Selective sensing of guanidinium and tetraalkylammonium ions using lipophilic cyclodextrins

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A range of lipophilic cyclodextrin derivatives, including 2,6-didodecyl-α- and -β-cyclodextrins, and 2,3,6-tri-*O*-ethyl-β-cyclodextrin, has been examined as ionophores in electrode response studies of the detection of guanidine, alkylated guanidines, creatinine and several tetraalkylammonium ions including choline, acetylcholine and the long-chain cationic surfactants $C_nH_{2n+1}NMe_3^+$ ($n = 8 \longrightarrow 16$). Interference from Group Ia and IIa cations is minimal for alkylammonium ions ($-\log k^{pot} > 4$ typically) and in certain cases binding of the ion has been further defined by electrospray mass spectrometric and NMR methods.

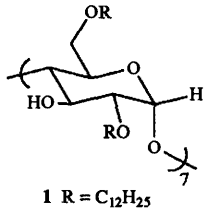
In order to detect cationic 'onium ions such as tetraalkylammonium or guanidinium derivatives using potentiometric sensors, a selective, neutral, lipophilic ionophore is required so that cation and pH interference is minimised. Most of the published work relating to the binding of tetraalkylammonium ions has focussed on anionic receptor molecules for which coulombic attraction dominates the generally non-selective binding interaction.[1-4] Although there are examples of neutral receptors designed to bind these charge-diffuse 'onium ions by 'cation–π' interactions,[5,6] none has been studied in detail for the purpose of selective analytical detection. We have previously reported that certain alkylated cyclodextrin derivatives are very good ionophores for the chemoselective detection of certain 'onium ions[7-9] and guanidinium cations.[10] Binding of the ion is considered to occur through a combination of hydrogen-bonding interactions and Van der Waals' forces. In the former case, according to the nature of the substrate, attractive –N–H • • • O and/or N–C–H • • • O interactions occur between the included ion and the array of oxygen lone-pairs on the cyclodextrin host. The attractive C–H • • • O hydrogen bonds may seem unusual, but in a recent analysis of the Cambridge database, 90% of the shortest measured C–H • • • O contacts involved $[N–C–H]^{\delta+}$ as the hydrogen bond donor.[11] Furthermore, in mass spectrometric measurements of the dissociation energies of gas-phase complexes between $\overset{+}{N}Me_4$ or $\overset{+}{N}Et_4$ with H_2O, MeOH, Me_2CO, DMF, Bu_2O and polyethers, the interaction between the 'onium ions and H_2O, MeOH and benzene was considerably weaker than with Bu_2O, and polyethers gave the highest values consistent with multiple, additive C–H • • • O interactions.[12,13] An approximate MNDO calculation on $\overset{+}{N}Me_4$ reveals that 72% of the positive charge resides on the peripheral hydrogen atoms, so that a fairly strong electrostatic component may be expected for the C–H • • • O interaction. Theoretical work has suggested that such hydrogen-bonding has a major electrostatic component that diminishes only slowly with increasing O • • • H separation.[14] Even with a C • • • O separation as long as 2.6 Å (and C • • • O separations as short as 2.39 Å have been reported in β-cyclodextrin inclusion complexes),[15] the interaction may be sufficient to force an sp^3 methyl group into an unfavourable eclipsing interaction.[16-18] Given the additional size-match between $\overset{+}{N}Me_4/\overset{+}{N}Et_4$ (7 and 8 Å ionic diameters) and the β-cyclodextrin cavity (*ca.* 8 Å in diameter), relatively weak inclusion complexes between these tetraalkylammonium ions and alkylated cyclodextrins are quite reasonable and may well involve multiple weak N–CH • • • O interactions.

There have been reports of the use of several neutral ligands as ionophores for the guanidinium ion. These include phosphoryl-containing podands,[19] bis-sulfonamide podands,[20] crown ethers[21] and functionalised calixarenes.[22] Interference from sodium or potassium ions is usually a problem but no reports discuss the problem of interference from the isoelectronic, neutral urea molecule.

With this background in mind, we now report extended electrode-response studies, targeting the selective detection of various substituted guanidinium and trimethylammonium ions, involving the use of dodecylated, octylated or perethylated α- and β-cyclodextrins each of which was prepared and characterised using standard methods.[7,8]

Results and discussion

The electroactive membranes which were tested contained 1.2%, by weight, of the appropriate cyclodextrin (*e.g.*, **1**, 2,6-di-*O*-dodecyl-β-cyclodextrin), 65.6% of the plasticiser, 2-nitrophenyl



1 R = $C_{12}H_{25}$

octyl ether, 32.8% of high molecular weight poly(vinyl chloride) and 0.4% of the lipophilic salt sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate. The membranes were prepared by standard methods[9] and were mounted in electrode bodies supplied by Fluka, usually using a 10^{-3} mol dm^{-3} NH_4Cl internal filling solution. Each electrode was conditioned in a 10^{-2} mol dm^{-3} analyte solution prior to usage. Calibration and selectivity data for each electrode were usually obtained at 310 K using the constant volume dilution method with selectivity coefficients being determined by the fixed interferent method. Recent work[10] has shown that in the detection of the guanidinium ion there is no significant difference in response between alkylated cyclodextrins with or without residual hydroxy groups. This indicates that the binding to the cyclodextrin ionophore involves N–H donation to the 2,6-ring oxygens.

The results obtained for the detection of guanidinium, **2**, and

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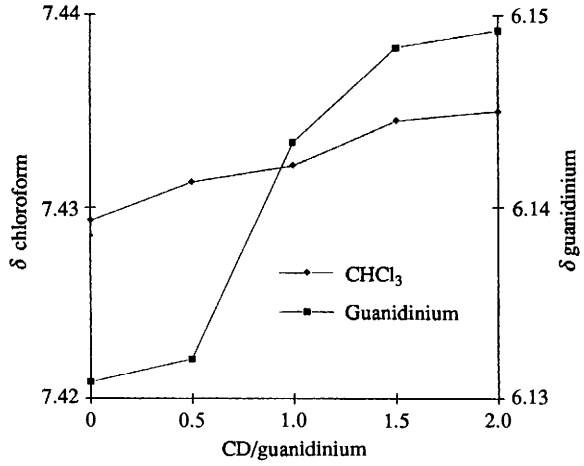
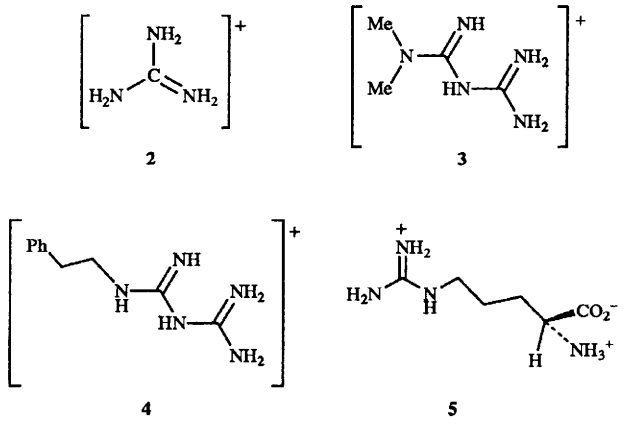


Fig. 1 Proton NMR titration of guanidinium triflate with 2,6-di-*O*-dodecyl-β-CD, **1**, in 12:7 $CDCl_3$–CD_3CN at 293 K. Chemical shifts of the guanidinium protons and the solvent chloroform protons are relative to the CHD_2CN proton chemical shift assigned the value of 1.93.



2 **3**

4 **5**

some *N*-substituted derivatives **3**, **4** and **5**, are consistent with those obtained earlier[10] and show good selectivity for the organic cations over the more charge-dense inorganic ions, Table 1. Over the pH range 2–5 there was less than a 5 mV change in the response of an electrode based on **1** to a 10^{-3} mol dm^{-3} solution containing the guanidinium ion. Using 'poly'-*O*-octyl-α-cyclodextrin, **13**, as the ionophore, the electrode response was almost *unaffected* by the presence of urea as interferent, $[NH_2CONH_2]$ = 5 mmol dm^{-3}, the slope was unchanged and the limit of detection only changed from $10^{-6.2}$ mol dm^{-3} to $10^{-5.4}$ mol dm^{-3}.

