Supramolecular Complex Formation: A Study of the Interactions between β-Cyclodextrin and Some Different Classes of Organic Compounds by ESI-MS, Surface Tension Measurements, and UV/Vis and ^{1}H NMR Spectroscopy

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The ability of several organic compounds [alcohols **1** and **2**, the corresponding phosphate esters **3** and **4** and their sodium salts **5** and **6** as well as some commercial surfactants such as C_{14}DMAO (**7**, zwitterionic), SDS (**8**, anionic) and CTABr (**9**, cationic)] to produce supramolecular complexes with β-cyclodextrin has been investigated by four different techniques (ESI-MS, surface tension measurements, UV/Vis and ^{1}H NMR spectroscopy). The data collected have allowed information about the formation of complexes and the location of the guest in the host to be gained. Comparison of the results obtained for phosphate esters and their salts, for both saturated and unsaturated derivatives, has made it possible to evaluate the factors that affect the stability of the supramolecular complexes under the different experimental conditions used.

Introduction

Our research group has for a long time been involved in studies on the reactivity of organic systems in "confined environments". So far we have measured the rates of decarboxylation[1] of several 5-amino-1,3,4-oxadiazole- and 5-amino-1,3,4-thiadiazole-2-carboxylic acids variously substituted at the exocyclic nitrogen atom, as well as the rates of rearrangement[2] of the (*Z*)-phenylhydrazones of some 5-substituted 3-benzoyl-1,2,4-oxadiazoles into the corresponding 4-acylamino-2,5-diphenyl-1,2,3-triazoles in the presence of Triton X-100 (a non-ionic surfactant).

In view of the increasing interest in the use of cyclodextrins (CDs) as hosts in drug, food and cosmetic chemistry[3] and the changes in the physicochemical properties[4] of many chemicals produced by the ability of cyclodextrins to interact strongly and stably with different guests, we have recently examined the course of the rearrangement of the (*Z*)-phenylhydrazone of 3-benzoyl-5-phenyl-1,2,4-oxadiazole (essentially insoluble in water) in water solutions of β-cyclodextrin (β-CD) at pH 9.6,[5] taking advantage of the ability of the β-cyclodextrin to dissolve organic compounds. On studying the reaction at different β-CD concentrations we observed a typical Michaelis−Menten trend of the reactivity as a function of the β-CD concentration. The value of the binding constant (3×10^3 mol·L^{-1}) has been estimated accordingly. A significant decrease in the reactivity with respect to the same rearrangement in a dioxane/water mixture at the same proton concentration was observed, and this could be ascribed to a medium effect of the lipophilic β-CD cavity as well as to the occurrence of some specific host−guest interactions.

In this context we have addressed our attention towards the study of modified CDs containing a functionalised aliphatic chain linked to the larger rim of the CD, which might act as catalysts (basic or acidic) of several organic reactions. Our first aim is the use of β-CD functionalised with phosphate groups, which at low and at high pH [that is, as free acids (R−OPO_3H_2) or as salts (R−OPO_3^{2-} 2 Na^+), respectively] might be able to exert acid and base catalysis, respectively. To gain some initial information on the possible interactions between the functionalised chains described above and the β-CD, we studied the host−guest interactions between β-CD and alcohols and some of their derivatives (phosphoric esters and their salts), as well as some commercial surfactants.

As a matter of fact, inclusion complex formation between linear molecules and CDs has been the subject of investigation of several papers.[6−8] Many guest molecules with end-functional groups (basic or acidic, such as amino or carboxylic groups)[9] capable either of reacting with or of associating with host molecules, have been studied. In some

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cases these systems are able to produce pseudorotaxanes,[7] characterized by a high molecular order.

Complexes between CDs and alkyl derivatives are particularly promising as pharmaceutical drugs because of their low toxicities and their high solubilities in water and organic solvents.[10,11] Despite the great amount of work about the inclusion phenomena, relatively few data on the properties of such complexes are available. It should be remarked that it is possible, through changing the structures of the host and of the guest molecules in a systematic manner, to correlate the chemical structure of the species involved with their binding affinities[12,13] and general properties.[4]

We now present some results on the interactions between β-CD and a series of linear alcohols [saturated (**1**) and unsaturated (**2**), such as oleyl alcohol as a typical (*Z*) isomer], the corresponding phosphate esters (alkylmonophosphoric acids **3** and **4**) and their sodium salts (**5** and **6**), as well as some commercial surfactants with very different chemical structures [tetradecyldimethylamine oxide (**7**, C_{14}DMAO, zwitterionic), sodium dodecylsulfate (**8**, SDS, anionic) and cetyltrimethylammonium bromide (**9**, CTABr, cationic)] characterized by hydrophobic appendices of different lengths and degree of unsaturation, with both charged and uncharged head groups, in order to evaluate the influence of the structure of the head group on the complexation (Figure 1).

The complexation processes were studied by four independent techniques: electrospray ionisation mass spectrometry (ESI-MS), surface tension measurement and UV/Vis and ^{1}H NMR spectroscopy. The first two techniques can provide qualitative information on the occurrence of complexation and on the stoichiometry of the inclusion complexes, while the other two allow the evaluation of the binding constants. Data deriving from ^{1}H NMR spectroscopy can be of special interest as they can also provide information concerning the location of the guest in the host.

Results and Discussion

The ESI-MS Data

ESI-MS is one of the most promising tools[14−16] for the characterization of supramolecular complexes; the study of host−guest interactions in the gas phase, in the absence of solvent effects, allows the intrinsic phenomena responsible for molecular recognition to be estimated. Analysis of the complexes in the gas phase is of help in, for example, the understanding of some specific host−guest interactions such as hydrogen bonding, because hydrophobic effects − which in the case of apolar guests represent the most important driving force for the formation of complexes in solution − are absent.[17]

The mildness of the electrospray ionization-desorption process enables weakly bound complexes to remain intact upon transition to the gas phase;[18] the process occurs in two stages: the formation of charged droplets and the production of gas-phase ions from the charged droplets.[19] This allows the determination of the molecular weights (and then the stoichiometries) of all the vaporised species (host, guest and host−guest complexes).

The main problem with the use of the ESI-MS technique in supramolecular chemistry is in evaluating whether the species present in the mass spectra correspond to those present in solution, or whether they are rather the result of processes occurring in the mass spectrometer.[20] Moreover, it is not clear whether the inclusion complexes still survive in the gas phase[17] or whether the molecular ions observed pertain to ion−dipole external adducts.[21]

Nevertheless, the problem can be solved by comparison of the results obtained by ESI-MS with those acquired by other independent and complementary methods such as the UV/Vis displacement technique and complex-induced ^{1}H NMR shift measurements.

