ChemComm
FEATURE ARTICLE

Selective complexation and sensitive analysis of charge diffuse cationic species using lipophilic cyclodextrins

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Lipophilic peralkylated cyclodextrins are useful ionophores for the selective binding of a variety of size-matched charge-diffuse cationic species. Chiral potentiometric sensors may be devised using plasticised membrane electrodes, while voltammetric sensors may be fabricated that allow sub-nanomolar levels of detection of electron-rich aromatic cations. The observation of the binding of size-matched tetraalkylammonium ions has led to the development of sensitive methods of detection for species such as acetylcholine. When lipophilic cyclodextrins are coupled to enzymes in a disposable biosensor, a highly specific method of analysis is achieved giving sub-picomolar levels of detection.

Introduction

Classical coordination chemistry focuses on the binding of metal ions by ligands and is an area of research activity that is relatively mature.[1] For example, the principles that govern how a ligand binds to a given ion are well established and selectivity in complexation may be quite accurately predicted from an appreciation of the preference of the ion for a particular type of donor atom, coordination number and geometry. Metal ions are usually charge dense species with large metal–ligand free energies of complexation. On the other hand, many organic cations—such as 'onium ions (of O, P, S and N)—are best considered to be charge diffuse species whose interaction with a ligand or receptor relies upon multiple, weaker binding forces. In the case of ammonium ions for example, the positive charge is usually delocalised over the adjacent carbon and hydrogen atoms. Complexation of such charge diffuse species usually requires both size and shape complementarity between the receptor and the ion and the presence of stabilising hydrogen-bonding interactions. Size and shape complementarity is often associated with a favourable solvophobic interaction, while directed hydrogen-bonding involves the cationic 'onium ion acting as a hydrogen-bond donor to proximate electron-rich acceptors. An early example of such an interaction with a synthetic neutral ionophore involves the complexation of a primary ammonium ion, $R\overset{+}{N}H_3$ with the lone-pairs of C_3-related ether oxygen atoms in an 18-crown-6 derivative.[2] Receptors that are rich in π-electron density may also serve as the hydrogen-bond acceptor:[3] in the enzyme acetylcholine esterase the interactions between the cationic NMe_3 head group of acetylcholine and the hydrophobic π-electrons of Trp-84 are believed to contribute over 20 kJ mol^{-1} to the overall free energy of binding.[4]

Whilst cation-π interactions are undoubtedly an important binding interaction in supramolecular chemistry, for an $\overset{+}{N}$–CH group as a hydrogen bond donor the interaction with ether oxygens is more energetically favourable. The gas-phase dissociation enthalpies for $\overset{+}{N}Me_4$ with various n and π donors have been measured.[5] The interaction with H_2O, MeOH and benzene was considerably weaker than that found for the more polarisable dialkyl ethers, and polyethers gave the highest measured values consistent with multiple additive $\overset{+}{N}C$–H···O interactions. Ionic C–H···O hydrogen bonds are well-documented structurally in the solid-state, and 9 out of 10 of the shortest C–H···O contacts involve $[NCH]^{\delta+}$ as the hydrogen bond donor.[6] Multiple weak C–H···O interactions between alkylammonium ions and size-matched receptors may therefore make a very significant contribution to the overall free-energy of binding of 'onium ions.

