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Observation of microscopic patterning at the air/water interface by mixtures of amphiphilic cyclodextrins: a compression isotherm and Brewster angle microscopy study

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ABSTRACT: Mixed monolayers of per-6-ammonium-per-6-deoxy-per-O^2, O^3-hexyl-β-cyclodextrin (**1**) and per-O^2, O^3-hexanoyl-β-cyclodextrin (**2**) were studied at the air/water interface by Langmuir balance techniques and Brewster angle microscopy. The mixed monolayers show non-ideal mixing with formation of a 3:2 complex between **1** and **2**. Brewster angle microscopy showed the formation of microscopic patterning at the interface by the complex and that the spaces within the two-dimensional cage structures formed are occupied by excess of 2 which can undergo phase changes within the cage structures. Copyright © 1999 John Wiley & Sons, Ltd.

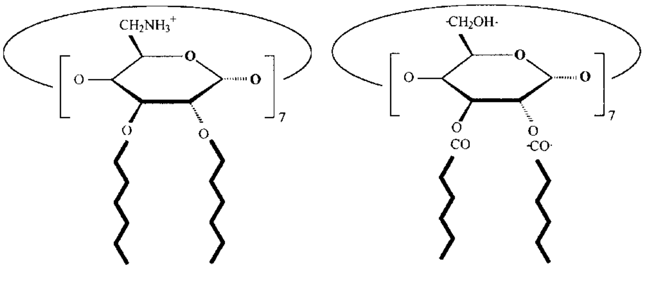
KEYWORDS: microscopic patterning; air/water interface; amphiphibic cydodextrins; compression isotherm; Brewster angle microscopy

INTRODUCTION

The cyclodextrins (CDs) are a family of water-soluble cyclic oligosaccharides produced by the action of the enzyme cycloglucosyl transferase (CGTase) on amylose. They consist of six, seven or eight α, 1–4-linked glucopyranose units and are known respectively as α-, β- and γ-cyclodextrin (α-CD, β-CD and γ-CD). Stabilized by a network of intramolecular hydrogen bonds, they present a rigid shape possessing a hydrophobic cavity. The presence of this hydrophobic cavity makes possible the inclusion of guest molecules to form water-soluble complexes and has led to their widespread use in pharmaceutical and separations applications.[1]

The pseudo 'face-to-face' symmetry of the cyclodextrins arises from the presence of the primary and secondary hydrophilic faces surrounding the hydrophobic cavity. Complete or selective modification at one face can lead to a change in physico-chemical properties of these molecules. The introduction of hydrophobic groups (such as long-chain ethers, esters, amines thiols or amides) at only one face offers a range of amphiphilic molecules already widely studied and which are capable of forming supramolecular assemblies. Other groups,[2,3] and ourselves,[4–6] have synthesized and studied the interfacial properties of a range of amphiphilic cyclodextrins.

Per-6-ammonium-per-6-deoxy-per-O^2,O^3-hexyl-β-cyclodextrin (**1**) (Fig. 1) has seven positively charged ammonium head groups and exhibits good interfacial properties. It can act as a potential biosensor for negatively charged biological macromolecules. Per-O^2,O^3-hexanoyl-β-cyclodextrin (**2**), in addition to its good interfacial properties, has the ability to form highly stable nanoparticules,[7] alone and in the presence of small quantities of **1**. The stability of the nanoparticles is highest for a chain length of six carbon atoms and in view of this we have limited our studies to **1** and **2**. It is thus of interest to study the interfacial behaviour of mixtures of



1

2

Figure 1. Schematic representation of the amphiphilic cyclodextrin derivatives **1**

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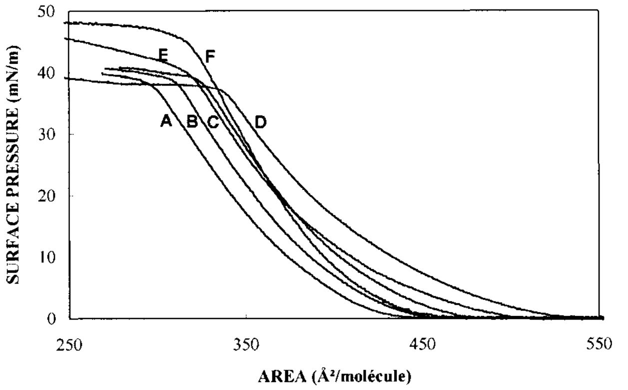