The ESI-MS signals are detected in the negative mode and the data obtained are collected in Table 1. Attempts to

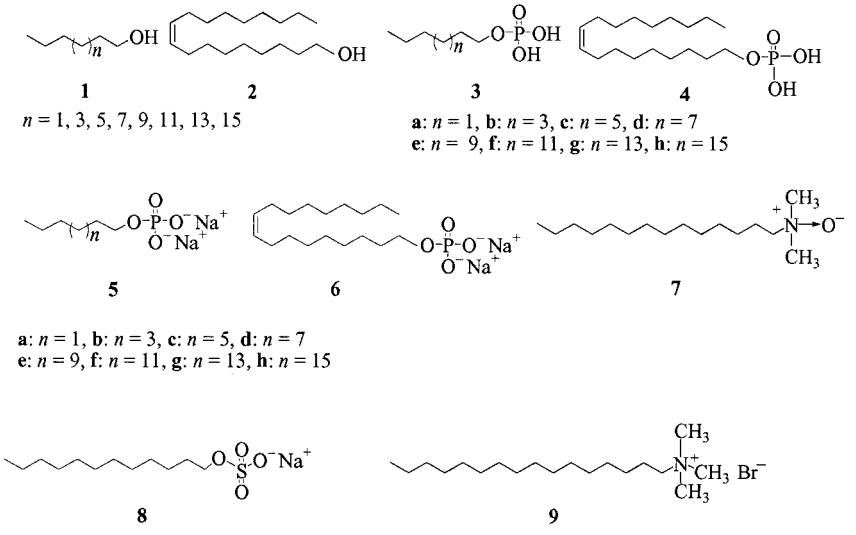

Figure 1. Guest molecules

Table 1. Major peaks and relative abundance for phosphate and commercial surfactants

Host	Guest	Major peak observed	Host/guest ratio	Relative abundance (%)
β-CD	C_8P	1133.9 $[\beta\text{-CD}]^-$	1:0	88
		1344.3 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2479.0 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	7
β-CD	$C_{10}P$	1134.4 $[\beta\text{-CD}]^-$	1:0	80
		1372.7 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2508.3 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	7
β-CD	$C_{12}P$	1133.9 $[\beta\text{-CD}]^-$	1:0	75
		1400.3 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2536.0 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	8
β-CD	$C_{14}P$	1133.9 $[\beta\text{-CD}]^-$	1:0	65
		1428.3 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2564.1 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	6
β-CD	$C_{16}P$	1133.8 $[\beta\text{-CD}]^-$	1:0	63
		1456.3 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2592.4 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	7
β-CD	$C_{18}P\Delta C^{9-10}$	1133.9 $[\beta\text{-CD}]^-$	1:0	75
		1482.4 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2618.0 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	8
γ-CD	$C_{18}P\Delta C^{9-10}$	1296.4 $[\gamma\text{-CD}]^-$	1:0	100
		1644.7 $[\gamma\text{-CD}\cdot S]^-$	1:1	25
		2942.6 $[(\gamma\text{-CD})_2\cdot S]^-$	2:1	2
β-CD	$C_{18}P$	1133.8 $[\beta\text{-CD}]^-$	1:0	45
		1484.4 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2619.9 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	8
β-CD	$C_{20}P$	1133.8 $[\beta\text{-CD}]^-$	1:0	40
		1512.4 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2648.1 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	8
β-CD	C_{14}DMAO	1158.1 $[\beta\text{-CD} + Na]^+$	1:0	100
		1392.2 $[\beta\text{-CD} + Na\cdot S]^+$	1:1	2
		2529.3 $[(\beta\text{-CD})_2\cdot S]^+$	2:1	4
β-CD	SDS	1134.3 $[\beta\text{-CD}]^-$	1:0	14
		1400.8 $[\beta\text{-CD}\cdot S]^-$	1:1	100
		2535.3 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	3
β-CD	CTABr	1134.1 $[\beta\text{-CD}]^-$	1:0	100
		1216.0 $[\beta\text{-CD} + Br]^-$	1:0	92
		1579.5 $[\beta\text{-CD} + Br\cdot S]^-$	1:1	9
		2634.4 $[(\beta\text{-CD})_2\cdot S]^-$	2:1	2

detect signals in the positive mode were unsuccessful for almost all the guest investigated.

Table 1 reports the most intense peaks for $[\beta\text{-CD}\cdot S]^-$ and $[(\beta\text{-CD})_2\cdot S]^-$ anionic species, where S refers to the guest molecules. Measurements made on different samples of the same β-CD complex provided reproducible spectra; all the samples being mixtures of the starting β-CD and its supramolecular complexes with host/guest ratios of 1:1 and 2:1.

We started with a study evaluating the behaviour of alcohols **1**−**2**. Under the experimental conditions adopted (negative and positive modes, cone voltage 20 V and 80 V), no peaks corresponding to the molecular weights of the corresponding complexes with β-CD were observed in the mass spectra.

In contrast, compounds **3**−**6** showed high-abundance ion peaks, indicating the formation of supramolecular complexes.

As an example, Figure 2 shows the spectrum of the $C_{12}P$(**3d**)/β-CD system. The most intense peak (100 %), at m/z = 1400.3, supports the formation of a 1:1 complex $[\beta\text{-CD}\cdot S]^-$, while the peak (75 %) at m/z = 1133.9 corresponds to the free β-CD, and the peak (8 %) at m/z = 2536.0 gives evidence of the formation of a 2:1 complex ($[(\beta\text{-CD})_2\cdot S]^-$). Other peaks can be attributed to a monodeprotonated dimer of β-CD (2269.6), to the complex of β-CD and diester of $C_{12}P$ present as an impurity (1569.5), to some complexes of β-CD with phosphoric fragments deriving from $C_{12}P$ (1232.1 and 1214.0), and to the complex of β-CD with chlorine (1170.0).

The spectra of alkylmonophosphoric acids (**3b**−**h**; C_nP, n = 8, 10, 12, 14, 16, 18 and 20) are characterized by high-abundance negative ion peaks corresponding to the 1:1 complexes between deprotonated C_nP and β-CD (cone voltage 80 V). A comparison between the abundance of $[\beta\text{-CD}\cdot C_nP]^-$ and β-CD peaks shows a monotonic increase in the relative abundance of the supramolecular complex with increasing alkyl chain length in C_nP; the increasing ability of β-CD to accommodate the guests with elongation of the alkyl chains seems to be a good indication of formation of inclusion complexes and not of external adducts. Weak signals corresponding to 2:1 complexes were also observed for all the samples.

