Functionalized α-Cyclodextrins as Potentiometric Chiral Sensors

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Octylated cyclodextrins have been synthesized and characterized by mass spectrometry (+ fast atom bombardment, + field desorption) and 500 MHz ^{1}H nuclear magnetic resonance spectroscopy. These highly lipophilic, enantiomerically pure molecules have been incorporated into solvent polymeric membranes and investigated as electrochemical sensors for chiral molecules incorporating aryl rings. Bis(1-butylpentyl) adipate (BBPA) and *ortho*-nitrophenyl octyl ether (*o*-NPOE) were used as plasticizers. Electrodes using BBPA as the plasticizer were stable and well defined with a limit of detection for ephedrine of $-\log[c] = 6.5$. Interference from serum levels of Na^+, K^+ and Ca^{2+} is minimal; the best value obtained for $-\log k^{pot}$ (the over-all selectivity coefficient) was 3.9 with BBPA as plasticizer and 1×10^{-3} mol dm^{-3} NH_4Cl as inner filling solution. The electrodes were highly enantioselective in binding ephedrine (enantioselectivity $k^{pot}_{(+)/(-)}$, 2.7). The *o*-NPOE-based electrodes, although enantioselective with minimal interference from serum levels of Na^+, K^+ and Ca^{2+}, behaved in a time-dependent manner.

Keywords: *Cyclodextrin; sensor; potentiometry; chiral; enantioselective*

Cyclodextrins (CDs) are optically active oligosaccharides consisting of 6–12 D-glucose units with an α-D(1–4) linkage. They form inclusion complexes in aqueous solution and in the solid state with various aromatic molecules and are toroidal in shape with each of the chiral glucose residues possessing a rigid $^4C_1$ chair conformation (Fig. 1). Complex formation requires that both the host and the guest molecules are complementary (*i.e.*, they possess a favourable match of the aryl moiety with the CD cavity). The interior of the CD cavity is highly hydrophobic and non-polar, and the hydroxy groups, which are directed away from the molecular cavity, are readily accessible for chemical modification.

Cyclodextrins have been used for the resolution of racemates by stereoselective complex formation.[1–3] They have also been used in aqueous liquid membranes for the enrichment of racemic mixtures.[4] More recently, they have been used as chiral stationary phases in gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) analyses.[5,6] The peroctylation of α- and β-CDs renders them highly lipophilic and suitable for incorporation into solvent polymeric membranes.

The primary aim of this work was to investigate the feasibility of using peroctylated α-CD **1a** as a sensing ionophore in potentiometric ion-selective electrodes for monosubstituted arylammonium ions. The enantiopure host was envisaged as forming diastereoisomeric complexes with chiral arylammonium analytes, allowing the possibility of selective detection of one particular enantiomer.

Experimental

Reagents and Chemicals

The synthesis and characterization of the octylated α-CDs **1a** and **1b** used in the membrane preparation has been reported elsewhere.[7]

Ephedrine hydrochloride (Eph·HCl), norephedrine hydrochloride (norEph·HCl) and pseudo (ψ) ephedrine hydrochloride (ψEph·HCl) were obtained from Sigma (Poole, Dorset, UK). High relative molecular mass poly(vinyl chloride) (PVC), *ortho*-nitrophenyl octyl ether (*o*-NPOE), bis(1-butylpentyl)adipate (BBPA) and potassium tetrakis(*p*-chlorophenyl)borate were obtained from Fluka (Buchs, Switzerland). Chloride salts of sodium, potassium and ammonia were obtained from BDH (Poole, Dorset, UK) and were of

