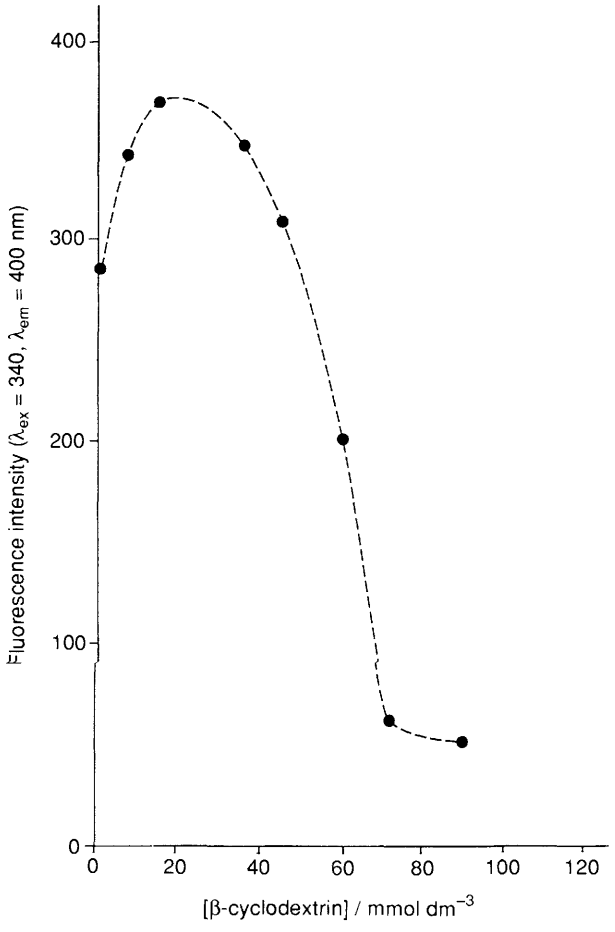


Fig. 1 The effect of β-cyclodextrin on the fluorescence intensity of 1-naphthylNHPh **1** in the presence of taurodeoxycholic acid (50 mmol dm^{-3}) at pH 7.2