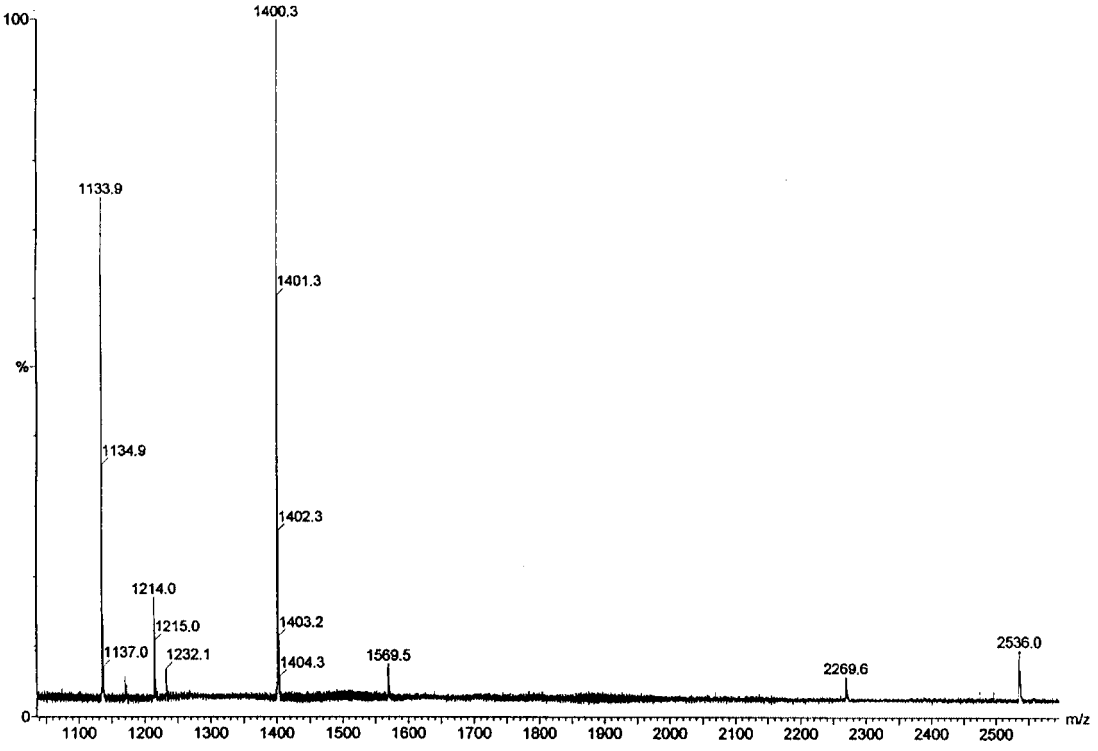

Figure 2. Negative-ion ESI mass spectra of the complex of $C_{12}P$ and β-CD

Moreover, a competition experiment carried out by treatment of $C_{12}P$ (**3d**) with a mixture of α-, β- and γ-CD (α-CD/β-CD/γ-CD/$C_{12}P$, 1:1:1:1) showed that the most abundant peak is that relating to the complex with β-CD (the probability of formation of the different complexes is 1.7:2.5:1.0 for α-, β- and γ-CD, respectively). Similar behaviour occurs in solution for other surfactants (*n*-alkanesulfonates and *n*-alkyl sulfates) containing alkyl chain of the same length,[22] thus confirming that the relationship between the sizes of the surfactants and the cavity dimension observed in solution is maintained under electrospray conditions, this relationship appearing to be a necessary premise for an inclusion process.

Comparison between the ESI-MS spectra of the β-CD complexes with $C_{18}P$ (**3g**) and with $C_{18}P\Delta C^{9-10}$ [**4**, (*Z*) isomer] − that is, for the complexes of β-CD with two phosphates (alkyl and alkenyl, respectively) containing chains of equal length but with very different steric requirements − allows some interesting information to be gained. Compound **4** shows an affinity for β-CD very similar to that observed for $C_{12}P$, but much lower than that measured for $C_{18}P$ (see also the discussion on NMR spectroscopic data below).

With the folded chain of **4**, which could result in higher steric requirements for the complexation process, in mind, we also investigated the complexation between **4** and γ-CD (a host with a larger cavity, in which the folded chain might be better accommodated), and observed an even less favourable formation of the 1:1 and 2:1 complexes. That is, the cavity of γ-CD appears to be too large for effective interactions with the guest under consideration and this allows the inclusion of the folded chain in the gas phase to be excluded.

We have also recorded the ESI-MS spectra of C_nPNa_2 (**5a**−**h** and **6**) in the presence of β-CD.

In this case different conditions (cone voltage 20 V) were used in order to reveal the presence of the complexes with the salts. The spectra showed intense signals of dianionic species corresponding to molecular ion peaks of the β-CD/$C_nPO(O^-)_2$ aggregates together with the doubly charged peak of free β-CD.

In examination of the behaviour of commercial surfactants, we observed that the anionic SDS (**8**) shows an high-abundance signal corresponding to the 1:1 complex. In contrast, for the zwitterionic C_{14}DMAO (**7**) and the cationic CTABr (**9**) we observed a much weaker tendency to give a supramolecular complex than seen with the corresponding **3**, with the same carbon chain.

The overall results collected allow some conclusions to be drawn, firstly in analysis of the nature of the occurring hydrogen bond. The presence of the polar head $PO(OH)_2$ group together with its conjugate base $PO(OH)O^-$ seems to be essential for a gas-phase inclusion aggregate to be detected, reasonably because of the formation of hydrogen bonds between the hydroxy groups of the larger rim of the β-CD (as hydrogen bond donors) and the $PO(OH)O^-$ (Figure 3, **b**). Similar considerations apply for the behaviour of SDS (**8**). In contrast, the commercial C_{14}DMAO and CTABr displayed much lower affinities of the relevant head groups for the hydroxy groups of the larger rim of β-CD.

In solution (see the NMR part), linear alcohols give complexes with β-CD; their formation can reasonably be as-

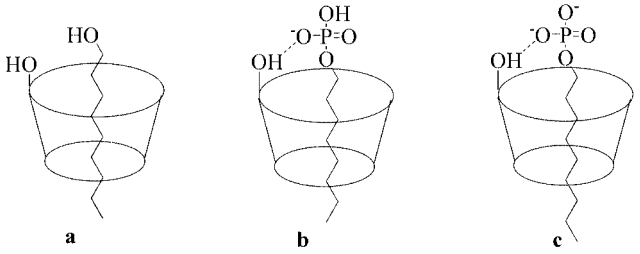

Figure 3. Interactions of guest molecules with the hydroxy groups of the larger rim of cyclodextrins

cribed to hydrophobic interactions. The absence of supramolecular complex formation between alcohols and β-CD, in contrast with observations made in ESI-MS measurements, indicate that the contribution of hydrogen bonds (the strengths of which depend on the acidity/basicity of the species involved) to the binding in the gas phase is not strong enough to compensate for the hydrophobic interactions operative in solution.

It should be remembered that the lack of solvent effects in the gas phase allows the detection of specific interactions, not present in solution, that give a complementary picture of the host−guest relationship. Actually, detection of doubly charged signals for β-CD complexes with C_nPNa_2 surfactants is indicative of the presence in the gas phase of hydrogen bond interactions (Figure 3, **c**) that are not detectable in water due to the effective solvation of the phosphate moiety occurring in solution.

In conclusion, the ESI-MS studies provide evidence that both the nature of the head group and the length of the alkyl chain (an increase in this causes a parallel increase in the van-der-Waals forces) are responsible for the presence of signals corresponding to complexes between the surfactants and β-CD in the mass spectra. These qualitative results have been strongly confirmed by data from ^{1}H NMR and UV/Vis measurements (see below), indicating that the innovative ESI-MS techniques can be useful for investigation of inclusion complexes usually detected and studied only in solution.