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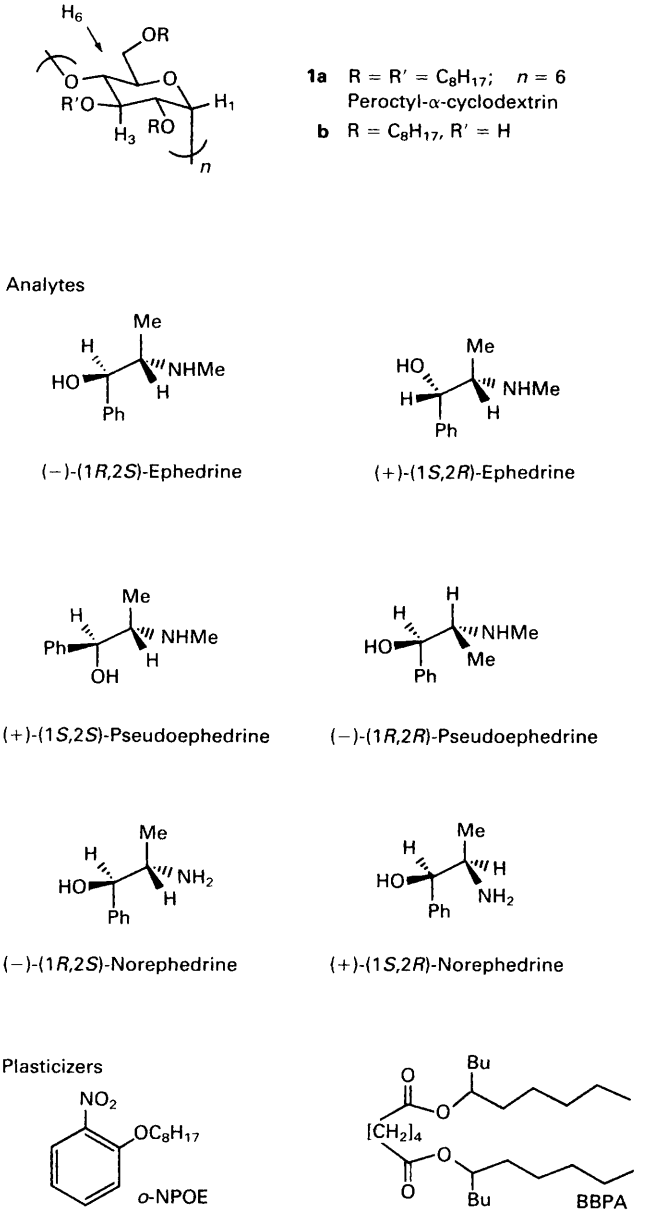

Fig. 1 Structures of the compounds discussed in the text

AnalaR grade. A 1.0 mol dm^{-3} calcium chloride solution (BDH AnalaR) was used.

All standard solutions were prepared in de-ionized water (Milli-Q; Millipore-Waters, Milford, MA, USA) and their cation concentrations were checked by atomic absorption spectometry.

Some of the membrane components are shown in Fig. 1 giving the structures of the peroctylated CD, the two plasticizers used, *o*-NPOE and BPPA, and of the lipophilic anion.

Membrane Preparation

The membrane composition for the *o*-NPOE-based membranes was 1.2% ionophore, 65.6% *o*-NPOE, 32.8% PVC and 0.4% potassium tetrakis(*p*-chlorophenyl)borate in 6 cm^3 of tetrahydrofuran (THF). For the BBPA-based membranes, the composition was 2.0% ionophore, 65.6% BBPA, 32.0% PVC and 0.4% potassium tetrakis(*p*-chlorophenyl)borate in 10 cm^3 of THF.

The membranes were cast by a controlled evaporation method according to the published procedure.[8] Unless otherwise stated compound **1a** was the ionophore used in these studies.

Calibration and Selectivity Measurements

A Philips IS (561) electrode body (Philips Analytical, Eindhoven, The Netherlands) was used to mount the electroactive membranes. The reference electrode was a Philips double junction RE3/DJ electrode. The electrochemical cells were set up using two different inner filling solutions for the ion-selective electrode.

(1) Ag,AgCl | 0.01 mol dm^{-3} Eph·HCl | PVC membrane | Analyte ‖ 0.1 mol dm^{-3} Li acetate (salt bridge) | KCl(salt) | Hg_2Cl_2(s); Hg

(2) Ag,AgCl | 1.0 mmol dm^{-3} NH_4Cl | PVC membrane | Analyte ‖ 0.1 mol dm^{-3} Li acetate (salt bridge) | KCl(salt) | Hg_2Cl_2(s); Hg

A constant dilution technique was used for calibration and selectivity measurements as described previously.[9] The selectivity measurements were performed with a background of 150.0 mmol dm^{-3} NaCl, 4.3 mmol dm^{-3} KCl and 1.26 mmol dm^{-3} $CaCl_2$.