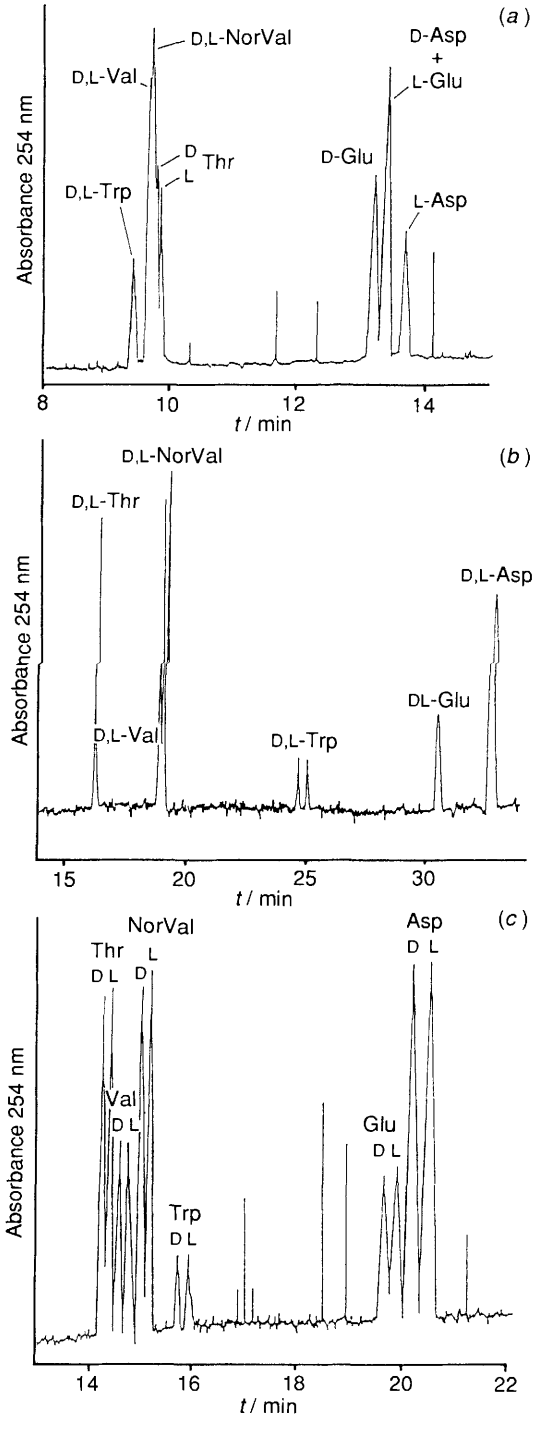
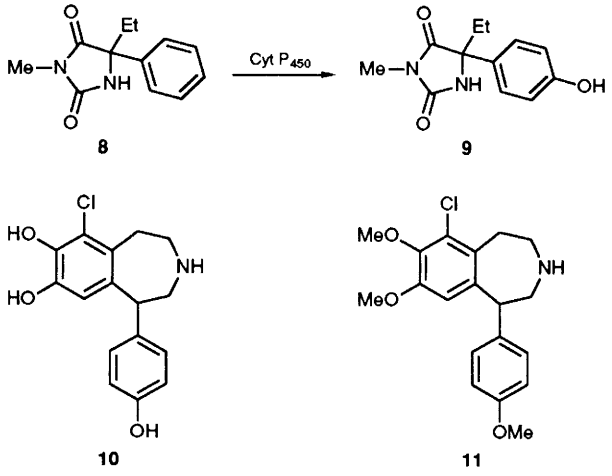


Fig. 2 MECC analysis of a mixture of six dansylamino acid derivatives in the presence of (*a*) β-cyclodextrin (20 mmol dm^{-3}) alone; (*b*) taurodeoxycholic acid (50 mmol dm^{-3}) alone; (*c*) a mixture of β-cyclodextrin (20 mmol dm^{-3}) and taurodeoxycholic acid (50 mmol dm^{-3}). Electrophoresis conditions: Beckman P/ACE 2100; capillary, 57 cm × 50 μm (i.d.); buffer (pH 7.2) sodium dihydrogen phosphate (30 mmol dm^{-3}), boric acid (10 mmol dm^{-3}); separation voltage 9 kV for (*a*) and (*c*), and 20 kV for (*b*); UV detection: 254 nm.

of molecules between two chiral phases gives optimum enantiomeric resolution of a variety of racemic analytes (anionic, neutral or cationic at pH *ca.* 7).

At the start of our study we examined the effect of different amounts of β-cyclodextrin on the fluorescence intensity of a commonly used[10] probe, *N*-phenylnaphthalene-1-amine **1** incorporated in a taurodeoxycholic acid micelle. Experiments were carried out in a buffer (pH 7.2) made up of sodium dihydrogen phosphate (30 mmol dm^{-3}) and boric acid (10 mmol dm^{-3}). Results are shown in Fig. 1. In the presence of taurodeoxycholic acid alone the fluorescence intensity of **1** increases considerably compared to that in the absence of micelle most probably owing to restriction in mobility. This effect has been observed[10] previously with **1** incorporated in other bile salts. In these experiments homogeneous solutions of mixtures of β-cyclodextrin and taurodeoxycholic acid were obtained at concentrations far above the saturation solubility (15.8 mmol dm^{-3}) of β-cyclodextrin. The fluorescence intensity–β-cyclodextrin profile at low levels of β-cyclodextrin may be due to a loose association between β-cyclodextrin and the hydrophilic face of taurodeoxycholic acid molecules within the micelle *via* hydrogen bonding. The fall in fluorescence intensity of **1** with higher concentrations of β-cyclodextrin is almost certainly due to disruption of the micelles. If one assumes that the role of **1** in these studies is to probe the constitution of the micelles formed by the bile acid molecules, then the variation of the fluorescence intensity on the addition of different concentrations of β-cyclodextrin appears to be similar to that reported for the effect of short-chain alcohols.[11,12] Low concentrations of these alcohols have been found to enhance micelle formation whereas higher concentrations disrupt micelles.