CMC Data

C_nPNa_2 surfactants in water give micelles when the CMC is exceeded: if significant concentrations of a host molecule (such as β-CD) are added to water an increase in the CMC values is to be expected.[23] To gain further information on the complexation between C_nPNa_2 and β-CD we measured the CMCs of some C_nPNa_2 [24] species (**5d**−**g**, n = 12, 14, 16, 18) in water and in water solutions containing variable amounts of β-CD (Table 2).

As far as the CMC values in pure water are concerned, we observed the expected decrease with increasing number of carbon atoms in the alkyl chain, in agreement with the fact that the tendency to form micelles is higher for more hydrophobic surfactants.[25] As a matter of fact, the data collected fit the relationship between the hydrocarbon chain length and CMC for ionic surfactants (Klevens equation):

$$\log_{10} \mathrm{CMC} = A - B\, n_C$$

where A and B are constants specific for each homologous series (under constant conditions of temperature, pressure and other parameters) and n_C is the number of carbon atoms in the chain. The values at 25 °C are A = 1.30 ± 0.45 and B = 0.21 ± 0.03 (r = 0.98), in quite good agreement with the A value for *n*-alkyl-1-sulfates (1.42) and *n*-alkyl-1-sulfonates (1.59) and the typical B values (0.27−0.30) for all paraffin chain salts possessing a single ion head group.[26]

The CMC values of C_nPNa_2 increase with addition of increasing amounts of β-CD, confirming a decrease in thermodynamic activity probably due to the formation of surface-inactive inclusion complexes with cyclodextrins. An attempt to measure the binding constants of β-CD with the surfactants accurately by a reported methodology[23] gave very poor results. This is probably a consequence of our experimental procedure; the necessity to neutralise the acids in order to obtain the corresponding salts could leave a slight excess of electrolytes in solution. The presence of small amounts of electrolytes would not alter the values of the CMCs but it could significantly change the values (used to fit the equation above)[23] of the surface tension in very dilute solutions.

Table 2. CMC values at 25 °C, determined by the tensiometric method

Surfactant	Maximum surfactant concentration [mol·L^{-1}]	Equivalent of NaOH [mol·L^{-1}]	10^3[β-CD] [mol·L^{-1}]	CMC
$C_{12}P$	0.15	2	−	0.074
$C_{14}P$	0.15	2	−	0.018
$C_{14}P$	0.15	2	5	0.026
$C_{14}P$	0.15	2	10	0.027
$C_{14}P$	0.15	2	20	0.031
$C_{16}P$	0.05	2	−	0.007
$C_{16}P$	0.05	4	0.5	0.007
$C_{16}P$	0.05	4	20	0.020
$C_{18}P$	0.01	20	−	0.004
$C_{18}P$	0.01	4	20	0.018

However, it is noteworthy that the effect of addition of the same amount of β-CD to solutions of different surfactants (at concentrations above the β-CD concentration) increases the CMC value of each surfactant by a similar amount, independently of the initial concentration of the surfactant and of its CMC. This gives support to the assumption that the association between the host and the guest is tighter than that between the monomers to form the micelles. In other words, the addition of a surfactant to the cyclodextrin solution results in initial inclusion of the surfactant apolar tail into the cyclodextrin cavity, and only when all the cyclodextrin molecules have hosted C_nPNa_2 molecules does the addition of more surfactant give rise to the formation of micelles.[27] The same conclusion could be drawn by analysis of the surface tension data in Figure 4. Increasing amounts of β-CD increase the minimum concentration of surfactant necessary for determination of a surface activity.

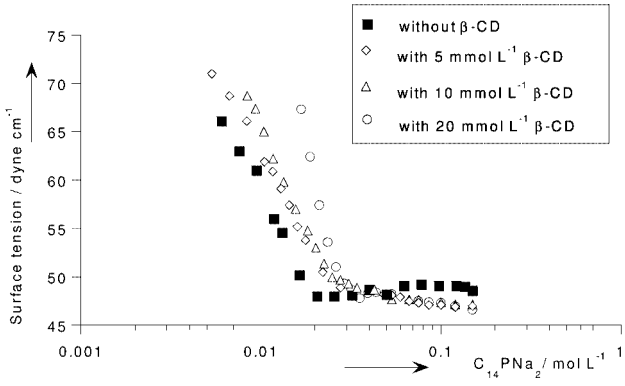

Figure 4. Variation of the surface tension determined for solutions of $C_{14}PNa_2$: a) without β-CD (■ data); b) in the presence of 5 mmol·L^{-1} β-CD (◇ data); c) in the presence of 10 mmol·L^{-1} β-CD (△ data); d) in the presence of 20 mmol·L^{-1} β-CD (○ data), as a function of surfactant concentration (logarithmic abscissa)

Moreover, in Figure 4 it can be also observed that for [surfactant] > apparent CMC (micellar region) all the curves present the same trend and are characterized by a similar and constant value of surface tension. Since all the changes in surface tension can be assigned to the features of the surfactant monomer, this fact would suggest that the complexed surfactant does not interfere with the formation of the micelles.

The UV/Vis Data

Taguchi pointed out[28] that phenolphthalein salts give "stable" complexes that do not absorb at 550 nm (the typical absorption wavelength of phenolphthalein at pH > 10) in aqueous solutions of β-CD. The relevant binding constant of phenolphthalein (I) with β-CD [K_I = (3.0 ± 0.2) × 10^4 L·mol^{-1}] was measured by a conventional spectrophotometric method.[29] On addition of different quantities of a second guest the phenolphthalein salt is displaced from the host−guest complex and its concentration in water can be measured by visible spectroscopy, allowing the determination of the second binding constant (K_{II}).

$$\beta\text{–CD} + \text{I} \rightleftharpoons \beta\text{–CD•I} \overset{(S)}{\rightleftharpoons} \beta\text{–CD•S} + \text{I}$$

I = phenolphathalein, S = surfactant

A graphical procedure[30] was used to determine the 1:1 binding constants (K_{II}) between surfactants and β-CD by plotting of S_t/P against Q [Equation (1)], where P is obtained from Equation (2). The experimental data do not fit for a 2:1 complex formation. On the other hand, the overall results from all the techniques used indicate the prevalent formation of 1:1 complexes.

$$\frac{S_t}{P} = \frac{K_I}{K_{II}} Q + 1 \quad (1)$$

In Equation (1): S = free surfactant, S_t = [S] + [β-CD·S], $K_I = 3.0 \times 10^4$, $Q = (\varepsilon - \varepsilon_{\beta\text{-CD·I}})/(\varepsilon_I - \varepsilon)$, where ε_I and $\varepsilon_{I\cdot\beta\text{-CD}}$ are the molar absorbances of free and complexed indicator, respectively, and ε is the measured molar absorbance at different surfactant concentrations.

$$P = \frac{S_t K}{QK_I + K_{II}} \quad (2)$$

From the data collected from the ESI-MS and CMC techniques and in view of the fact that the recording of UV/Vis data must be carried out at pH > 10, we determined the binding constants by the displacement technique only for some C_nPNa_2 (n = 8, 12, 14), for $C_{18}P\Delta C^{9-10}Na_2$ and for the anionic surfactant SDS.