Chiral sensors

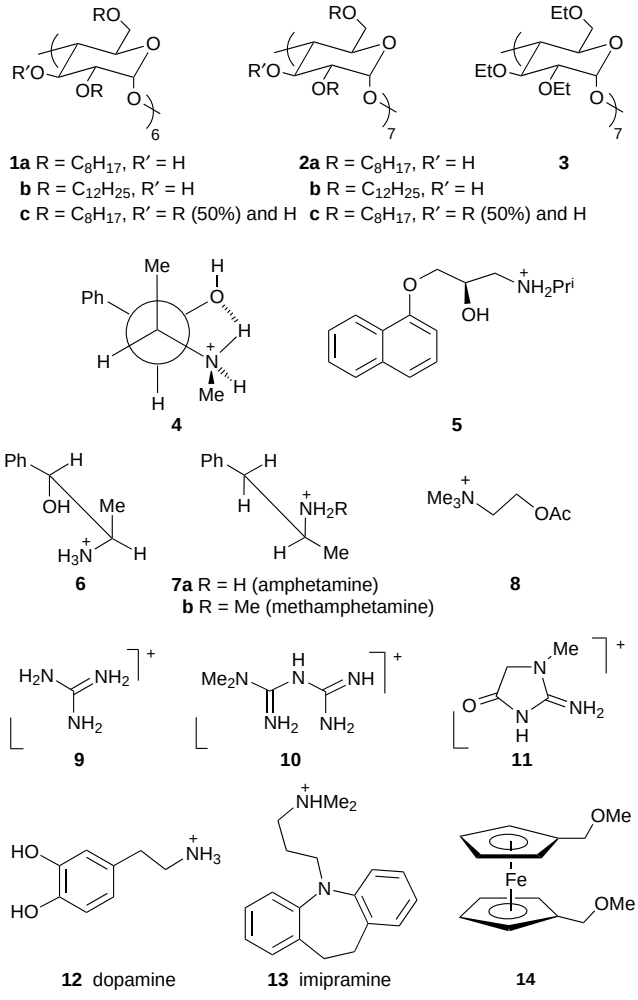
Our interest in the binding of charge diffuse organic species was sparked by the challenge of devising an enantioselective ion-selective electrode that exhibited reasonable chemoselectivity for the target ion. If analysis is to be carried out with a membrane-based potentiometric sensor, then a neutral ionophore is required that can reversibly bind the target ion, with fast exchange kinetics, *i.e.* a free energy of activation for exchange between free and bound forms of the analyte of less than, say, 65 kJ mol^{-1}. Chemoselectivity in binding the target analyte is important—rejecting competing charge-dense ions for example—and stereoselectivity is also required so that the potential difference that builds up at the aqueous-membrane interface is proportional to the activity of the ion and its enantiomeric purity. Earlier work on chiral sensors had examined certain lipophilic chiral crown ethers as ionophores[2] and although enantioselective sensors for chiral primary amines were defined, chemoselectivity was very modest (severe Na^+/K^+ interference) and *N*-alkyl substitution compromised the N–H···O binding interaction.

Given the developing role of alkylated cyclodextrins in GC[7] and HPLC[8] analysis, we were attracted by the possibility of devising lipophilic ionophores by modifying the 2, 3 and 6-OH groups of these chiral host molecules. Initial work therefore focused on the synthesis and characterisation of the lipophilic ionophores[9] and the evaluation of their use as chiral ionophores for size-matched arylammonium ions.[10] A series of alkylated cyclodextrins was prepared, **1**, **2** and **3**, systematically varying the size of the cyclodextrin cavity and the nature of the alkyl chain. α-Cyclodextrin derviatives, comprising six glucose sub-units, define a toroidal cavity that is sufficiently large to include monosubstituted benzene derivatives. The free energy of binding may be attributed to a favourable solvophobic interaction, with inclusion leading to the displacement of relatively 'high-energy' solvent molecules, and to attractive van der Waals' and dispersion forces in the complex. The β- and γ-cyclodextrin analogues containing seven and eight glucose units respectively define a larger cavity suitable for inclusion of larger substrates such as naphthyl derivatives in the former case and tricyclic molecules or ions in the latter.

Stepwise alkylation procedures were devised that allowed preferential 2,6-dialkylation, and the more hindered 3-OH group could be alkylated under more forcing conditions (*e.g.* NaH–THF–18-crown-6–RX). The precise degree of alkylation was assessed by a combination of NMR, reductive depolymerisation–GCMS and direct electrospray mass spectrometric methods[9] and the three independent methods of analysis showed excellent agreement. The per-*O*-ethyl β-cyclodextrin

3[11] is perhaps the simplest lipophilic cyclodextrin and is very soluble in non-polar organic solvents and almost completely insoluble in water.