All e.m.f. measurements were made at 25 °C (± 0.1 °C).

Bias Potential Measurements

The bias potential between two peroctylated α-CD–BBPA electrodes, both containing 1.0 mmol dm^{-3} NH_4Cl as the inner filling solution, one conditioned in (+)-Eph·HCl and the other in (−)-Eph·HCl, was measured in the cell shown in Fig. 2. One arm was filled with (+)-EpH·HCl and the other with (−)-Eph·HCl (0.1 mol dm^{-3}). The tap was carefully rotated to allow the solution of the (−)-enantiomer to move half way up the capillary tube. The second arm was filled with the (+)-enantiomer and this solution was forced down the capillary with the aid of a syringe fitted with a flattened needle. Care was taken to prevent the two solutions from mixing. The electrodes were immersed in the appropriate solutions and the potential difference was monitored over 4 h.

Behaviour of the Electrodes in Solutions of Varying Enantiomeric Excess

A range of solutions was prepared containing 0–100% of the (+)- and (−)-enantiomers of Eph·HCl, respectively. The behaviour of the electrochemical cell containing 1.0 mmol dm^{-3} NH_4Cl inner filling solution and with the ion-selective electrode mounted with an α-CD–BBPA electroactive membrane conditioned either in (−)-Eph·HCl or (+)-Eph·HCl, was observed.

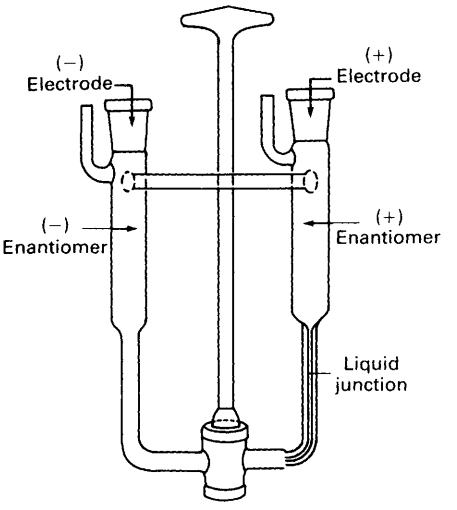

Fig. 2 Cell used for measurement of bias potential

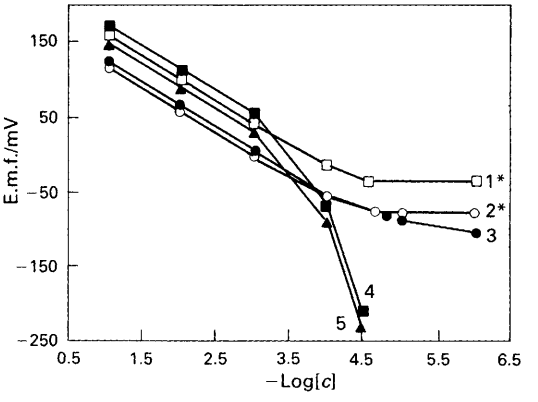

Fig. 3 Calibration graphs for peroctylated α-CD as the sensing ionophore with *o*-NPOE as plasticizer and a 1 × 10^{-2} mol dm^{-3} Eph·HCl inner filling solution. 1*, (−)-Eph·HCl; 2*, (+)-Eph·HCl; 3, (+)-Eph·HCl; 4, (−)-Eph·HCl; and 5, (±)-Eph·HCl. * Background of serum levels of Na^+, K^+ and Ca^{2+} (see Table 1)

Results

Calibration of Electrodes

Using o-NPOE as plasticizer

The first set of electrodes to be tested had *o*-NPOE as plasticizer with **1a** as the sensing ionophore and used a 0.01 mol dm^{-3} solution of either (+)-Eph·HCl, (−)-Eph·HCl or (±)-Eph·HCl as the inner filling solution. The electrodes were conditioned in 0.01 mol dm^{-3} solutions of the appropriate enantiomer and were calibrated by continuous dilution. The (+)-enantiomer showed a normal Nernstian response with a detection limit $-\log[c] = 4.8$. The (−)-enantiomer and the racemic mixture behaved in a Nernstian manner down to a concentration of 1 × 10^{-3} mol dm^{-3}. On further dilution an unusual hyper-Nernstian behaviour was observed. In a background of serum levels of Na^+, K^+, and Ca^{2+} this behaviour was not observed (Fig. 3 and Table 1). Both electrodes functioned satisfactorily with over-all selectivity coefficients of $-\log k^{pot} = 3.82$, and 3.68 for the (+)- and (−)-enantiomers, respectively. In the absence of added inorganic cations, the initial difference in measured electrode