The binding constant values measured (K_{II}) (n = 8, r = 0.992−0.994) are collected in Table 3. They are in the 7.5−26.0 × 10^3 L·mol^{-1} range and increase with the alkyl chain length of the surfactant, as already also found by ESI-MS and CMC results.

Table 3. Binding constants obtained from spectral displacement studies

Surfactant	10^3K [L·mol^{-1}]
SDS	18.0 ± 5.0
C_8PNa_2	8.3 ± 0.8
$C_{12}PNa_2$	14.3 ± 0.9
$C_{14}PNa_2$	26.0 ± 1.0
$C_{18}P\Delta C^{9-10}Na_2$	7.5 ± 0.9

The relatively small K_{II} value measured for the $C_{18}P\Delta C^{9-10}Na_2$, similar to that of C_8PNa_2, confirms the ESI-MS data and agrees with the prevision that the complexation involves only a proportion of the carbon atoms

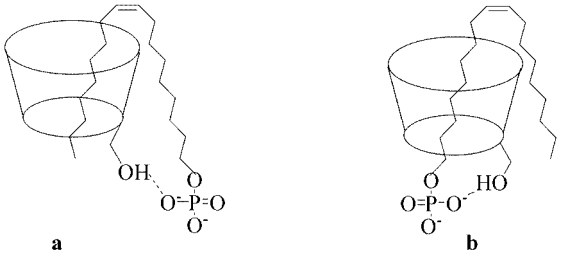

Figure 5. Possible complexes of the $C_{18}P\Delta C^{9-10}Na_2$ with β-CD

of the alkyl chain of the surfactant (Figure 5, **a**). However the formation of the complex **b** does not occur, as ESI-MS and ^1H-^1H NOE data indicate that the phosphate group (no matter whether ionised or not) is, because of its hydrophilicity, unable to enter the host.

Moreover, SDS and $C_{12}PNa_2$, anionic surfactants with different head groups but identical hydrophobic tails, show similar K_{II} values, once more confirming that the hydrophobic effect is a significant contributor to the binding affinity between surfactants and β-CD.

^1H NMR Data

The above data (ESI-MS, CMC, UV/Vis) clearly showed the tendency of C_nP and C_nPNa_2 to give supramolecular complexes with β-CD. When host−guest complexes from β-CD are obtained, the signals of the inner proton H(3) and H(5) of the host[31] undergo complex-induced shifts (CISs) that can be used both to obtain information on the formation of a complex and on the location of the guest in the host and to calculate the relevant binding constants.

In this context, the binding affinities between phosphate surfactants and β-CD were measured by ^1H NMR titrations in D_2O at 25 °C, by monitoring of the chemical shift changes of the inner protons of the host in the presence of different amounts of surfactant (C_nP or C_nPNa_2). The proton signals of H(3) and H(5) of β-CD undergo the most significant CISs, ranging from 0 to 0.08 ($\Delta\delta_{H3}$) and from 0 to 0.16 ($\Delta\delta_{H5}$) ppm, while guest CH_3 or CH_2 protons show smaller and in some cases negligible variations. Moreover, the individual CH_2 signals, especially in long *n*-alkyl chain surfactants, were not resolved; on the other hand the methylene protons of **3**−**4** and **5**−**6** close to the oxygen atom of the phosphate head (ca. 3.9 ppm for C_nP and 3.7 ppm for C_nPNa_2) shows signals overlapping with those of the sugar host.

Binding constants were estimated by nonlinear regression of the spectroscopic CIS data, the observed H(3) and H(5) chemical shifts of the solutions containing host and guest species being regarded as weighted averages between the chemical shift of the free host and of the complex (fast exchange on the NMR timescale). The chemical shift contributions of the aggregates are consistent with the formation of 1:1 complexes and not with that of 2:1 complexes (see above). Satisfactory fits were obtained (*r*, 0.961−0.994): Figure 6 shows a typical one.

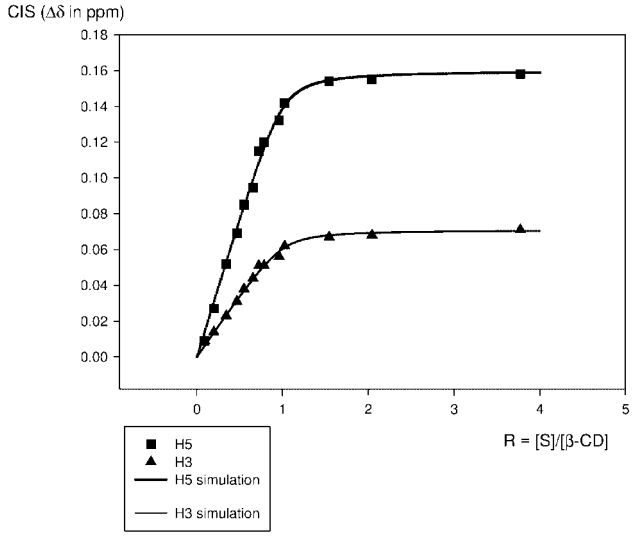

Figure 6. ^1H NMR spectroscopic data of the β-CD/$C_{14}PNa_2$ system at t = 25 °C; CIS (in ppm) for H(3) and H(5) protons of the host against mol ratio (R)

Table 4. Binding constants at 25 °C, obtained from ^1H NMR CIS for H(3) and H(5) protons of the hosts of some β-CD/C_nP complexes

Surfactant	10^3K_{H3} [L·mol^{-1}]	10^3K_{H5} [L·mol^{-1}]
C_6P	0.35 ± 0.18	0.18 ± 0.08
C_8P	1.3 ± 0.3	1.5 ± 0.3
$C_{12}P$	67.0 ± 30.7	49.7 ± 16.6

Table 5. Binding constants at 25 °C, obtained from ^1H NMR CIS for H(3) and H(5) protons of the hosts of some β-CD/ C_nPNa_2 complexes

Surfactant	10^3K_{H3} [L·mol^{-1}]	10^3K_{H5} [L·mol^{-1}]
C_6PNa_2	0.35 ± 0.08	0.39 ± 0.05
C_8PNa_2	1.5 ± 0.3	3.1 ± 0.7
$C_{12}PNa_2$	13.1 ± 6.0	14.1 ± 4.8
$C_{18}P\Delta C^{9-10}Na_2$	34.3 ± 16.0	14.5 ± 4.4
$C_{14}PNa_2$	75.6 ± 13.4	98.8 ± 18.0
$C_{16}PNa_2$	62.9 ± 33.8	133.3 ± 87.0

Table 4 and 5 list the values of the binding constants calculated by monitoring of the surfactant concentration dependence for δ_{H-3} or δ_{H-5} of β-CD.[32]

The general observation that complexation induces a change in the chemical shifts of the inner protons of the macrocycle suggests that the guest is located inside the cavity and the host−guest interaction is generated by hydrophobic effects. CIS values obtained by monitoring of H(5) are larger than those from H(3) because the former proton is located at the narrower edge of the macrocycle and probably gives closer intermolecular contacts with the alkyl chain of the surfactant.