Poly-*O*-octyl-α-cyclodextrin (containing an average of 15.4 octyl groups per cyclodextrin), when incorporated into a plasticised PVC membrane, gave an ion-selective electrode that showed a different response (in slope and in $E°$ values) to the (−)-(1*R*, 2*S*)-ephedrinium ion **4**, compared to its enantiomer and its diastereoisomers, the (+)- or (−)-pseudoephedrinium cations. The electrode could be calibrated to measure the enantiomeric purity of samples of ephedrinium salts (Fig. 1) even in the presence of the pseudo-ephedrinium diastereoisomers.[10] In order to measure the total ephedrinium concentration parallel measurements are needed using an achiral or non-selective ionophore: per-*O*-octyl maltose serves as a suitable ionophore for the latter purpose. Moreover, the electrode response was not significantly impaired by the presence of a simulated clinical electrolyte containing Na^+ (150 mm), K^+ (4.3 mm), Ca^{2+} (1.26 mm) and Mg^{2+} (0.9 mm), and an overall selectivity coefficient $-\log K^{pot}_{clin}$ of 3.7 was determined. The difference in the free energy of complexation for binding of the ephedrinium enantiomers was only 2.5 kJ mol^{-1} with **1b** and **1c**, but this corresponds to a 26 mV differential response that is sufficient to be measured quite accurately. Using the size-matched β-cyclodextrins **2b** or **2d** as the ionophore, similar experiments were conducted with propranolol **5**, as the analyte. The observed enantiodifferentiation was also about 25 mV or 2.4 kJ mol^{-1}, but was sufficient to allow enantiomeric purity determinations to be effected using a calibrated electrode.



Probing complexation

Complex formation was monitored by NMR methods and by electrospray mass spectrometry, and was probed further by variation of the host and guest structures. Confirmation of the formation of a 1 : 1 complex with ephedrinium or propranolol cations was provided by positive-ion ESMS spectra (Fig. 2), and measurements using pulsed-gradient spin echo ^1H NMR methods of the diffusion coefficient of the (+)-ephedrinium ion in the presence and absence of **1b** in $CDCl_3$ were consistent with 1 : 1 complex formation with an equilibrium constant of 137 (288 K).[12] The (−)-enantiomer was bound more weakly and when all of the OH groups in the 3-position were capped with methyl groups both enantiomers bound with the same affinity. Capping of the residual OH groups in the α-cyclodextrin hosts also markedly reduced the enantioselective response of the electrode and its sensitivity to the ephedrinium analyte, highlighting the importance of these groups *either* in rigidifying the CD structure through intramolecular hydrogen bonding *or* in acting as an orienting hydrogen-bond donor to the cationic guest. Variation of the structure of the β-arylammonium ion was also examined: the norephedrinium ion **6** behaved in a parallel manner to ephedrinium, while species lacking the α-hydroxy group—such as the amphetamines **7**—also gave an enantioselective response, suggesting that the configuration of the chiral centre β to the aryl ring was most important in defining enantioselectivity.[10]

Further details of the solution complexation process were provided by measurements of the ^1H NMR relaxation rates of the cyclodextrin and of the included ion. Changes in relaxation rate dependent upon which enantiomer was bound by **1c** were observed for both the cyclodextrin and the β-arylammonium ion. The rate of relaxation of the cyclodextrin H^3 proton, for example, increased differentially by 1.45 s^{-1} on complexation