Table 1 Behaviour of electrodes with **1a** using *o*-NPOE as plasticizer and 1×10^{-2} mol dm^{-3} Eph·HCl inner filling solution

Sensor	Slope/mV decade^{-1}	Limit of detection, $-\log[c]$	Selectivity, $-\log k^{pot}$
(+)-Eph·HCl*	60.0	4.64	3.82
(−)-Eph·HCl*	60.0	4.54	3.68
(−)-Eph·HCl	NQ†	NQ†	—
(±)-Eph·HCl	NQ†	NQ†	—
(+)-Eph·HCl	59.0	4.80	—

* Background of serum levels of Na^+, K^+, Ca^{2+}.

† NQ = The slope and limit of detection have not been quoted because of the unusual behaviour of the electrode.

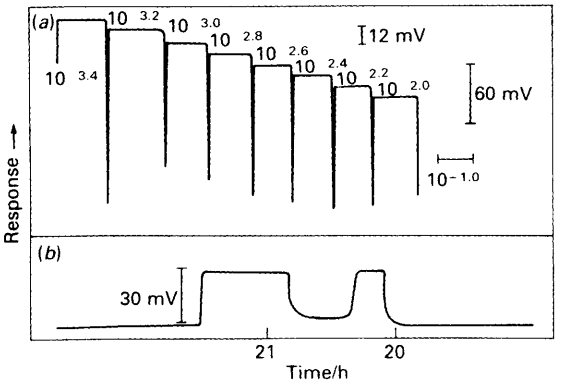

Fig. 4 Behaviour of electrode based on peroctylated α-CD (**1a**), *o*-NPOE, 0.01 mol dm^{-3} (−)-Eph·HCl inner filling solution, conditioned in 0.01 mol dm^{-3} (−)-Eph·HCl. (*a*) Measurement of discrete solutions in the vicinity of 1×10^{-3} mol dm^{-3}. Further dilution revealed no hyper-Nernstian behaviour. (*b*) Electrode potential measured over 24 h in 0.1 mol dm^{-3} (−)-Eph·HCl solution. The time-dependent behaviour is evident after 20 h

potentials between the (+)-electrode immersed in 0.1 mol dm^{-3} (+)-Eph·HCl and the (−)-electrode immersed in 0.1 mol dm^{-3} (−)-Eph·HCl was 50 mV. This value was not constant with time and had reduced to 20 mV after further conditioning for 18 h in the same solutions (Fig. 3 and Table 1). Thereafter it remained constant.

In discrete solutions, calibrations in which the electrode was transferred into solutions of decreasing concentration within 60 s, the hyper-Nernstian behaviour was not observed (Fig. 4). However, on transferring the (−)-electrode from the conditioning solution into a 0.1 mol dm^{-3} solution of (−)-Eph·HCl, an abrupt and reversible drop in potential was observed after about 20 h (Fig. 4).

Using BBPA as plasticizer

The plasticizer was changed to BBPA. The concentration-dependent hyper-Nernstian response that was evident when *o*-NPOE was used as plasticizer was no longer observed (Table 2). The (−)-Eph·HCl electrode showed a slope 13 mV decade^{-1} less than the (+)-Eph·HCl sensor and the difference in electrode potentials, $\Delta E[(+) - (-)]$ was 14 mV. In a background of serum levels of Na^+, K^+ and Ca^{2+} the over-all selectivity coefficient, $-\log k^{pot}$ was 2.73 for the (+)-enantiomer.