A general trend emerging from the analysis of the binding constants is that the affinity increases with the number of carbon atoms in the surfactant (Entrys 1−3 for C_nP, Table 4; Entrys 1−3, 5 for C_nPNa_2, Table 5); the experiments also indicate that measurements of binding constants are affected by large errors for alkyl chains longer than 14 carbon atoms.

Moreover, comparison of the affinities of β-CD with the two series of surfactants shows different behaviour: similar binding constant values are obtained for C_nP and C_nPNa_2 for $n = 6-8$, while an increase of n up to 12 generates a significantly larger binding constant for the neutral acid relative to its salt, thus reinforcing both qualitative and quantitative results obtained from the other techniques.

Two questions arise from the data analysis: how does the phosphate group affect the inclusion mode[33] of the surfactant into the host, and why are the constant values affected by large errors when $n > 14$?

In water solution, the phosphate group [$-PO(OH)_2$] and its dissociated form $-PO(OH)O^-$ may be hydrogen-bonded to the hydroxy groups of the β-CD: these extra bonds, which add to lipophilic interactions of the hydrocarbon within the cavity, affect the magnitudes of binding affinity constants, causing an important increase in their values. Comparison between data for C_nP and C_nPNa_2 containing the same chain (compare the data for C_nPs and C_nPNa_2s of Table 4 and 5, respectively) shows the higher ability of $C_{12}P$ than of $C_{12}PNa_2$ to be included in the β-CD: the significant differences in the assembly ability can confidently be ascribed to the different interactions of the heads of the two guests with the host. In fact, the $-PO(ONa)_2$ moieties seem to prefer to be bound (solvated) to external water molecules rather than to rim hydroxy groups, and this decreases the inclusion constant. In the case of the smaller homologues (C_6P and C_8P, in relation to C_6PNa_2 and C_8PNa_2, respectively), the similar binding constants obtained for the acid and the corresponding salt indicate that water becomes competitive with cyclodextrin in solubilising the surfactants. Water solubility is also the reason for a smoother increase in the binding constants of the salts relative to the free acids (Table 4 and 5) with increasing alkyl chain lengths.

Some ^1H-^1H NOE experiments were carried out in order to elucidate the geometry of the complexes of the acid and of its salt with β-CD. ROESY data for solutions containing C_6P or C_6PNa_2 in a 1:1 host/guest molar ratio (0.012 mol·L^{-1}) show the formation of aggregates in which the polar group is presumably situated near the larger rim of cyclodextrin. This assumption was indirectly confirmed by the appearance, in the spectra of both complexes, of cross-peaks linking the $C(6)H_3$ protons of the surfactant with H(6) of the β-CD, which indicate that the hydrophobic portion of the guest is located close to the smaller edge of the β-CD.

From analysis of the cross-peaks' volumes it is also possible to estimate the extents of host−guest interaction, which are different for the acid C_6P and for the salt C_6PNa_2 (Figure 7). In the case of C_6P, a stronger interaction occurs between $C(6)H_3$ (guest) and H(6) (host) than for $C(6)H_3$ and H(5); in the case of C_6PNa_2 the main cross-peak is detected for $C(6)H_3$ and H(5), followed by weaker correlations of $C(6)H_3$ with H(6) and H(3), respectively. These results indicate that C_6P is more deeply inserted into the cavity than its salt. Though no analysis may be carried out on guest $C(1)H_2$ protons, the signals of which ($\delta = 3.90$ and 3.78 ppm for C_6P and C_6PNa_2, respectively) overlap with β-CD protons, host correlations with $C(2)H_2$ signals (ca. 1.60 ppm) are easily detected. The $C(2)H_2$ protons of the acid are connected with both H(3) and H(5) (the second giving a weaker interaction), while no connection is observed for the corresponding protons of the salt. In neither case is $C(2)H_2$ connected to H(6). The conclusion is that C_6PNa_2 shifts the two adjacent methylene carbon atoms [C(1) and C(2)] outside the larger rim of the cavity relative to C_6P, its alkaline polar head group being better solvated by water molecules.

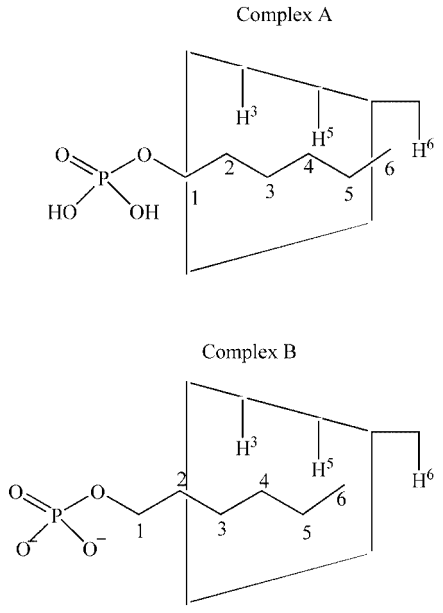

Figure 7. Influence of head group solvation on inclusion geometry of β-CD-surfactant complexes

The indications obtained by ROESY experiments also allow some results reported in Table 5 to be explained. Despite the number of carbon atoms, $C_{18}P\Delta\ C^{9-10}Na_2$ shows an association constant value (Entry 4, Table 5) close to that of $C_{12}PNa_2$ (Entry 3, Table 5). This behaviour, also observed in the case of the other techniques used (see above), is presumably due to the (*Z*) geometry of the C^{9-10} double bond, which forces the molecule to bend in the middle of the chain; in this case the inclusion is possible only for one of the two parts of the molecule, one ending with the methyl and the other with the phosphate group. As lipophilic effects favour the inclusion of the aliphatic (ten carbon atoms) and not the phosphate portion of the chain, the obtained value agrees well with that of $C_{12}PNa_2$, the polar head of which draws the closest C(1) and C(2) outside the cavity and leaves the remaining ten carbon atoms inside (see structure **a** in Figure 5).

Some considerations reported by Wilson and Verral[32] in a ^{1}H NMR study of surfactant complexes with CDs may be useful for understanding of why the binding affinity in Table 5 does not increase further with *n*-alkyl surfactant chains longer than C_{14}. They pointed out that β-CD is a better host than α-CD for linear hydrocarbons with a chain of more than five carbon atoms because of its increased cavity size (6.3 Å against 4.5 Å): the chains can be arranged in a coil fashion rather than being fixed in an all-*trans* conformation, thus increasing the number of carbon atoms included in the cavity and intensifying the van der Waals interactions inside the macrocycle. It has been calculated that approximately eight methylene groups[22] in a *gauche* conformation could enter into the β-CD cavity.