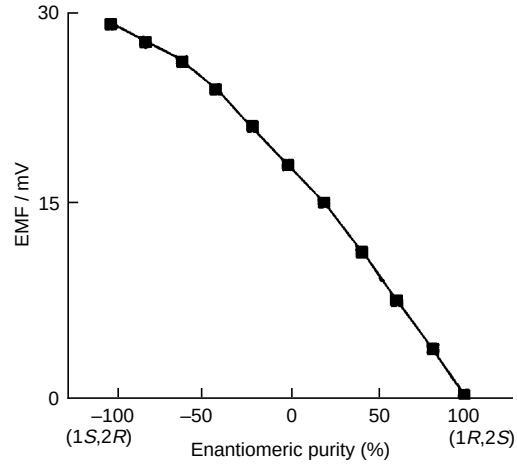


Fig. 1 Calibrated electrode response for an ISE based on poly-*O*-octyl-α-cyclodextrin with the enantiomeric purity of the ephedrinium analyte (310 K, I = 0.1)

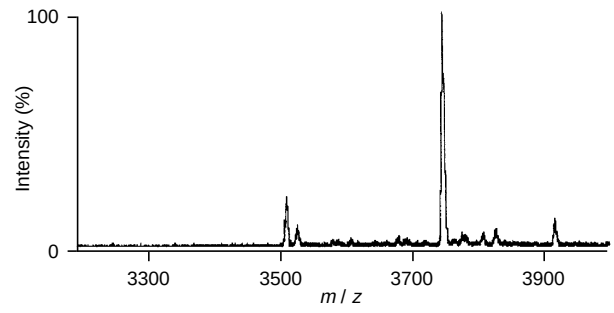


Fig. 2 Electrospray mass spectrum of the complex formed between 2,6-didodecyl-β-cyclodextrin and (*R*)-propanolol

of (+)-ephedrinium compared to (−)-ephedrinium. It is well known that this proton is oriented towards the centre of the cyclodextrin cavity, and similar (albeit smaller) changes in rate have been observed following inclusion of (+)-Trp by α-cyclodextrin.[13] More marked enantiodependent changes in relaxation rate were measured for the diastereotopic NH_2 protons of the guest ephedrinium ion. A model has been proposed that accounts for the observed changes.[10] In the complex of the more strongly bound (+)-ephedrinium ion, the *C*-methyl (in the 2-position) group is oriented away from the H-3 and H-5 protons of the cyclodextrin. With the (−)-enantiomer, in order to avoid an unfavourable steric interaction the complex adopts an alternative structure lacking a stabilising N–H···O hydrogen-bond. Small differences in hydrogen-bonding (*i.e.* one H-bond mismatch) have recently been invoked to explain free-energy differences of the order of 2.5 kJ mol^{-1} in DNA binding to intercalating molecules.[14]

Binding and detection of 'onium ions

During the course of electrode-response experiments aimed at examining the effect of ionic strength on complexation, we quickly became aware of an *unusual* response in the presence of tetramethylammonium ions, when using poly-*O*-octyl-β- or tri-*O*-octyl-β-cyclodextrin as the membrane ionophore. The *absence* of any response was traced to competitive binding by the alkylammonium ion,[11,15] and 1:1 cyclodextrin: NR_4^+ complexes were observed by ESMS (Fig. 3) and were further characterised by ^{14}N, ^2H and ^1H NMR measurements.[16] Both NMe_4^+ and NEt_4^+ have ionic diameters that are matched quite closely in size to a peralkylated cyclodextrin,[17] and complex formation is expected to be driven by a favourable solvophobic interaction and may be aided by multiple, weak $\overset{\delta+}{N}CH\cdots O$ interations.[5,6] This binding interaction allows the lipophilic cyclodextrins to be used as ionophores for the potentiometric detection of a variety of substituted alkylammonium ions (Table 1). Long-chain trimethylammonium ions may be detected sensitively at sub-micellar concentrations, and selectivity over Na^+/K^+ was found to reach a maximum at chain lengths that matched those of the alkyl groups in the cyclodextrin receptor.[11] Favourable hydrophobic interactions between alkyl chains may afford an extra stabilising interaction. Indeed, the lipophilic cyclodextrins **1a**, **1b**, **2a** and **2b** themselves form well-defined bilayers at an air–water interface.[18] Calculations of the surface area per molecule in these assemblies suggest that not only are the 'primary' alkyl chains (those bound to the 6-position) directed away from the aqueous phase but also that the chains linked to the 2-position are folded back tightly along the sides of the cyclodextrin. At a clean silicon or gold surface, atomic force microscopic measurements provided clear evidence for aggregation of the lipophilic cyclodextrins and aggregates varying in diameter from 2 to 0.1 μm were observed.[18]