The drug metformin, **3**, which has been used to treat diabetes mellitus, responded well and like phenformin, **4**, exhibited improved selectivity in a simulated clinical background compared with the guanidinium ion itself. Arginine, the natural amino acid incorporating a guanidinium group in the side chain, **5**, responded much less well, although it showed a modest selectivity over lysine of about 8:1. A proton NMR titration was carried out (293 K 12:7 $CDCl_3$–CD_3CN) involving **1** and guanidinium trifluoromethanesulfonate (triflate). Although the observed shift in the guanidinium N–H resonance was small (Fig. 1), the variation of $\Delta\delta_H$ with added cyclodextrin concentration is consistent with 1:1 complexation and the shift to higher frequency may be associated with an enhanced degree of hydrogen-bonding. Further support for 1:1 complexation came from electrospray mass-spectrometric studies. In the presence of a 50-fold excess of NH_4OAc, a 5 × 10^{-5} mol dm^{-3} solution of **1** in propan-2-ol in the presence of 10 × 10^{-5} mol

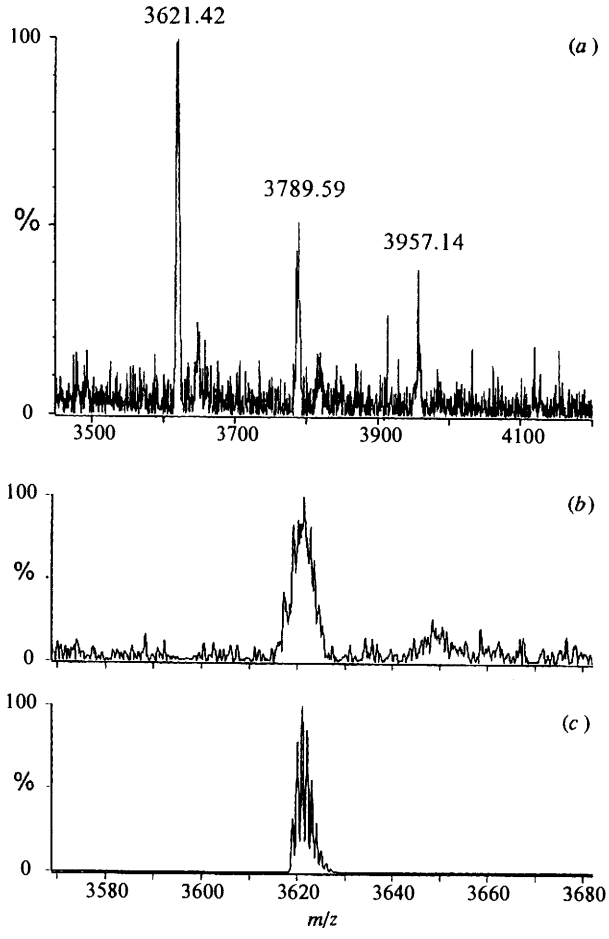
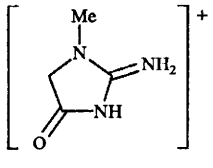


Fig. 2 Electrospray mass spectrum of the complex of metformin **3** with 2,6-di-*O*-dodecyl-β-CD **1**: (*a*) main peak (*m*/*z* = 3621.42) corresponds to **1** + **3**; secondary peaks at 3789.59 and 3957.14 correspond to **1** with 15 and 16 dodecyl groups, respectively; (*b*) experimental peak at *m*/*z* = 3621.42 of **1** + **3**; (*c*) calculated isotope pattern for the complex of **1** + **3**

dm^{-3} guanidinium chloride, gave rise to an ES mass spectrum in which a 1:1 guanidinium complex was observed (observed mass 3551.5; calculated 3551.9). Neither peaks due to the ammonium adduct at lower mass nor those expected at approximately half the mass, corresponding to a 2:1 complex, was discerned. Similarly 1:1 complexes with other guanidinium ions could be defined by the electrospray method, and for the complex between metformin, **3**, and **1** there was a reasonably good agreement between the observed and calculated isotope patterns (Fig. 2).

Creatinine, **6**, formally bears a structural resemblance to guanidinium ions but because of amide conjugation it is much less basic (pK_a = 4.7, 298 K, I = 0.1 mol dm^{-3}).[23] It is an important metabolite—being the end-product of creatine



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catabolism—which is produced in the liver and kidneys by the transfer of the guanidine moiety of arginine, **5**, to glycine followed by *N*-methylation. The clinical assay of creatinine is

Table 1 Response of ISEs incorporating 2,6-di-*O*-dodecyl β-cyclodextrin, **1**, to guanidinium ions in the absence and presence of interfering ions at 310 K. Selectivity coefficients for interferent ions are given as $-\log K_{i,j}^{pot}$; for individual ions the interferent concentration is 0.1 mol dm^{-3}

	Calibration		Interferent				
	Slope/mV	Limit of detection/ mol dm^{-3}	Clinical background[a]	Na^+	K^+	Ca^{2+}	NH_4^+
Guanidine, **2**	60.2	$10^{-5.7}$	2.7	2.9	1.3	3.9	1.7[d]
Guanidine, **2**[b]	61.5	$10^{-5.8}$	2.5	2.9	1.3	3.5	1.6
Metformin, **3**	61.4	$10^{-5.6}$	3.1				
Phenformin, **4**	55.6	$10^{-4.3}$	3.3				
Arginine,[c] **5**	60.2	$10^{-4.8}$	0.6				

[a] Clinical background is a simulated background of clinical ions (c/mmol dm^{-3}: Na^+ 145; K^+ 4.3; Ca^{2+} 1.26). [b] Using tri-*O*-octyl-β-cyclodextrin, **7**, as the ionophore. [c] Selectivity of arginine over lysine: $pK^{pot} = 0.9$. [d] Selectivity of guanidine over H_3O^+: $pK^{pot} = 1.9$.

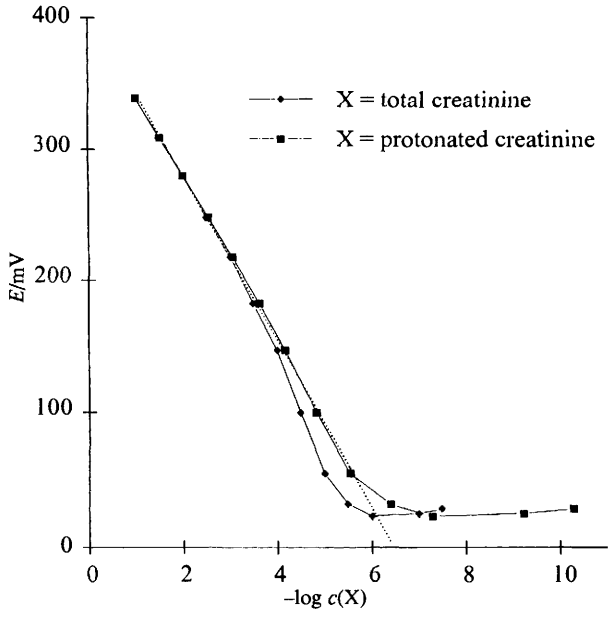


Fig. 3 Response of an ISE incorporating 2,6-di-*O*-dodecyl-β-cyclodextrin, **1**, to creatinine hydrochloride solutions in deionized water. Experimental results are plotted as total creatinine; protonated creatinine values are corrected for dissociation owing to the low pK_a of creatinineH^+. The correction results in a Nernstian response, shown by linear regression fit ($R^2 = 0.999$).