As shown in Fig. 1 optimum fluorescence intensity occurs at concentrations of 20 mmol dm^{-3} β-cyclodextrin and 50 mmol dm^{-3} taurodeoxycholate. This molar ratio of these two components was incorporated in buffer solutions (pH 7.2) and



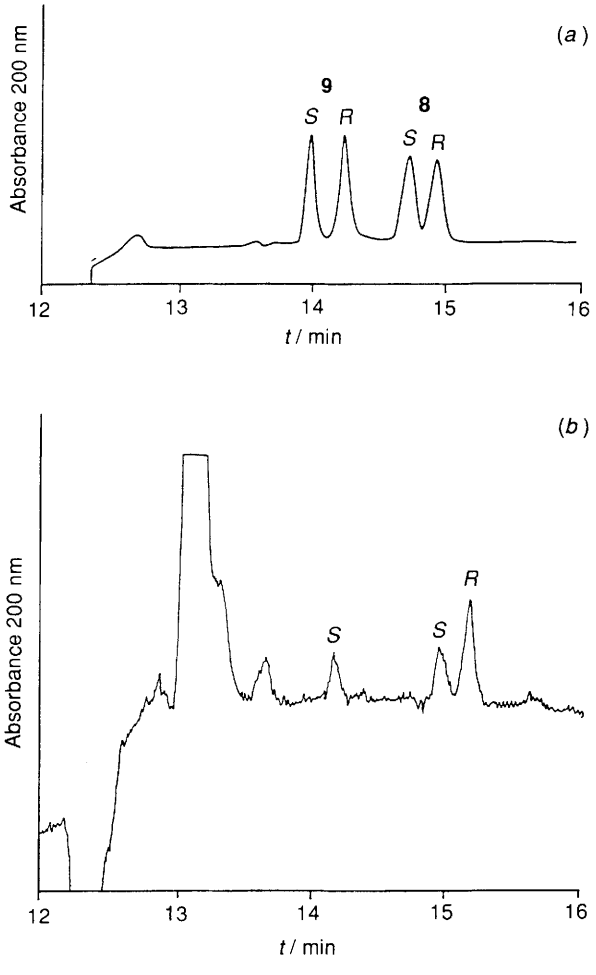


Fig. 3 Analysis of the enantiomers of mephenytoin **8** and its hydroxy metabolite **9** in a test mixture and (*b*) in a biological fluid extract. Electrophoresis conditions are the same as in Fig. 2 except UV detection at 214 nm.

was used in MECC studies. Fig. 2 compares the analysis of pairs of enantiomers of the dansylamino acid derivatives of threonine **2**, valine **3**, norvaline **4**, tryptophan **5**, glutamic acid **6** and aspartic acid **7**, using either β-cyclodextrin or taurodeoxycholic acid alone or the above mixture. Resolution obtained in the presence of the mixture is far superior to that obtained with either of the two chiral additives. Moreover, the order of migration of the various amino acid derivatives obtained by using β-cyclodextrin alone is different from that