Various other types of charge diffuse cations have been examined as analytes. For example, the strongly basic guanidinium ions such as **9** and **10** may be sensitively detected by potentiometric methods,[19] and the related metabolite creatinine **11** (pK_a = 4.75) may also be assayed, provided that the electrode response is corrected to allow for dissociation of this weak acid.[11] With these hydrogen-bond donating cations, the independence of the observed electrode response to the structure of the sensing cyclodextrin (3-OH *vs.* 3-OR groups, α *vs.* β derivatives) indicated that the primary binding interaction involves $\overset{+}{N}–H\cdots O$ hydrogen-bonding.

Voltammetric methods of analysis, such as differential pulse voltammetry, allow lower concentrations of analytes to be measured, provided that the analyte possesses an accessible redox couple. The catecholamine oxidation of dopamine **12** occurs at +200 mV (*vs.* Ag/AgCl) on a screen-printed carbon electrode that has been coated with 2,3,6-tri-*O*-ethyl-β-cyclodextrin **3**.[20] Dopamine concentrations as low as 10^{-11} mol dm^{-3} may be detected in this way, comparing favourably to the levels found in samples of cerebrospinal fluid (10^{-8} mol dm^{-3}). The more sterically bulky cation, imipramine—an anti-depressive drug with a toxic level in blood of 5 μg per ml—is more effectively analysed using a sensor incorporating a poly-*O*-octyl-γ-cyclodextrin ionophore. In this case oxidation of the electron-rich aromatic moiety may be monitored by differential pulse voltammetry, and a linear current response was observed in the range 10^{-4} to 10^{-9} mol dm^{-3} (Fig. 4).

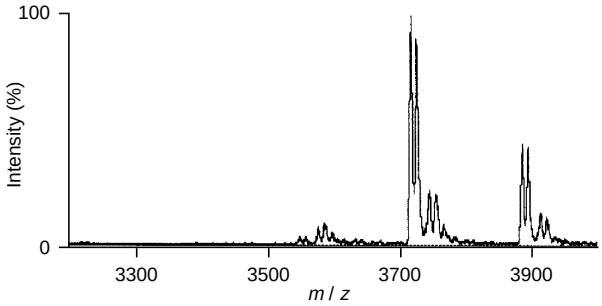


Fig. 3 Electrospray mass spectrum of the 1:1 complexes between equimolar amounts of $C_{12}H_{25}N(CD_3)_3^+/C_{12}H_{25}NMe_3^+$ and 2,6-didodecyl-β-cyclodextrin (Pr^iOH; cone-voltage 30 V)

Table 1 Response of ISEs to alkylammonium cations in the absence and presence of interferent ions using plasticised PVC-based membrane electrodes[a] (310 K)

Analyte	Ionophore	Slope	Detection limit −log[c]/mol dm^{-3}	pK_{pot}^{clin}
NMe_4^+	**1b**	58.8	6.0	4.3
$C_{12}H_{25}NMe_3^+$	**2d**	61.5	6.6	5.2
acetylcholine **8**[b]	**1c**	61.4	6.5	4.2
choline **9**	**1c**	61.4	6.4	3.4
methacholine **10**	**1c**	60.3	6.6	4.4

[a] pK^{pot} values were determined in a simulated clinical electrolyte (refs. 11,16). [b] Protein interference (*e.g.* 40 g dm^{-3} serum albumin) did not perturb the slope but raised the limit of detection to $10^{-5.1}$ mol dm^{-3}.