important in monitoring renal function, and so its selective detection is a significant challenge. Neutral hosts for creatinine that rely on directed hydrogen bonding in non-polar media have been sought, as possible sensing components in optical sensors.[24,25] To date, there are no neutral ionophores for creatinine that have been developed for the potentiometric analysis of this important clinical metabolite whose concentration in blood plasma is of the order of 50 μmol dm^{-3}. A preliminary experiment was carried out using **1** as the lipophilic cyclodextrin in a conditioned membrane electrode. The electrode response, both in dip-type and in flow experiments, revealed apparent super-Nernstian behaviour as the concentration of creatinine‡ fell below 0.1 mmol dm^{-3} (Fig. 3). This behaviour is explained by the low pK_a of protonated creatinine: the electrode responds only to the protonated (charged) species and as the total concentration of creatinine (protonated and non-protonated) decreases, the equilibrium in eqn. (1) is driven to the right so that the concentration of protonated creatinine

‡ Creatinine solutions were always prepared freshly, so that on the timescale of the experiments less than 1% hydrolysis to creatine (an amino acid) had occurred, as deduced by UV analysis.

$$[\mathbf{6}\cdot H]^+ + H_2O \rightleftharpoons [\mathbf{6}] + [H_3O^+] \quad (1)$$

$$K_a = \frac{[\mathbf{6}][H_3O^+]}{[\mathbf{6}\cdot H]^+} = 10^{-4.7} \text{ mol dm}^{-3}$$

decreases more rapidly than the total concentration of both creatinine species. After correcting for this effect and with the assumption that $[\mathbf{6}] = [H_3O^+]$ (Fig. 3), a linear response is observed down to micromolar concentrations. In order to maintain creatinine in its protonated form, dilution experiments were carried out using 10^{-3} mol dm^{-3} hydrochloric acid solution in place of neutral water. The results obtained (Table 2), reveal that creatinine sensing was more or less independent of the nature of the cyclodextrin used (**1**, **7**, **8** or **9**: α *vs.* β, octyl *vs.* dodecyl) and the interference from clinical ions was of a similar order of magnitude to that observed with the guanidinium ion (Table 1). This similarity of response again implies that the primary binding interaction involves N–H donation to the cyclodextrin ether lone-pairs and the analyte must not be significantly included within the cavity.

Alkylammonium ions

A quite different situation arises with alkyltrimethylammonium ions because the cyclodextrin cavity has a diameter that corresponds closely to the ionic diameter of these ions. Evidence for size-selective 'onium inclusion has already been reported.[9] As the size of the alkylammonium ion is increased, the cation is unable to be included within or 'perch' on the cyclodextrin and the binding interaction is weakened. Such an effect has been studied in the following cell:

$$\text{Ag, AgCl} \left| \begin{matrix} 10^{-2} \text{ mol dm}^{-3} \\ NR_4^+\ Cl^- \end{matrix} \right| \text{membrane} \left| \begin{matrix} 10^{-3} \text{ mol dm}^{-3} \\ NH_4^+\ Cl^- \end{matrix} \right| \text{AgCl} \left| \text{Ag} \right|$$

$$-\Delta G = nFE$$

using a variety of tetraalkylammonium ions **14a–14f** (R = Me ⟶ R = Hexyl) as the analyte. The cell free energies were compared using 10^{-2} mol dm^{-3} analyte solutions and the fall of $-\Delta G$ with increasing size of the 'onium ion (Fig. 4) is consistent with the preferred (stronger) binding of the smaller, size-matched NMe_4^+ and NEt_4^+ ions (Table 3). This general pattern of behaviour is also exhibited in the variation of the overall cation selectivity coefficient, in a simulated clinical electrolyte background, (c/mmol dm^{-3}: Na^+, 145; K^+, 4.3; Ca^{2+}, 1.26) with 'onium ion chain length, **14a–14f** (R = Me ⟶ R = *n*-hexyl), Table 4. With an electrode based on tri-*O*-octyl-β-cyclodextrin, **7**, overall selectivities of $10^{4.9}$:1 were observed with NEt_4^+, falling to $10^{3.3}$ for the larger tetra-*n*-hexylammonium ion.

Acetylcholine, **17**, is an important neurotransmitter and lipophilic neutral organic molecules that can bind to it

Table 2 Response of ISEs incorporating alkylated cyclodextrins to creatinine in the absence and presence of interfering ions at 310 K

	Deionized water	10^{-3} mol dm^{-3} HCl		Clinical background[a]	10^{-3} mol dm^{-3} HCl in clinical background
	$Slope_{initial}$/mV	Slope/mV	Limit of detection at pH 3/mol dm^{-3}	pK^{pot}	pK^{pot}
2,6-Didodecyl β CD, **1**	57.4	57.4	$10^{-5.0}$	2.7	2.5
2,3,6-Trioctyl β CD, **7**	57.6	54.5	$10^{-5.0}$	2.4	—
2,6-Dioctyl α CD, **8a**	58.2	57.1	$10^{-5.1}$	2.5	—
'poly'octyl α CD,[b] **9**	55.7	55.6	$10^{-5.1}$	2.5	2.5

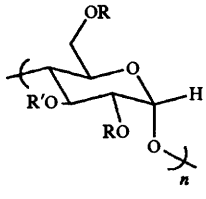
[a] pK^{pot} = −log (selectivity coefficient) in a simulated clinical background (c/mmol dm^{-3}: Na^+ 145, K^+ 4.3, Ca^{2+} 1.26). [b] Only partially octylated at the 3 position; 2.6 free OH groups remaining, on average.[8]

Table 3 Response of ISEs incorporating alkylated cyclodextrins to NMe_4^+ in the presence of a simulated background of clinical ions (310 K; c/mmol dm^{-3}: Na^+ 145; K^+ 4.3; Ca^{2+} 1.26)

Cyclodextrin ionophore	Slope/mV	pK^{pot}_{clin}
Triethyl-β, **10**	59.3	3.4
Dioctyl-β, **8b**	54.6	4.3
Trioctyl-β, **7**	56.5	4.0
Didodecyl-β, **1**	56.1	4.1
Me-didodecyl-β, **11**	57.1	4.9
Didodecyl-α, **12**	58.8	4.3

Table 4 Response of ISEs incorporating tri-*O*-octyl-β-cyclodextrin, **7**, to various tetraalkylammonium ions, **14a–14f**, in a simulated clinical electrolyte background

	NR_4^+ R	pK^{pot}_{clin}
14a	Methyl	4.0
14b	Ethyl	4.9
14c	*n*-Propyl	4.9
14d	*n*-Butyl	4.4
14e	*n*-Pentyl	4.7
14f	*n*-Hexyl	3.3



7 R = R′ = C_8H_{17}; n = 7
8a R = C_8H_{17}; R′ = H; n = 6
b R = C_8H_{17}; R′ = H; n = 7
9 R = C_8H_{17}; 50% R′ = C_8H_{17}, 50% R′ = H; n = 7
10 R = R′ = Et; n = 7
11 R = $C_{12}H_{25}$; R′ = Me; n = 7
12 R = $C_{12}H_{25}$; R′ = H; n = 6
13 R = C_8H_{17}; 57% R′ = C_8H_{17}, 43% R′ = H; n = 6
14 R_4N^+
14a R = Me **b** R = Et
c R = Pr^n **d** R = Bu^n
e R = *n*-pentyl **f** R = *n*-hexyl

selectively are of interest not only from the viewpoint of analysis but also with regard to cation transport. Transport of acetylcholine through cellular lipid membranes exemplifies this process *in vivo*. The electrode response of **17** and of the related ions choline, **16**, and methacholine, **15**, has been studied comparatively (Table 5). The electrode response characteristics