The next experiment was performed in an attempt to minimize the standard electrode potential differences. Electrodes were chosen that had 1.0 mmol dm^{-3} NH_4Cl as the inner filling solution instead of the appropriate Eph·HCl solution. The *o*-NPOE-based electroactive membrane behaved unusually again with (−)-Eph·HCl as analyte, after dilution beyond a concentration of $10^{-2.8}$ mol dm^{-3}. The (+)-Eph·HCl electrode functioned satisfactorily, showing

Table 2 Behaviour of electrodes with **1a** using BBPA as plasticizer and 1×10^{-2} mol dm^{-3} Eph·HCl as inner filling solution

Sensor	Slope/mV decade^{-1}	Limit of detection, $-\log[c]$	Over-all selectivity, $-\log k^{pot}$
(+)-Eph·HCl	59.0	5.05	—
(−)-Eph·HCl	46.0	5.40	—
(+)-Eph·HCl*	56.0	3.55	2.73

* Background of serum levels of Na^+, K^+, Ca^{2+}.

Table 3 Behaviour of electrodes with **1a** using *o*-NPOE as plasticizer and 1.0 mmol dm^{-3} NH_4Cl inner filling solution

Sensor	Slope/mV decade^{-1}	Limit of detection, $-\log[c]$	Over-all selectivity, $-\log k^{pot}$
(+)-Eph·HCl	56.0	5.25	—
(+)-Eph·HCl*	58.0	4.70	3.91
(−)-Eph·HCl*	52.0	5.27	4.45
(−)-Eph·HCl	NQ†	NQ†	—

* Background of serum levels of Na^+, K^+, Ca^{2+}.

† NQ = The slope and limit of detection have not been quoted because of the unusual behaviour of the electrode.

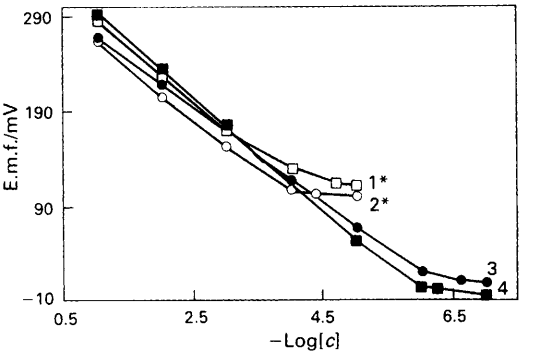

Fig. 5 Calibration graphs for electrodes with α-CD–BBPA membranes with NH_4Cl inner filling solutions. 1*, (+)-Eph·HCl; 2*, (−)-Eph·HCl; 3, (−)-Eph·HCl; and 4, (+)-Eph·HCl. * Background of serum levels of Na^+, K^+ and Ca^{2+} (see Table 4)

the expected Nernstian response. In a background of serum levels of Na^+, K^+ and Ca^{2+} it showed very little interference, $-\log k^{pot} = 3.9$ (Table 3).

The more well defined electrode had BBPA as plasticizer and 1.0 mmol dm^{-3} NH_4Cl as inner filling solution. The unexpected behaviour observed with *o*-NPOE was again not evident. The slope of the (−)-Eph·HCl sensor was 10 mV decade^{-1} lower than that of the (+)-Eph·HCl sensor. The difference in electrode potentials between enantiomeric electrodes $\Delta E[(+) - (-)]$ in the appropriate 0.1 mol dm^{-3} solutions was 26.0 mV in aqueous solutions and 21.0 mV in a background of serum levels of Na^+, K^+ and Ca^{2+} corresponding to $-\log k^{pot}_{(+)/(-)}$ of 2.7 and 2.3, respectively $\{-\log k^{pot}_{(+)/(-)} = [E_{(+)} - E_{(-)}]/S$, where S is the electrode slope$\}$. The over-all selectivity coefficients were $-\log k^{pot}$ 3.9 and 3.5 for the (+)- and (−)-enantiomers, respectively (Fig. 5, Table 4).