With the above results (^{1}H-^{1}H NOE experiments) in mind, we think that sodium *n*-alkyl phosphates (Table 5), featuring part of the molecule (polar head group and some adjacent carbon atoms) out of the larger rim of β-CD, might be characterized by an increase in the binding constants with increasing chain length. When the number of methylene groups of the sodium *n*-alkyl phosphate accommodated in the β-CD reaches approximately eight units the binding constant for a 1:1 complex is affected by relatively low errors. In contrast, the difficulty in measuring equilibrium constants for 1:1 complexes of *n*-alkyl hydrocarbons larger than 14 units is presumably due to the formation of other aggregates, namely 2:1 (β-CD/surfactant) complexes.[32] The result may be compared with the ESI-MS data, in which the 1:1 complex peak abundance increases with increasing alkyl chain length and the 2:1 complex is also detected at very small percentages for $n \geq 14$. In solution, the hydrophobic effects are more important for 2:1 complex formation than for 1:1 complex formation, while in the gas phase the same effects are absent. Consequently, on going from the solution to the gas phase, a disruption of the 2:1 complex (for $n \geq 14$) in favour of the formation of a 1:1 complex may occur.

Quantitative analysis of the binding affinities of β-CD with the alcohols used to synthesize phosphate surfactants was not carried out, owing to solubility problems regarding the heavier homologues. A rough analysis of the ^{1}H NMR spectra of solutions containing β-CD and 1-hexanol or 1-octanol show displacements of the host signals H(3) and H(5) of degrees comparable to those recorded for the related alkylphosphates; this result was to be expected on the basis of the hydrophobicity of the alcohol alkyl chains.

The solution structure of a β-CD complex with 1-hexanol was estimated by the measurement of ROESY spectra in a D_2O solution (0.01 mol·L^{-1}). The recorded intermolecular interactions indicate that 1-hexanol penetrates from the larger rim of β-CD, with the hydrophobic part of the molecule close to the H(5) and H(6) host protons (the narrower rim) and with the relatively hydrophilic hydroxy group close to the wider rim. This situation has already been observed by other authors in the study of complexes of smaller linear alcohols with α-CD.[33]

As the geometries of complexes of linear alcohols and of the corresponding *n*-alkylmonophosphates are similar (see above), the hydrophobic interactions apparently also play a predominant role in the choice of the orientation of these guests within the β-CD cavity in solution.

The overall results obtained by ^{1}H NMR measurements appear very useful for providing information about the structures of supramolecular complexes, also revealing interesting differences with the results obtained by ESI-MS, and this appears to be in line with the environmental differences associated with the two techniques: one working in the gas phase, the other in water solution.

Conclusions

Supramolecular complex formation by addition of several classes of organic compounds [alcohols **1**, **2**, the corresponding phosphate esters **3**, **4** and their sodium salts **5**, **6**, and some commercial surfactants **7** (C_{14}DMAO, zwitterionic), **8** (SDS, anionic) and **9** (CTABr, cationic)] to solutions of β-CD has been investigated by different techniques. From ESI-MS and surface tension data the formation of inclusion complexes in the cases of **3**−**9** has been established.

The ESI-MS data have highlighted: (a) the prevalence of 1:1 complexes for all the investigated surfactants, (b) the importance of the polar interaction between the head group of the surfactant and the larger rim of the β-CD, and (c) the significance of the carbon chain length. As complex formation in the gas phase does not benefit from hydrophobic interactions, because there is no water surrounding the complex, van der Waals forces and hydrogen bonding contribute to the detection of complex peaks in the mass spectra.

CMC, UV/Vis and ^{1}H NMR spectroscopic data, on the other hand, have furnished a picture of the supramolecular interactions in water solution.

The CMC data have confirmed the importance of the association process between surfactants and β-CD, which can overwhelm the affinity occurring in the micellisation process.

Both UV/Vis measurements and ^{1}H NMR spectroscopic data have been used to calculate the binding constants, and the observed trends are in good agreement.

The CIS data obtained from ^{1}H NMR measurements have been of primary importance for identification of the exact positions of the surfactants in the β-CD cavity, thus ascertaining that the hydrophobic part of the guest is located close to the smaller rim and the polar head group close to the larger edge of the β-CD, because of the formation of strong hydrogen bonds. The presence of such a head group, together with the hydrophobic interactions of the alkyl chains, thus cause the formation of stable supramolecular complexes. Geometric factors (for example, the folded chain of **4** and **6**) play an important role.

Interestingly, the combination of several physicochemical methods used has revealed some differences between gas-phase and water solution measurements. Comparison of the results allows the different contributions to the supramol-

ecular complex formation (hydrophobic, van der Waals and hydrogen bond interactions) to be distinguished.

Experimental Section

Synthesis of Alkyl Phosphoric Acids: Alkylmonophosphoric acids (C_nP, n = 6, 8, 10, 12, 14, 16, 18 and 20)[34,35] were prepared by phosphorylation of the corresponding alcohols with phosphorus oxychloride by application of minor modifications to a reported procedure.[35] Phosphorus oxychloride was placed in a dry, nitrogen-purged flask, and the alcohol was then slowly added. The resulting mixture was stirred for two hours at 0 °C, water (250 mL) was then added, and the mixture was heated at reflux for five hours. The alkylmonophosphoric acids containing six and eight carbon atoms are liquid, while those containing ten or more carbon atoms and the alkyldiphosphoric acids are solid.

C_6P and C_8P were purified by distillation, while solid compounds were recovered from the reaction mixture by extraction with diethyl ether and finally purified by crystallization from hexane. The degrees of purity of the products obtained (yield 90−95 %) were evaluated by ESI-MS and ^{1}H NMR spectroscopy.

Compound 3a: m/z = 181.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 5.85 [br. s, 2 H, $PO(OH)_2$], 4.03 (q, $^3J_{H,H}$ = 6.3 Hz, 2 H, CH_2OP), 1.59−1.67 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 6 H, CH_2), 0.89 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3b: m/z = 209.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.86 [br. s, 2 H, $PO(OH)_2$], 4.00 (q, $^3J_{H,H}$ = 6.3 Hz, 2 H, CH_2OP), 1.58−1.67 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 10 H, CH_2), 0.87 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3c: m/z = 236.8 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.40 [br. s, 2 H, $PO(OH)_2$], 4.01 (q, $^3J_{H,H}$ = 6.3 Hz, 2 H, CH_2OP), 1.60−1.68 (m, 2 H, CH_2CH_2OP), 1.20−1.42 (m, 14 H, CH_2), 0.87 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3d: m/z = 265.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 8.46 [br. s, 2 H, $PO(OH)_2$], 4.01 (q, $^3J_{H,H}$ = 6.6 Hz, 2 H, CH_2OP), 1.62−1.72 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 18 H, CH_2), 0.88 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3e: m/z = 293.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.40 [br. s, 2 H, $PO(OH)_2$], 4.01 (q, $^3J_{H,H}$ = 6.6 Hz, 2 H, CH_2OP), 1.62−1.72 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 22 H, CH_2), 0.88 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3f: m/z = 321.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.00 [br. s, 2 H, $PO(OH)_2$], 4.03 (q, $^3J_{H,H}$ = 6.0 Hz, 2 H, CH_2OP), 1.62−1.70 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 24 H, CH_2), 0.87 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3g: m/z = 349.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.50 [br. s, 2 H, $PO(OH)_2$], 4.06 (q, $^3J_{H,H}$ = 6.3 Hz, 2 H, CH_2OP), 1.62−1.70 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 26 H, CH_2), 0.87 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 3h: m/z = 377.3 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.60 [br. s, 2 H, $PO(OH)_2$], 4.01 (q, $^3J_{H,H}$ = 6.3 Hz, 2 H, CH_2OP), 1.62−1.72 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 28 H, CH_2), 0.88 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, CH_3) ppm.