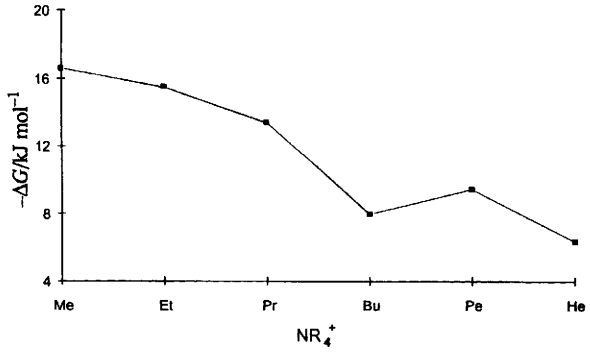
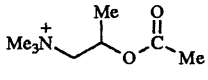


Fig. 4 Comparative $-\Delta G$ ($= -nEF$) values at 298 K of ion-selective membranes incorporating tri-*O*-octyl-β-cyclodextrin, **7**, in their response to tetraalkylammonium ions in the cell

Ag, AgCl | 10^{-2} mol dm^{-3} NR_4Cl (**14a–f**) | Membrane | 10^{-2} mol dm^{-3} NH_4Cl | AgCl, Ag



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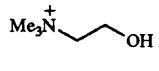

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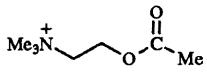

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towards these ions were very good, with very low (sub-micromolar) limits of detection and Nernstian slopes. Selectivity over a simulated clinical electrolyte background was in line with the results obtained for $\overset{+}{N}Me_4$ and $\overset{+}{N}Et_4$, with overall values of the order of 10^4:1 being found. The selectivity of a poly-octyl-β-cyclodextrin based electrode towards acetylcholine over the more hydrophilic ion, choline, **16**, is notable. With this electrode, protein interference (40 g dm^{-3} of bovine serum albumin) in solution was also only modest (Fig. 5), suggesting that appropriately treated clinical samples could be analysed using such methods.[26]

Long-chain alkyl trimethylammonium ions are cationic surfactants which also possess important antibacterial and antifungal properties, which have promoted their use in household and industrial disinfectant formulations. There are no reports of ion-selective electrodes for such cationic surfactants operating *via* the selective binding of the cation to a neutral ionophore. In contrast, there are innumerable reports of surfactant-selective membrane electrodes, functioning by an ion-exchange principle.[27,28] Typically, plasticised PVC membranes have been used in which 'anionic' impurities in the PVC matrix promote ion-pairing and lead to selective ion-exchange. The ion-exchange site excludes a co-ion from the membrane and thus generates permioselectivity to the objective surfactant ion, through the membrane. Cyclodextrins are well known to form 1:1 and 2:1 complexes with long-chain cationic and anionic surfactants,[29] involving a conventional 'hydrophobic

Table 5 Response of ISEs incorporating lipophilic cyclodextrins to various choline ions **15**, **16** and **17** (310 K)

Analyte	Membrane[b,c]	Slope/mV	Limit of detection/ mol dm^{-3}	pK^{pot} clinical background	pK^{pot} choline
Methacholine chloride	'Poly'octyl α CD, **13**/*o*NPOE	60.3	6.7	4.4	—
	Didodecyl β CD, **1**/*o*NPOE	61.4	6.2	4.3	—
Choline chloride	'Poly'octyl α CD, **13**/*o*NPOE	61.4	6.4	3.4	—
	Didodecyl β CD, **1**/*o*NPOE	61.1	5.9	3.4	—
Acetylcholine chloride	'Poly'octyl α CD, **13**/*o*NPOE	61.6	6.5	4.2	1.2
	'Poly'octyl β CD, **9**/*o*NPOE[a]	60.0	5.1	—	1.8
	'Poly'octyl β CD, **9**/BBPA[a]	52.0	3.1	—	—

[a] Electrode internal filling solution: 10^{-2} mol dm^{-3} acetylcholine chloride. [b] BBPA is bis(butylpentyl)adipate; *o*NPOE is *o*-nitrophenyl octyl ether. [c] 'Poly'-octyl-α-CD, **13**, contains 15.4 octyl groups on average; 'poly'-octyl-β-CD, **9**, contains 17.4 octyl groups on average.[8]

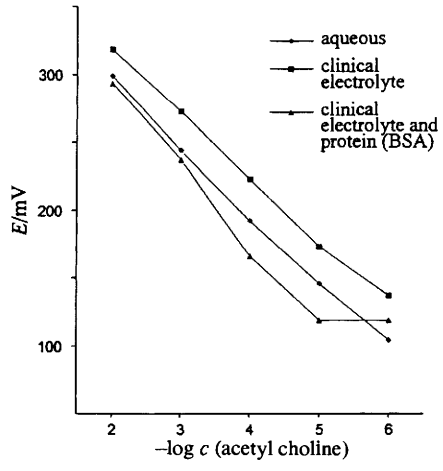

Fig. 5 Response of a 2,6-di-*O*-dodecyl-β-cyclodextrin, **1**, membrane to acetylcholine chloride solutions in deionized water, simulated clinical electrolyte background (*c*/mmol dm^{-3}: Na^+, 145; K^+, 4.3; Ca^{2+}, 1.26) and simulated electrolyte background plus 40 g dm^{-3} bovine serum albumin (295 K)

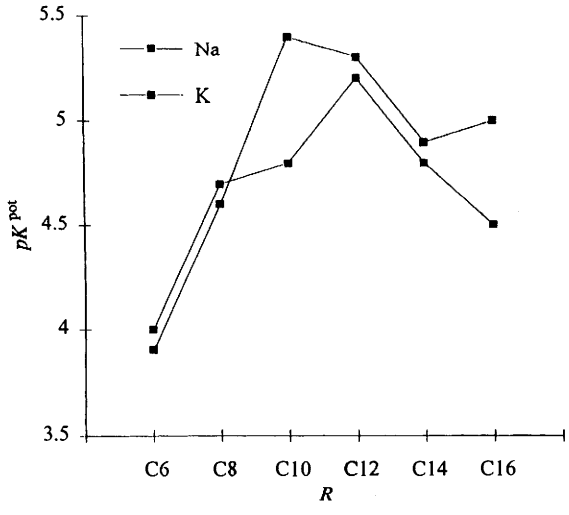

Fig. 6 Selectivity of response, pK^{pot} (= $-\log K^{pot}_{i,j}$), of 'poly'-*O*-octyl-β-cyclodextrin, **9**, membrane electrodes to cationic surfactants of various chain lengths, **18a–f**, in the presence of 0.1 mol dm^{-3} NaCl or KCl as interferents (298 K)

effect' interaction, for which a plateau of complex stability has been observed with chain lengths of 10 or more carbon atoms. Given this, and the observations of 'onium ion binding by lipophilic cyclodextrins, electrode response studies involving the detection of a series of alkyltrimethylammonium ions, **18a–18f**, **19** and **20** were undertaken, Table 6, working at 298 K.

$$R\overset{+}{N}Me_3Br^-$$

18a R = *n*-C_6H_{13} **b** R = *n*-C_8H_{17} **c** R = *n*-$C_{10}H_{21}$
d R = *n*-$C_{12}H_{25}$ **e** R = *n*-$C_{14}H_{29}$ **f** R = *n*-$C_{16}H_{33}$

$$R_2\overset{+}{N}Me_2Br^- \qquad H_{29}C_{14}\text{–}\overset{+}{N}(CD_3)_3I^-$$

19 R = $C_{10}H_{21}$ **20**

The results obtained were very encouraging. Using the less polar plasticiser, 'di-octyl' sebacate (DOS), and working at concentrations *below* the critical micelle concentration of the surfactant, Nernstian responses were obtained for all of the ions examined with selectivities often in excess of 10^5:1 over Na^+, K^+, Ca^{2+} and Mg^{2+} (Table 6). Sub-micromolar limits of detection were observed in all cases, except for the bis-C_{10} dialkylammonium analyte, **19**, which showed response characteristics more typical of a *short*-chain alkylammonium ion, suggesting that the binding interaction did *not* involve chain inclusion or 'host–guest' hydrophobic chain interactions.