Figure 2. π–A isotherms of (**A**) **2** and mixtures of **1** and **2** at different molar ratios: (**B**) 0.2, (**C**) 0.4, (**D**) 0.6 and (**E**) 0.9 as mole fractions of **1** and compound **1** (**F**)

Table 1. Properties of monolayers of **1** and **2** and their mixtures at different molar ratios of **1**[a]

Molar ratio X_1	A_c	π_c
0.00	295.5	37.4
0.10	303.6	38.3
0.20	309.9	38.4
0.30	316.1	38.2
0.39	323.5	38.5
0.49	327.4	38.2
0.59	336.8	37.0
0.70	327.3	38.0
0.80	319.3	39.6
0.91	313.0	39.9
1.00	300.1	45.3

[a] A_c, apparent molecular area at the collapse point; π_c, collapse pressure.

these molecules in order to attempt to extrapolate the phenomena to studies of three-dimensional aggregates.

Miscibility studies of amphiphilic CDs either with cholesterol[8] or with phospholipids have already been reported.[9–11] This is the first paper to report a complete study of the mixing behaviour of two amphiphilic cyclodextrins. The understanding of the mixing properties of tensioactive molecules is of crucial importance for the comprehension of the physical properties and biological functions of cell membranes. These studies show that there is an almost infinite number of possibilities ranging from ideal miscibility to complete immiscibility. In this paper it is shown that the formation of a 3:2 aggregate complex between **1** and **2** occurs, that in the presence of excess of **2** patterned two-dimensional micro-domains are formed within which the excess of **2** is enclosed and that this free amphiphilic molecule can undergo phase changes within the domains.

EXPERIMENTAL

Synthesis. The synthetic routes to the OH-β-CD-OCOC$_5$ (**2**) and NH$_3$-β-CD-OC$_6$ (**1**) have been described previously along with their compression isotherms.[4,5] To summarize, the synthesis of **2** involves silyl protection of the primary face, hydrophobic substitution at the secondary face and deprotection. The route to **1** involves coupling azide functions at the primary face (via bromination), hydrophobic substitution by at the secondary face and then reduction of the azide groups to ammonium functions. Analytical and physical data were consistent with literature values.[4,5]

Interfacial studies. Amphiphilic molecules were spread from a pure solution (CHCl$_3$) by use of a positive displacement micropipette (Gilson Microman, 50 µl) at the surface of deionized water. The typical concentration range of the CD derivatives was from 0.6 to 1 mg ml^{-1}. Water was purified using a Milli-Q system (ELGA) and its resistance was >18 MΩ. Isotherms were recorded using a computer-controlled NIMA 601 balance for isotherm studies and a KSV 5000 for Brewster angle microscopy experiments. The Compression rate was continuous at 3 cm^2 min^{-1}. The Brewster angle microscope was a commercial BAM1 manufactured by NFT (Göttingen, Germany) equipped with a 5 mW He–Ne laser. The real-time images obtained were recorded on a VCR and digitized using a video acquisition card. All experiments were carried out several times with different solutions to ensure reproducibility (errors in the apparent molecular areas were less than 2% and in the surface pressure less than 2%).

RESULTS AND DISCUSSION

The compression isotherms of pure **1** and **2** and of some of the studied mixtures are shown in Fig. 2.

The two molecules exhibit similar behaviour except for the collapse pressures. Compound **1** has an apparent molecular area of 300 Å^2 per molecule versus 295 Å^2 per molecule for **2** but the latter forms a less stable film than **1** (collapse pressure 45.3 mN m^{-1} for **1** versus 37.4 mN m^{-1} for **2**). This is surprising, as it would be expected that intermolecular repulsions between the cationic head groups of **1** would destabilize films of this compound.