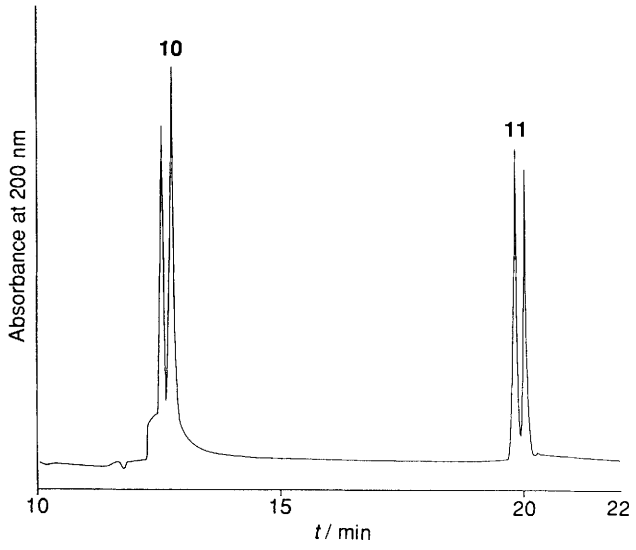


Fig. 4 Analysis of fenoldopam **10** and the closely related derivative **11**, using electrophoresis conditions as in Fig. 3

observed with the mixture, once again indicating the formation of a 'pseudo-stationary' phase involving both β-cyclodextrin and the bile salt. Moreover, in the presence of taurodeoxycholic acid alone only the enantiomers of the dansyl derivative of tryptophan are resolved and migration times are about twice as long as those shown in Fig. 2(*b*) when the voltage is set at 9 kV. Terabe *et al.*[8] recently reported the enantiomeric resolution of some dansylamino acid derivatives at pH 3 employing taurodeoxycholate. Here again, resolution is worse than that shown in Fig. 2(*c*) and migration times are considerably longer.

To test the robustness and the extent of applicability of the above mixture of β-cyclodextrin and taurodeoxycholic acid for chiral separations by MECC we examined the potential for the system to resolve enantiomers from neutral and cationic racemates at pH 7. Fig. 3(*a*) shows the resolution of the enantiomers of the antiepileptic drug mephenytoin **8** and its principal metabolite 4-hydroxymephenytoin **9**. Good resolution of the enantiomers of **8** and **9** was only obtained in the presence of β-cyclodextrin and taurodeoxycholic acid in the molar ratio of 20:50. The MECC analysis of a biological extract of **8** after treatment with human hepatic microsomes is shown in Fig. 3(*b*). As expected[13,14] only the (*S*)-enantiomer is largely oxidised to give the (*S*)-4-hydroxymephenytoin. The slight shift in the migration times of the enantiomers of **8** and **9** obtained with the biological extract is due to extraneous material affecting electroosmotic flow and hence mobility.

The resolution of the optical isomers of fenoldopam **10**, a drug used to increase renal blood flow, and a closely related derivative **11** is shown in Fig. 4. Unlike the dansyl derivatives **2**–**7** and compounds **8** and **9**, which are negatively charged and neutral, respectively at pH around 7, molecules **10** and **11** are positively charged under the same conditions. The common situation in all the cases studied is that the order of migration is primarily dependent on the hydrophobicity of the analytes. The higher the hydrophobicity the longer is the migration time. This is the case for the singly negative charged species **2**–**5** where the threonine derivative **2**, containing a hydroxy group, migrates first whereas the tryptophan derivative (the most hydrophobic) elutes last. The situation is reversed in the case of β-cyclodextrin in the absence of taurodeoxycholic acid [Fig. 2(*a*)]. A similar situation exists for the other compounds reported in this study, mephenytoin **8** is more hydrophobic than its hydroxy metabolite **9** and phenoldopam **10** is less hydrophobic than the fully methoxylated derivative **11**.

To conclude, we have shown that the mixture of the two chiral constituents β-cyclodextrin and taurodeoxycholic acid in the molar ratio of 20 : 50 dissolved in buffer at pH 7.2 forms a 'pseudo-stationary phase' which is particularly useful for the resolution of enantiomers of a wide variety of analytes by MECC. We are currently further evaluating this system for the chiral separation of a number of other racemates. These studies have so far proved very successful and will hopefully lead to a better understanding of the mechanism of chiral recognition by this system.

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