Highest selectivities over interfering Na^+ and K^+ ions were found for the C_{10} and C_{12} long chain 'onium ions (Fig. 6), and this *peak* selectivity may be attributed to more favourable 'hydrophobic interactions' between the alkyl chains of the analyte and the alkylated cyclodextrin. That discrete 1:1 complexes are formed between these long-chain ammonium ions and the lipophilic cyclodextrin ionophore has been established by electrospray mass spectrometry. For example, with tetradecyl(trimethyl)ammonium bromide, **18e**, a 1:1 complex was observed with 'poly'-octyl-β-cyclodextrin, **9**, and peaks at 3186.9 (calc. 3186.9), 3298.3 (3299.0) and 3411.3 (3411.4) were observed corresponding to the complexes in which the cyclodextrin has 16, 17 and 18 octyl groups, respectively. When 2,6-di-*O*-dodecyl-β-cyclodextrin, **1**, containing some over-alkylated impurity (*i.e.*, 15 dodecyl chains instead of 14), was challenged with a 50:50 mixture of $(CH_3)_3\overset{+}{N}C_{12}H_{25}$ and $(CD_3)_3\overset{+}{N}C_{12}H_{25}$ in wet propan-2-ol solution, the resultant electrospray mass spectrum (Fig. 7) revealed two equally intense sets of peaks 9 mass units apart. Given that $(CD_3)_3\overset{+}{N}C_{12}H_{25}$ also responded identically with its protiated analogue in electrode response studies (Table 6), it is evident that deuterium substitution has no obvious effect on complex formation. This is expected because a C–D bond is very similar in length to a C–H bond[30] and they are electronically identical. Given that the major electrostatic potential component of hydrogen bonding in C–H···O interactions only varies as $1/r$, no significant changes in binding energy are likely to be evident in these sorts of experiment. Of course, these two experiments do allow the $-\overset{+}{N}(CD_3)_3$ grouping to be confidently used as an NMR binding probe

Table 6 Response of ISEs incorporating 'poly'-octyl-β-cyclodextrin, **9**, to various long-chain alkylammonium ions ($R\overset{+}{N}Me_3$; R = C_nH_{2n+1}, **18a–18f**) at 298 K

	R								
	C_6	C_8	C_{10}	C_{12}[b,c,d]	C_{14}	C_{14}	C_{16}	C_{16}	$(C_{10})_2$[c]
cmc/mol dm^{-3}	—	1.4×10^{-1}	6.8×10^{-2}	1.6×10^{-2}	3.6×10^{-3}	3.6×10^{-3}	9.2×10^{-4}	9.2×10^{-4}	1.85×10^{-3}
Plasticiser	DOS	DOS	DOS	DOS	*o*NPOE	DOS	*o*NPOE	DOS	DOS
Calibration									
$c_{initial}$/mol dm^{-3}	$10^{-1.5}$	$10^{-2.0}$	$10^{-2.0}$	$10^{-3.0}$	$10^{-3.0}$	$10^{-3.0}$	$10^{-3.5}$	$10^{-3.5}$	$10^{-3.0}$
Slope/mV	59.6	59.6	58.6	58.4	58.4	58.8	52.8	58.6	59.1
Limit of detection (approximate)/mol dm^{-3}	$10^{-6.7}$	$10^{-6.1}$	$10^{-6.1}$	$10^{-6.6}$	$10^{-6.3}$	$10^{-6.2}$	$10^{-6.2}$	$10^{-6.6}$	$10^{-5.6}$
Selectivity,[a] pK^{pot}									
$c_{initial}$/mol dm^{-3}	$10^{-2.5}$	$10^{-2.5}$	$10^{-3.0}$	$10^{-3.0}$	$10^{-3.0}$	$10^{-3.0}$	$10^{-3.5}$	$10^{-4.5}$	10^{-4}
Na^+	4.0	4.7	4.8	5.2	4.0	4.8	3.6	4.5	4.0
K^+	3.9	4.6	5.4	5.3	4.0	4.9	—	5.0	3.9
Ca^{2+}	5.7	6.3	5.7	5.8	4.4	5.5	—	5.5	4.0
Mg^+	5.9	6.0	6.1	5.6	3.2	5.3	—	4.8	3.8

[a] pK^{pot} values calculated using the concentration corresponding to a deviation from linearity of *S* log 2 mV. [b] Using di-*O*-dodecyl-β-cyclodextrin with a DOS plasticiser, a limit of detection of $10^{-6.3}$ was observed with an electrode slope of 58 mV decade^{-1} and selectivity coefficients (0.1 mol dm^{-3} interferent), pK^{pot} = 4.9 (Na^+), 5.1 (K^+), 5.8 (Mg^{2+}) and 6.2 (Ca^{2+}). [c] Very similar response characteristics were observed using 'poly'-octyl-α-cyclodextrin, **13**, as the ionophore. [d] Identical response characteristics were found using $(CD_3)\overset{+}{N}C_{12}H_{25}$ as the analyte.

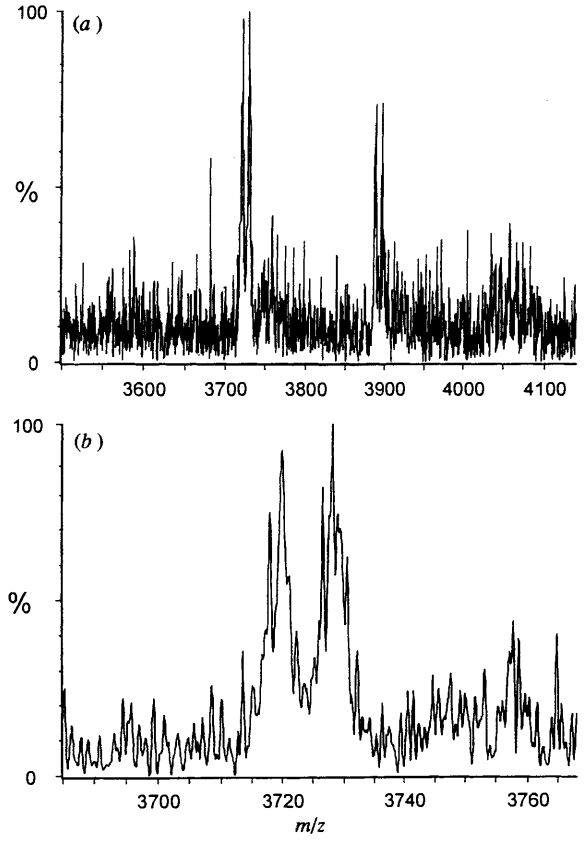

Fig. 7 Electrospray mass spectrum of a 1:1 molar mixture of dodecyltrimethylammonium bromide, **18d**, and its deuteriated analogue, **20**, with 2,6-di-*O*-dodecyl-β-cyclodextrin, **1**, in propan-2-ol: (*a*) shows two major sets of peaks corresponding to **18d** + 1 and **20** + 1 and a secondary pair corresponding to an additional dodecyl chain on the cyclodextrin; (*b*) shows in detail the main pair of peaks **18d** + 1 and **20** + 1

and ^2H NMR studies are underway to define further the nature of this cyclodextrin interaction.

The addition of non-ionic surfactants to aqueous solutions of cationic surfactants leads to a lowering of the critical micelle concentration (cmc) due to the formation of mixed micelles. The effect of adding Triton X-100 [isooctyl-*p*-phenoxypoly(ethoxy)-ethanol, **21**, cmc = 3.3×10^{-4} mol dm^{-3} at 298 K] to $C_{14}H_{29}\overset{+}{N}Me_3$, **18e** (cmc = 3.6×10^{-3} mol dm^{-3} at 298 K), has been examined, measuring the cmc of the mixed surfactant solution and examining the effect on electrode response studies.