The only difference between the (+)-Eph·HCl and the (−)-Eph·HCl sensors used in this experiment was that the electrodes had been conditioned separately in a 0.1 mol dm^{-3} solution of the appropriate enantiomer. The 'bias' potential between these two electrodes was measured in the cell shown in Fig. 2. This cell was used in order to eliminate any errors that may arise due to liquid junction potentials. The measured potential was observed to be constant over 4 h at ambient temperature:

$$\Delta E_{bias} = E_{(+)} - E_{(-)} = 24.5 \pm 0.5 \text{ mV}$$

Table 4 Behaviour of electrode with **1a** using BBPA and 1.0 mmol dm^{-3} NH_4Cl inner filling solution

Sensor	Slope/mV decade^{-1}	Limit of detection, $-\log[c]$	Over-all selectivity, $-\log k^{pot}$
(+)-Eph·HCl	60.0	6.3	—
(−)-Eph·HCl	50.0	6.6	—
(+)-Eph·HCl*	59.0	4.7	3.9
(−)-Eph·HCl*	59.0	4.4	3.5

* Background of serum levels of Na^+, K^+, Ca^{2+}.

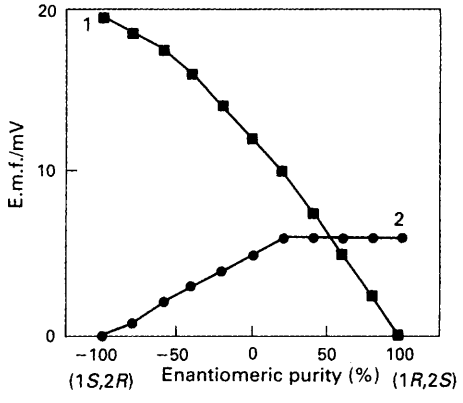

Fig. 6 Behaviour of electrodes in solutions of varying enantiomeric excess. Electroactive membrane: α-CD–BBPA; inner filling solution: 1.0 mmol dm^{-3} NH_4Cl; conditioned in 10 mmol dm^{-3} (+)- or (−)-Eph·HCl. 1, (−)-Eph·HCl–BBPA; and 2, (+)-Eph·HCl–BBPA

This corresponds to a free energy difference between the two diastereoisomeric complexes of 2.4 (0.05) kJ mol^{-1}.

Enantioselective Sensor

The performance of the α-CD–BBPA electrodes containing 1.0 mmol dm^{-3} NH_4Cl as inner filling solution was assessed in solutions containing ephedrine of varying enantiomeric purity. The electrodes were conditioned overnight in the appropriate 0.01 mol dm^{-3} Eph·HCl solution. The (−)-Eph·HCl electrode appears to be the more sensitive of the two (Fig. 6), exhibiting a near linear e.m.f. response with varying enantiomeric purity.

The stability of the BBPA-based ionophore was monitored over 5 weeks in 0.1 and 0.01 mol dm^{-3} solutions. The e.m.f. readings were reproducible to within ±0.5 mV.

Calibration and selectivity measurements were also performed with enantiomers of norEph·HCl and ψEph·HCl using BBPA as plasticizer and the appropriate enantiomer as the inner filling solution (Table 5). The (−)-norEph·HCl–BBPA electrode showed a slope 12 mV decade^{-1} less than that of the (+)-enantiomer. The other electrodes, although satisfactory in terms of slope, limit of detection and selectivity, did not show significant enantioselective behaviour.

When a racemic mixture of ψEph·HCl was used as inner filling solution, the electrodes functioned satisfactorily in terms of slope, selectivity and limit of detection (Table 5). However, they did not show enantioselective behaviour.

The less highly octylated α-CD **1b** (two octyl groups and one free hydroxy group per glucose residue) was not a good sensor for these β-hydroxyarylammonium salts. With BBPA as plasticizer and the appropriate enantiomer [*i.e.*, (+)-enantiomer when testing (+)-enantiomer as the analyte] as the inner filling solution, the (+)-electrode gave a slope of 24 mV decade^{-1}. The slope of the (−)-electrode was 59 mV decade^{-1} at a 1 × 10^{-2} mmol dm^{-3} dilution; however, the

Table 5 Behaviour of electrode with **1a** using BBPA as NorEph·HCl and ψEph·HCl sensor inner filling solution: 0.01 mmol dm^{-3} analyte

Sensor	Slope/mV decade^{-1}	Limit of detection, $-\log[c]$	Over-all selectivity, $-\log k^{pot}$
(+)-norEph·HCl	58.0	5.05	—
(−)-norEph·HCl	46.0	3.80	—
(±)-norEph·HCl	58.0	2.90	2.1
(+)-ψEph·HCl	56.0	4.70	—
(−)-ψEph·HCl	59.0	5.10	—
(±)-ψEph·HCl	59.0	5.20	—

* Background of serum levels of Na^+, K^+, Ca^{2+}.

limit of detection was $-\log[c] = 2.9$, much reduced compared with its peroctylated analogue **1a**.