Whilst lipophilic cyclodextrins are very attractive ionophores for the sensitive detection of a broad spectrum of charge diffuse and size-matched cationic species, their relative lack of specificity needs to be addressed if practicable sensors are to be devised for environmental or clinical applications. For example,

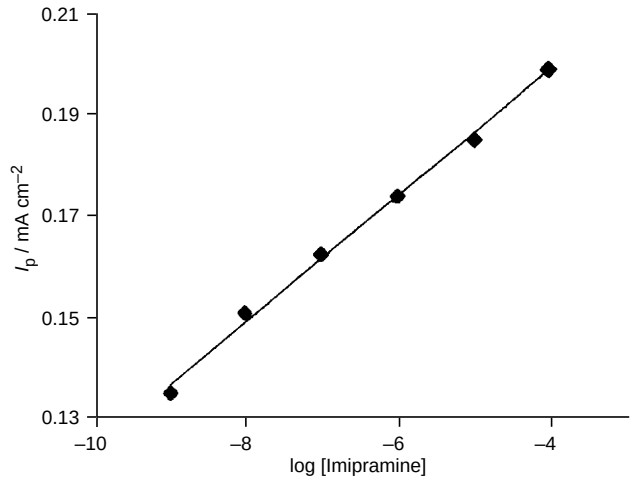
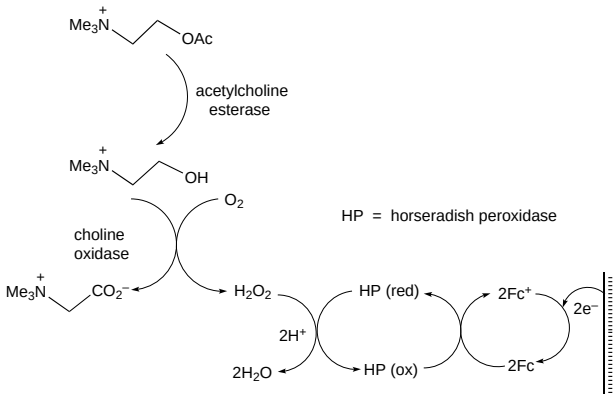


Fig. 4 Variation of current response, measured by differential pulse voltammetry, with concentration of the tricyclic anti-depressant drug imipramine **13**

while choline and acetyl choline are bound selectively by **1c** or **3**, compared to sodium, potassium, calcium or magnesium, various other cationic aryl species may compete in binding to the cyclodextrin host. By coupling the analysis into an enzymic relay, excellent *specificities* may be achieved with very low limits of detection. Typically biosensors based on this approach have allowed sub-micromolar levels of analytes to be measured, but lower limits of detection have only been achieved if 'preconcentration' techniques are used, *e.g.* microdialysis[21] or HPLC[22] separation. Generally these biosensors are not particularly robust devices that have a limited lifetime.

We have studied recently a biosensor based on the well-established[23] horseradish peroxidase–choline oxidase–acetylcholine esterase relay system (Scheme 1), which is known to allow sub-micromolar levels of acetylcholine to be assayed *via* the relayed amperometric detection of hydrogen peroxide. Using a screen-printed disposable electrode, successive layers were deposited, first the charge shuttle of 1,1′-bis(methoxymethyl)ferrocene **14** then the three enzymes in phosphate buffer and finally a thin encapsulating film of plasticised polyurethane containing 5% by weight of tri-*O*-ethyl-β-cyclodextrin and a lipophilic counterion. The resultant 'dip-type' sensor, after conditioning in aqueous buffer, allows *sub-picomolar* levels of acetylcholine to be measured with negligible interference from compounds such as ascorbic acid, dopamine and atropine (Fig. 5). The electrode was stable to dry storage in air for a period of at least four months and was active in solution for several weeks. Following continuous contact with a 10^{-10} mol dm^{-3} solution of acetylcholine, the measured current response had diminished only by 20%. Control experiments