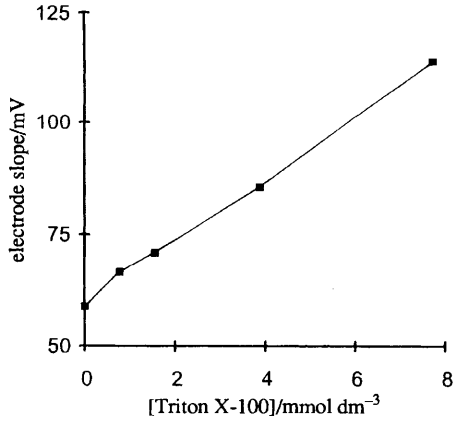

Fig. 8 Slope of electrode response of an ISE incorporating 'poly'-*O*-octyl-β-cyclodextrin, **9**, to $C_{14}H_{29}NMe_3Br$, **18e**, in the presence of various concentrations of the non-ionic surfactant, Triton X-100, **21**, at 298 K

iso-C_8H_{17}-$C_6H_4O(C_2H_4O)_nH$
(average $n \approx 10$)
21 (Triton X-100)

Addition of increasing amounts of Triton X-100 (0.05–0.5% w/v corresponding to concentrations of 10^{-3}–10^{-2} mol dm^{-3}) to solutions containing $C_{14}H_{29}\overset{+}{N}Me_3$, using an electrode involving poly-*O*-octyl-β-cyclodextrin, **9**, led to an increase in the slope of the electrode response (Fig. 8), from 59 mV (zero addition) to 114 mV (in the 'onium ion concentration range 10^{-3}–10^{-6} mol dm^{-3}). The effect of adding this non-ionic surfactant on the cell emf was also studied at three different concentrations of **18e** (10^{-3}, $10^{-3.5}$ and 10^{-4} mol dm^{-3}). Increasing the amount of the Triton X-100 led to a reduction in the measured cell emf ($\Delta G = -nFE$) and the effect was most

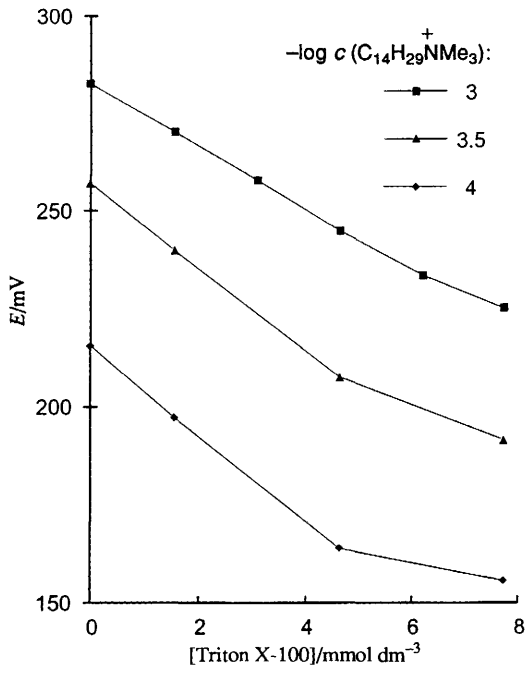

Fig. 9 Potentiometric response of an ISE incorporating 'poly'-*O*-octyl-β-cyclodextrin, **9**, to three concentrations of $C_{14}H_{29}NMe_3Br$, **18e**, in the presence of various concentrations of the non-ionic surfactant, Triton X-100, **21**, at 298 K

pronounced at lower 'onium ion concentrations (Fig. 9). Tensiometric methods were used to measure the cmc of the mixed micellar system. The cmc of a 1:1 mixture of Triton X-100 and **18e** was 4×10^{-4} mol dm^{-3}, while a 10:1 mixture gave a value of 2.3×10^{-4} mol dm^{-3}. It is clear that at concentrations *above* the cmc of the mixed micellle, the free 'onium ion concentration will be reduced and super-Nernstian slopes are expected because the total concentration of 'onium ion assumed (Fig. 7) is much more than that which is available to be sensed by the electrode. The more pronounced effect of adding Triton X-100 on the cell emf at lower 'onium ion concentrations is simply related to the changing composition of the mixed micelle at higher non-ionic surfactant concentrations accompanied by the diminution in cmc.

Conclusions

These studies indicate that lipophilic cyclodextrins are useful ionophores for the chemoselective detection of a variety of 'onium ions. The electrode response studies reveal a particularly good response to alkyltrimethylammonium ions, such as acetylcholine and cationic surfactants. The overall binding interaction is likely to be made up of a series of weak but additive C–H···O and/or N–H···O hydrogen-bonding interactions. The application of these findings, in the analysis of clinically important analytes, *e.g.*, cholines, or in the selective transport of cations such as acetylcholine is being pursued.

Experimental

Proton NMR spectra were recorded on a Bruker AC 250 (250.13 MHz) spectrometer. For the guanidinium titration, chemical shifts are quoted relative to the acetonitrile shift which was assigned the value of 1.93 ppm. The solvent system was 60:35 [^{2}H]chloroform–[$^2H_3$]acetonitrile. ^{13}C NMR spectra were recorded on a Varian VXR 400, a Bruker AC250 or a Bruker AMX 500 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1600 FT IR instrument.

Electrospray ionisation mass spectra were recorded with a VG Platform II (Fisons Instruments) employing MassLynx software. Samples were presented as solutions in propan-2-ol at a flow rate of 0.01 cm^3 min^{-1} (typical composition 50 μmol dm^{-3} cyclodextrin derivative, 100 μmol dm^{-3} analyte cation or 5 mmol dm^{-3} ammonium acetate). Measurements were made in the positive ion mode with a cone voltage ramp of 60–120 V. Mass scale calibration employed the ammonium adducts from polypropylene glycols 2000 and 3000 (1 g dm^{-3}).

Potentiometric studies

Membrane preparation. The membranes were prepared by dissolving 1.2% cyclodextrin, 65.6% plasticiser [2-nitrophenyl octyl ether or bis(2-ethylhexyl)sebacate], 32.8% PVC (high molecular weight, Fluka) and 0.4% lipophilic anion {sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate} in distilled tetrahydrofuran and casting the solution in a glass ring resting on a sheet of plate glass. Slow evaporation was achieved by weighting down a pad of filter papers on top of the ring. Discs of 7 mm diameter were cut from these master membranes and mounted in standard electrode bodies supplied by Fluka.

Dip type measurements. These were made in a thermostatted double walled glass vessel.

Constant volume dilution flow measurements. These were made using a small volume (approximately 2.3 cm^3) thermostatted double walled glass cell equipped with inlet and outlet capillaries and a miniature magnetic follower. Both methods employed a T-shaped thermostatted liquid junction configuration in which the analyte solution flowed, or was held stationary (dip type), over a capillary containing a saturated KCl bridge solution in contact with a saturated calomel reference electrode (Russell pH Ltd). The solution was drawn through tubing past the junction by a peristaltic pump (Gilson Minipuls 3).

The ion-selective electrode and reference electrode were connected to a digital multimeter (Keithley 197) or ion-selective meter (Unicam, model 9460) and chart recorder (Kipp & Zonen) *via* a buffer amplifier. The system was thermostatted using a Techne Tempette junior TE-85 Thermostat bath.

Analytes

Analar NaCl, KCl, $CaCl_2$ (1 mol dm^{-3} volumetric solution), $MgCl_2 \cdot 6H_2O$ and Convol HCl solution were obtained from BDH, Microselect NH_4Cl from Fluka. Guanidine (99+%), 1,1-dimethylbiguanide (metformin), phenformin and creatinine hydrochlorides were obtained from Sigma, L-arginine (> 99.5%) and L-lysine (> 99%) monohydrochlorides from Fluka. Tetramethyl- (> 98%), tetra-*n*-butyl- (> 97%) and tetra-*n*-hexyl- (> 98%) ammonium chlorides were obtained from Fluka, tetraethyl- and tetra-*n*-pentyl- (99%) ammonium chlorides from Aldrich and tetra-*n*-propylammonium chloride from Eastman Kodak. Choline (99+%) and acetylcholine (98%) chlorides came from Aldrich, acetyl-β-methylcholine (methacholine) chloride from Sigma. *n*-Hexyl- (98%), *n*-octyl- (97%) and *n*-decyl-trimethylammonium bromides were obtained from Lancaster Synthesis, and dodecyl-, myristyl- (99%) and cetyltrimethylammonium bromides from Aldrich. Didecyl(dimethyl)ammonium bromide (> 97%) came from Fluka. Triton X-100 was obtained from Aldrich.