Discussion

Electrodes that are based on peroctylated α-CD appear to show a highly pronounced enantioselective behaviour provided that the appropriate inner filling solution is used. With BBPA as plasticizer, this is evident both in the measured ΔE values and as a lower slope for the (−)-enantiomer. Based on the bias potential measurements, and assuming that the slopes of the electrodes are equivalent:

$$\log k^{pot}_{(+)/(-)} = 2.6; \{\log k^{pot}_{(+)/(-)} = [E_{(+)} - E_{(-)}]/S, \text{ where } S = \text{slope}\}$$

The (+)-enantiomer thus appears to form a more stable complex with the CD than the (−)-enantiomer. Yasaka *et al.*,[10] and Bussmann *et al.*,[11] have previously reported $-\log k^{pot}_{(+)/(-)}$, using chiral 18-crown-6 based-macrocyclic polyethers, as 1.5 and 2.6, respectively, for the α-phenylethylammonium ion determined by the separate solutions method. These sensors are limited by their sensitivity to Na^+ and K^+ and cannot be used in a clinical background of serum cations.

With *o*-NPOE as the plasticizer, the electrodes behaved in an unexpected manner. While detection of the (+)-enantiomer was normal with a Nernstian response in both the presence and absence of serum cations at clinical concentrations, the detection of the (−)-enantiomer was concentration- and time-dependent and was also sensitive to the absence or presence of added cations. This intriguing behaviour could, in principle, be related to competititve binding of the *o*-NPOE by the peroctylated CD. It is known, for example, that *o*-nitrophenol forms a 1:1 complex with α-CD in aqueous solution in which the aryl nitro group enters the 'cavity' first.[12–14] That such behaviour is not observed at all with the (+)-enantiomer (under any conditions of ephedrine concentration) suggests that this is unlikely particularly in the light of the apparent small difference in the free-energy of binding (about 2.4 kJ mol^{-1} at 298 K) of the two enantiomers. A more likely explanation may involve a concentration- and ionic strength-dependent aggregation phenomenon involving both the peroctylated CD and the plasticizer. When a charged arylammonium ion is bound by the peroctylated CD the complex may be regarded as amphiphilic. Enantioselective aggregation may occur beyond a critical concentration which is inhibited in the presence of added cations (*i.e.*, at higher ionic strength). Thus the modest enantioselectivity observed at the molecular level may be amplified in the chiral aggregate.

Preliminary ^1H NMR spectroscopic investigations with peroctylated α-CD and the trifluoroacetate salts of (+)- and (−)- Eph in $CDCl_3$ (298 K, 1:1 stoichiometry, 0.05 mol dm^{-3}) show that the chemical shift of certain of the CD resonances (3-H, 5-H, and C-6, CH_2O) is dependent on the nature and concentration of the enantiomer included, indicative of enantioselective binding. Further NMR and circular dichro-

ism experiments are in progress in order to define the structure and relative stability of the diastereoisomeric complexes, and will be reported subsequently.

Conclusions

The peroctylated α-CD–BBPA electrode using 1.0 mmol dm^{-3} NH_4Cl as the inner filling solution is suitable as a chiral sensor. It has an excellent sensitivity (60 mV $decade^{-1}$ at 25 °C), limit of detection ($-\log[c] \approx 6.5$), selectivity over serum levels of cations ($-\log k^{pot} = 3.9$) and enantioselectivity ($-\log k^{pot}_{+/-} = 2.7$). A calibrated electrode has been constructed that allows the enantiomeric purity of (−)-ephedrine (the pharmacologically active enantiomer) to be measured, even in the presence of its diastereoisomers, (*R*,*R*)- and (*S*,*S*)-pseudoephedrine.

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