Scheme 1

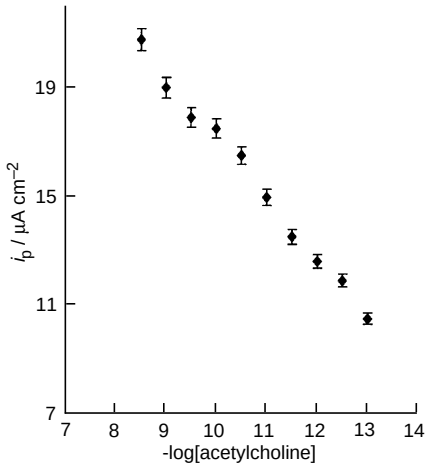


Fig. 5 Calibration curve for an amperometric enzyme electrode for acetylcholine based on a coated screen printed carbon electrode using the enzyme system, acetylcholine esterase–choline oxidase–horseradish peroxidase encapsulated with a plasticised polyurethane thin film containing 2,3,6-tri-*O*-ethyl-β-cyclodextrin (see Scheme 1)

using an electrode that did not contain, in turn, either the encapsulating thin film or the cyclodextrin derivative have been undertaken. An acetylcholine sensor without the lipophilic anion and the plasticised thin film responded to acetylcholine only at micromolar concentration levels and with nanoamp current levels. The sensor with the cyclodextrin included showed a much improved current response and could be accurately calibrated over a much wider range of substrate concentrations.

The β-cyclodextrin derivative may serve a dual function: not only does it mediate transport of the acetylcholine through the thin polymeric film, but it can also reversibly bind the ferrocene derivative[24] (especially in the ferricenium state) promoting the relay of charge to the electrode. Given that many compounds with widely different uses target the acetylcholine esterase enzyme, *e.g.* organophosphorus pesticides or various chemical warfare agents, then such species may also be detected readily and at low concentration using such a biosensor.[25] More generally, lipophilic cyclodextrins that are designed to bind with some selectivity to other important charge diffuse cations, *e.g.* dopamine, may be coupled into a variety of relayed biosensors and may give excellent specificity and sensitivity in detection.

Acknowledgements

It is a pleasure to thank the EPSRC for an Advanced Fellowship (R. K.), and the BBSRC for their support of this work.

David Parker was born in Consett, County Durham. He gained a first class honours degree in Chemistry from Oxford University in 1978 and a DPhil in 1980, working on asymmetric catalysis, under the supervision of Dr John M. Brown in the Dyson Perrins Laboratory. He was a NATO Fellow in 1980/81 working with Professor Jean-Marie Lehn at the Université Louis Pasteur, Strasbourg and was appointed to a Lectureship at Durham University in 1982. He was subsequently promoted to a Senior Lectureship in 1989 and to a Chair in Chemistry in 1992. At present he is the Chairman of the Department of Chemistry at Durham. He was awarded the Hickinbottom Fellowship by the Royal Society of Chemistry in 1988, the Corday Morgan Medal and Prize of the RSC for 1987 and the ICI Research Prize in Organic Chemistry in 1991. In 1996, he gained the Interdisciplinary Award of the RSC and his research interests embrace many aspects of complexation phenomena in solution.

Ritu Kataky was born in Shillong, Assam, India and took her degree at the Indian Institute of Technology, New Delhi, India. After lecturing at St. Mary's College, Shillong, she took a PhD under the supervision of Professor Arthur Covington working on ion-selective electrodes in clinical analysis. In 1988 she began a post-doctoral fellowship at Durham University and in 1992 was awarded an EPSRC Advanced Fellowship. Her research interests are in physical aspects of analytical chemistry, focusing on electroanalysis of clinically important species.

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