Hygroscopic salts were dried overnight on a freeze drier or vacuum line and solutions were made up in deionised water.

Syntheses—cyclodextrin derivatives

Hexakis(2,6-di-*O*-octyl)-α-cyclodextrin, **8a**, hexakis(2,3,6-'poly'-*O*-octyl)-α-cyclodextrin, **13**, heptakis(2,6-di-*O*-octyl)-β-cyclodextrin, **8b**, heptakis(2,3,6-'poly'-*O*-octyl)-β-cyclodextrin, **9** and heptakis(2,3,6-tri-*O*-octyl)-β-cyclodextrin, **7**, were synthesized by the procedures of Parker *et al.*[8] Hexakis(2,6-di-*O*-

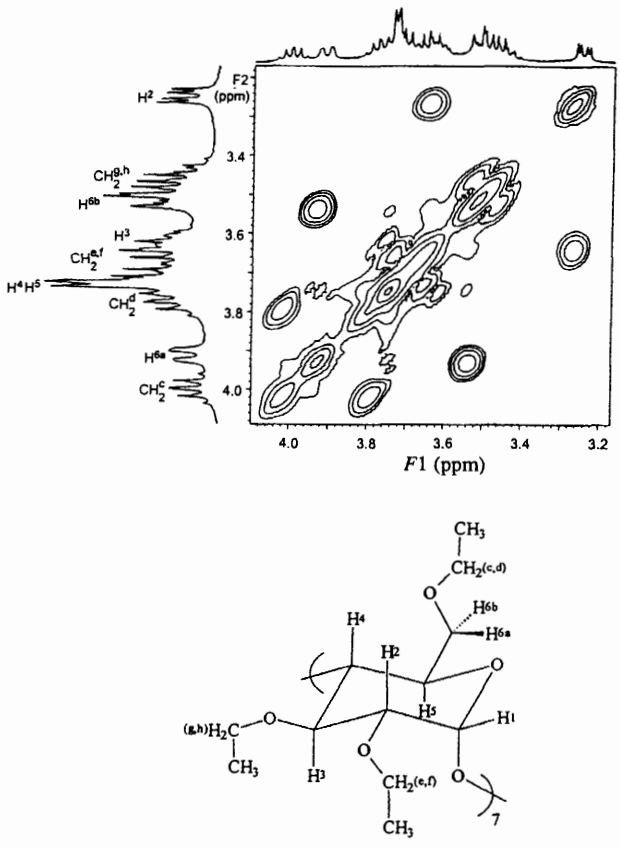

Fig. 10 Partial ^1H–^1H COSY NMR spectrum of 2,3,6-tri-*O*-ethyl-β-cyclodextrin, **10**

dodecyl)-β-cyclodextrin, **1**, was synthesized according to the method of Wenz.[31]

Heptakis(2,6-di-*O*-dodecyl-3-*O*-methyl)-β-cyclodextrin, 11 [heptakis(2,6-di-*O*-dodecyl-3-*O*-methyl)cyclomaltoheptaose]. Heptakis(2,6-di-*O*-dodecyl)-β-cyclodextrin (0.6 g) was treated with powdered NaH (500 mg) and methyl iodide (1.0 g) in stirred, dry tetrahydrofuran (30 cm^3) under N_2 at 50 °C for 3 days. After 24 h, additional NaH (500 mg) and methyl iodide (0.5 g) were added to the reaction mixture. After 3 days, methanol (5 cm^3) was added dropwise to destroy excess NaH, the solvent was removed under reduced pressure and the residue taken up in dichloromethane (80 cm^3). The solution was washed with distilled water (3 × 20 cm^3), dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure to give a yellow oil. Chromatography (100% *n*-hexane; SiO_2) of the yellow oil gave the desired product as a clear oil (0.56 g, 90%). *m/z* (ES^+, 50 μmol dm^{-3} cyclodextrin derivative, 5 mmol dm^{-3} ammonium acetate in propan-2-ol) 3607.6 $(M + 18)^+$, 1812.9 $(M/2 + 18)^+$ for 14 dodecyl groups, 3762.6 $(M + 18)^+$ for 15 dodecyl groups, minor peak; ν_{max}(thin film)/cm^{-1} 2923s, 2853s, 1465m, 1375w, 1142m and 1039m; $\delta_H(CDCl_3)$§ 5.10 (7 H, d, J = 3.0 Hz, H^1), 3.84* (7 H, m, H^4), 3.65* (7 H, m, H^5), 3.59* (7 H, m, H^{6a}), 3.56 (21 H, s, $-OCH_3$), 3.43 (7 H, m, H^3), 3.41* (7 H, m, H^{6b}), 3.32 (14 H, t, J = 7 Hz, $-CH_2O$), 3.31 (14 H, t, J = 7 Hz, $-CH_2O'$), 3.15 (7 H, dd, J = 9.5, 3.0 Hz, H^2), 1.50 (28 H, m, $-CH_2CH_2O$), 1.18 (252 H, br s, CH_2) and 0.82 (42 H, t, J = 7.2 Hz, CH_3); $\delta_C(CDCl_3)$§ 98.5 (C^1), 82.1 (C^3), 80.6 (C^2), 79.1* (C^5), 71.7 (CH_2O), 71.14* (C^6), 71.07* (C^4), 69.6 (CH_2O'–), 61.6 ($-OCH_3$), 32.1 (CH_2), 30.2 (CH_2CH_2O), 29.96, 29.91, 29.88, 29.83, 29.75, 29.72, 29.61, 29.53, 29.49, 29.46 (CH_2 and CH_2CH_2O'), 26.5, 26.3, 26.1 CH_2) and 22.8 (CH_3).

Heptakis(2,3,6-tri-*O*-ethyl)-β-cyclodextrin, 10¶ [heptakis-(2,3,6-tri-*O*-ethyl)cyclomaltoheptaose]. Powdered β-cyclodextrin hydrate (1.5 g, 1.3 mmol) was dried overnight under reduced pressure. Anhydrous DMF (30 cm^3) and sodium hydride (2.1 g, 88 mmol) were added sequentially and the solution was stirred under a nitrogen atmosphere for 1 h. Ethyl bromide (7 cm^3, 44 mmol) was added in three equal portions over 1 h, and stirring was continued for one week under a nitrogen atmosphere. Further daily additions of sodium hydride (0.3 g, 12 mmol) and ethyl bromide (3 cm^3, 19 mmol) were made to the reaction mixture until the fourth day. After 7 days, methanol (10 cm^3) was added and the solvent was evaporated off under reduced pressure. Dichloromethane (100 cm^3) and distilled water (30 cm^3) were added to the residue and the lower organic layer was separated, washed with distilled water (3 × 30 cm^3) and dried over anhydrous sodium sulfate. Filtration and removal of the solvent under reduced pressure gave a yellow oil.