Compound 4: m/z = 347.2 [M$^-$ − H]. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.00 [br. s, 2 H, $PO(OH)_2$], 5.30−5.40 (m, 2 H, HC=CH), 4.02 (q, $^3J_{H,H}$ = 6.6 Hz, 2 H, CH_2OP), 1.95−2.10 (m, 4 H, CH_2CH=CHCH_2), 1.60−1.68 (m, 2 H, CH_2CH_2OP), 1.20−1.40 (m, 22 H, CH_2), 0.87 (t, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3) ppm.

Commercial alcohols were used after purification by distillation at reduced pressure. Commercial surfactants **7**−**9** were used without further purification.

ESI-MS Measurements: ESI-MS were collected with a ZMD Micromass single quadrupole mass spectrometer operating at 4000 m/z. A Hamilton syringe driven by a Harvard pump was used for direct injection of the sample into the instrument. To minimize the influence of variations in instrumental conditions on the reliability of mass spectra, the ESI-MS parameters (i.e., the pressure of the gas, the desolvation temperature, the capillary and cone voltages, etc.) were kept rigorously constant from run to run in each series of solutions. In particular, a capillary voltage of 3.3 kV and a cone voltage of 80 V were applied; a desolvation temperature of 150 °C was used. The sample solutions in acetonitrile/water (50:50, v/v) were introduced at a flow rate of 15 μL·min^{-1}. For all the experiments, 2.0 × 10^{-4} mol·L^{-1} solutions of CDs and 5.0 × 10^{-5} mol·L^{-1} solutions of surfactant were used (host/guest molar ratio being 4:1).

In the ESI-MS experiments, the uncomplexed surfactants gave very intense signals that can even mask the peaks of the other species present in the mixture. We have therefore examined the 1000 m/z to 3000 m/z spectral range, renormalising the relative abundance accordingly.

CMC Measurements: The CMC values were determined by the surface tension method. The surface tensions of the solutions of the sodium phosphate surfactants were measured at 25 °C with a SensaDyne QC6000 Surface Tensiometer by the maximum bubble pressure method.[36] To solubilise the longer-chain derivatives ($C_{16}P$, $C_{18}P$) an excess of NaOH was added to deprotonate the acid completely. Surface tension values (dyne cm^{-1}) were plotted against the decimal logarithm of molar concentration of the surfactant. A typical example is shown in Figure 8.

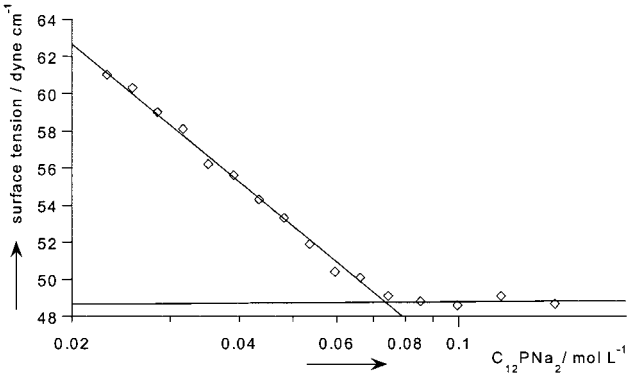

Figure 8. Variation of the surface tension determined for $C_{12}PNa_2$ in the absence of β-CD, as a function of surfactant concentration (logarithmic abscissa)

UV/Vis Measurements: A spectroscopic displacement technique[29,37] was used to determine the binding constants (1:1 complexes) of the spectroscopically transparent (250−600 nm) guests described above with β-CD.

Absorbance measurements were collected with a double-beam spectrophotometer (Kontron Uvikon 860). All solutions were pre-

pared in a 0.1 mol·L^{-1} Na_2CO_3 buffer adjusted to pH 10.6. To determine the binding constants the concentration of phenolphthalein was 2×10^{-5} mol·L^{-1} in all experiments; the concentration of β-CD was kept constant at 3×10^{-4} mol·L^{-1} and the concentrations of surfactants [SDS, C_nPNa_2 (n = 8, 12, 14) and $C_{18}P\Delta C^{9-10}Na_2$] were changed within a significant range ($0-1 \times 10^{-2}$ mol·L^{-1}). The concentration of surfactant was always kept below the critical micellar concentration (see CMC values in the presence of β-CD in Table 2). A stock solution of phenolphthalein in ethanol was prepared and aliquots were used to prepare ethanol/water (0.04 %) solutions of phenolphthalein in buffer. All sodium phosphate surfactants were prepared from the corresponding acids by addition of NaOH. Absorption measurements were carried out at 550 nm and at room temperature.

NMR Measurements: All the NMR spectra (1D and 2D experiments) were recorded with a Varian Inova 300 (300 MHz) and Varian Mercury 400 (400 MHz) spectrometers. The titrations were performed at constant cyclodextrin concentration ([β-CD] ca. 5×10^{-4} mol·L^{-1}) and with variation of the concentrations of phosphate surfactants ($0-3 \times 10^{-3}$ mol·L^{-1}, always below the CMC value) in each sample. The chemical shift data are expressed in terms of Δδ, defined as the difference between the chemical shift of the free β-CD and the observed chemical shift of the host in the presence of the surfactant. The data are referred to the water peak at 25 °C (δ_{HDO} = 4.76 ppm). Binding affinities for the alkylphosphates of lower molecular weight were measured both in neutral (C_nP) and in alkaline conditions (C_nPNa_2); in the latter case the salts were obtained by use of a 40 % solution of NaOD in D_2O. The solubility of the guests of higher molecular weight [C_nPNa_2 (n = 14, 16)] was so small that the measurements were performed only under alkaline conditions.

The ROESY experiments were recorded with a Varian Mercury 400 (400 MHz) spectrometer at 25 °C with mixing times of 50−500 ms in the phase-sensitive mode, with a CW spin-lock field of 2 kHz. The data were collected by use of a 90° pulse width of 12 μs and a spectral width of 4000 Hz. A total of eight repetitions were collected for 256 time increments. The relaxation delay was 2.5 s.

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