Column chromatography (0.5% methanol–dichloromethane; silica) gave a white glassy solid (1.1 g, 49%); (R_f = 0.6, 10% methanol–dichloromethane); mp 68–71 °C; ν_{max}(thin film)/cm^{-1} 2972s, 2926s, 1376m, 1350w, 1143s, 1111s and 1030s (**NB** OH absent) (Found: C, 58.23; H, 9.26. $C_{84}H_{154}O_{35}$ requires C, 58.5; H, 9.0%); $\delta_H(CDCl_3)$ 5.10 (7 H, d, J = 3.6 Hz, H^1), 4.00 (7 H, m, 6-OCH^cCH_3), 3.92 (7 H, m, H^{6a}), 3.75 (7 H, m, 6-OCH^dCH_3), 3.73 (14 H, m, $H^4 + H^5$), 3.69 (7 H, m, 2-OCH^eCH_3), 3.65 (7 H, m, 2-OCH^eCH_3), 3.62 (7 H, m, H^3), 3.51 (7 H, m, H^{6b}), 3.47 (14 H, m, 3-$OCH_2^{g,h}CH_3$), 3.25 (7 H, dd, J = 10, 3.6 H^2) and 1.19 (63 H, m, $-OCH_2CH_3$); $\delta_C(CDCl_3)$ 98.4 (C^1), 80.2 (C^3), 80.0 (C^2), 78.6 (C^4), 71.0 (C^5), 69.3 (C^6), 68.9 (6-OCH_2–), 66.6 (2-OCH_2–), 66.5 (3-OCH_2–), 15.7, 15.6 and 15.2 (3 × $-CH_2CH_3$).

Commentary.—In the ^1H spectrum of 2,3,6-tri-*O*-ethyl-β-cyclodextrin, the acetal hydrogen, H^1 (see numbering scheme in Fig. 10) appeared as a single resonance at 5.10 ppm and C^1 also resonated as a singlet (at 98.4 ppm) in the ^{13}C spectrum. The remaining ring CHO and CH_2O resonances were assigned from the ^1H–^1H COSY, ^{13}C DEPT and ^{13}C–^1H HETCOR spectra. NOE experiments were used to confirm these assignments. The diastereotopic methylene protons, H^{6a} and H^{6b}, showed a significant chemical shift non-equivalence (0.41 ppm), while the diastereotopic methylene CH_2O resonances of the ethyl groups were also all shift non-equivalent to varying degrees, the largest difference (0.25 ppm) being for the ethyl group attached to the 6-position. Similar observations in other alkylated cyclodextrins have been previously reported.[8,31,32] Irradiation of the ^1H signal elicited a strong positive NOE response for H^2 (3.25 ppm) and signals to a lesser extent for H^4 and for the $-CH_2$ (e, f) multiplet were also observed. Irradiation of the H^2 signal led to a strong positive NOE response for H^1 and the $-CH_2$ (e, f) multiplet and a weaker response from H^4. In addition, a strong negative response was observed from H_1[3] and the CH_2 (g, h) multiplet. Irradiation of the multiplet centred at δ 4.00 (attributed to 6-OCH^eCH_3) gave a positive response from the multiplet centred at δ 3.75 (attributed to 6-OCH^dCH_3) and a negative response from the multiplet centred at δ 3.92 (attributed to H^{6a}). Irradiation of the signal attributed to H^{6a} (δ 3.92) gave a negative response from the $-CH_2$ (c, d) multiplets and positive responses from the H^{6b} signal (δ 3.51) and H^5 (δ 3.73) signals. The NOE experiments also confirmed the coincidence of the H^4 and H^5 resonances.

§ As a result of considerable spectral overlap of the ^1H NMR and ^{13}C NMR spectra, several assignments are only tentatively reported (indicated by *).

¶ This compound has been described but not characterised in the literature.[32]

Hexakis(2,6-di-*O*-dodecyl)-α-cyclodextrin (12) [hexakis(2,6-di-*O*-dodecyl)cyclomaltohexaose]. Powdered α-cyclodextrin hydrate (1.0 g, 1.03 mmol) was dried overnight at 120 °C, over P_2O_5 under reduced pressure. Anhydrous DMSO (20 cm^{-3}) and fused, dried, powdered sodium hydroxide (2.2 g, 55 mmol) were added sequentially under an argon atmosphere and the mixture stirred vigorously. 1-Bromododecane (13.8 g, 55 mmol) was then added and the complete reaction mixture stirred at room temperature. The reaction was continued until TLC analysis (20% methanol–80% dichloromethane; silica) showed complete consumption of the α-cyclodextrin (R_f = 0.25). After 4 weeks, the DMSO was removed under reduced pressure. Dichloromethane (50 cm^{-3}) was added to the residual yellow oil and the solution was then successively washed with distilled water (3 × 20 cm^{-3}), dried over anhydrous potassium carbonate and the solvent removed under reduced pressure, leaving a yellow oil which was further treated under high vacuum (0.5 mmHg) at 110 °C for 6 h (Kugelrohr) to remove residual 1-bromododecane.

Column chromatography of the viscous pale yellow oily residue (silica; 0–2% methanol–dichloromethane) gave a colourless viscous oil (1.74 g, 58%) [R_f(0–4% methanol–dichloromethane) = 0.1 (SiO_2)]; ν_{max}(thin film)/cm^{-1} 3408s, 2923s, 2853s, 1466m, 1358m, 1153s, 1089s, 1045s, 859w, 768w and 721w; *m/z* (ES^+, 50 μmol dm^{-3} cyclodextrin derivative, 1 mmol dm^{-3} ammonium acetate in propan-2-ol) 3101 (M + 18)$^+$ for 12 dodecyl groups, 3179 (M + 18)$^+$ for 13 dodecyl groups, minor peak (Found: C, 72.2; H, 11.9. $C_{180}H_{348}O_{30}$ requires C, 72.2; H, 11.7%); $\delta_H(CDCl_3)$ 4.89 [12 H, s, H^1 and OH(3)], 4.05 (6 H, t, J = 9.0 Hz, part of an A′M′N′ system, H^3), 3.92 (6 H, m, H^{6a}), 3.84 (6 H, m, H^5), 3.64 (12 H, m, CH_2O), 3.62 (6 H, m, H^{6b}), 3.47 (6 H, m, H^4), 3.45 (12 H, m, CH_2O), 3.35 (6 H, dd, J = 9.0, 3.2 Hz, part of an A′M′X′ system, H^2), 1.52 (24 H, m, $-CH_2CH_2O$), 1.25 (216 H, br s, CH_2) and 0.78 (72 H, t, J = 7.2 Hz, CH_3); $\delta_C(CDCl_3)$ 101.3 (C^1), 83.5 (C^4), 79.8 (C^2), 73.7 (C^3), 72.7 (C^6), 71.7 (CH_2O), 70.4 (C^5), 69.3 (CH_2O^1), 31.8 (CH_2), 29.69, 29.65, 29.60, 29.51, 29.45, 29.33, 29.30 (CH_2 and CH_2CH_2O), 26.0 (CH_2), 25.7 (CH_2), 22.6 (CH_2) and 14.0 (CH_3).

Guanidinium triflate. Silver triflate (1 g, 3.9 mmol) solution (5 cm^3) was added to an aqueous solution (5 cm^3) of guanidine chloride (0.39 g, 4.1 mmol) with vigorous stirring. Silver chloride was removed by centrifugation and the solution was pumped to dryness. The solid was dissolved in acetonitrile and filtered through a PTFE membrane. Concentration and vacuum drying gave a white solid, mp 138 °C; $\delta_H(CD_3CN)$ 6.25. Combustion analysis confirmed the absence of chloride in the salt.

Dodecyl-tri-(deuteriomethyl)ammonium iodide. CD_3I (1 cm^3, 2.33 g, 0.016 mol), dodecylamine (0.60 g, 3.2 mmol), anhydrous K_2CO_3 (1.11 g) and dry acetonitrile (20 cm^3) were boiled under argon for 1 h. The solvent and excess CD_3I were removed under reduced pressure. The product was taken up in dichloromethane (30 cm^3), filtered and evaporated to dryness to give $C_{12}H_{25}N(CD_3)_3I$ as a white solid, mp 221 °C; $\delta_C(CDCl_3)$ 66.74 (CH_2N), 52.62 (septet, J_{CD} = 21 Hz, NCD_3), 31.76 (CH_2CH_2N), 29.46, 29.34, 29.26, 29.20, 29.11, 25.92, 23.03, 22.55 (CH_2C) and 14.01 (CH_3); $\delta_D(CHCl_3)$ 3.28 (s).

Acknowledgements

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