Table 1 summarizes the data obtained for the studied compression isotherms.

The Defay–Crisp phase rule,[12,13] applied to two-dimensional systems, makes possible the prediction of the miscibility of a two-component mixture at the air water interface. From the degree of liberty of the two variables (area and surface pressure) arises an additivity rule which is also applicable to surface pressure:

$$A_{1,2} = X_1A_1 + X_2A_2 \qquad (1)$$

where A_1 and A_2 are the molecular areas in the single-

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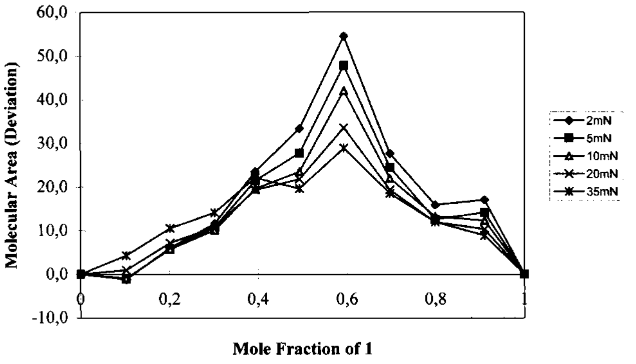

Figure 3. Deviation, for different surface pressures, of the experimental mean molecular area and the expected behaviour according to the additivity rule for different compositions of the mixture of **1** and **2** as a function of the molar fraction of **1**

component monolayers and X_1 and X_2 are the mole fractions of each component. We can thus determine the deviation of the experimental values from the ideal behaviour. This deviation is plotted for different surface pressures in Fig. 3.

A strong positive deviation from ideal behaviour is observed at all pressures. This deviation signifies an apparent expansion of the film compared with that expected for an ideal mixture. This phenomenon decreases with increasing pressure, hence under an applied force the molecules tend to mix more ideally. Two regions may be defined for the mixtures, the first part would consider $X_1 < 0.6$ and the second $X_1 > 0.6$, with a clear maximum for an apparent ratio of 3:2 in **1** and **2**. Reports on positive deviations from the additivity rules are not common, and have been interpreted in terms of specific molecular interactions. Such interactions might include mainly dipole–dipole or ion–dipole repulsions between the polar head groups of the molecules in the film; here the interactions are likely to arise from modification of the H-bonding between the head groups of the amphiphiles and the water networks between adjacent molecules.

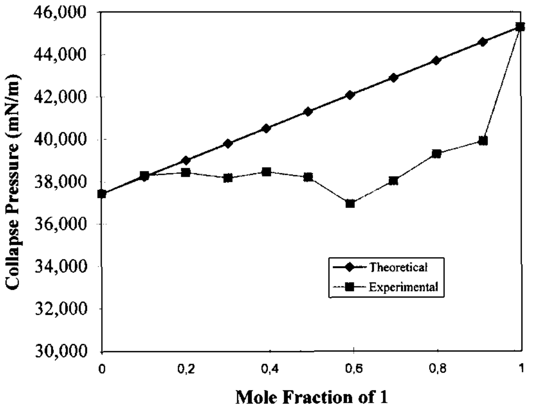

Figure 4. Experimental collapse pressures for different compositions of the mixture of **1** and **2** as a function of the molar fraction of **1** and the predicted behaviour for an ideal mixture

Table 2. Interaction parameters (α) and interaction energies (Δh) for mixtures of **1** and **2** at 35 mN m^{-1} at different molar ratios of **1**

Molar ratio X_1	α	Δh
0.10	−1.5	−145
0.20	0.6	56
0.30	−0.1	−6
0.39	−0.1	−5
0.49	1.3	129
0.59	3.3	320
0.70	1.5	150
0.80	1.1	106
0.91	2.8	274

In Fig. 4 are plotted the experimental collapse pressures. When two components are immiscible, domains of both molecules co-exist. Thus the collapse pressure of the mixture is independent of the concentration and is determined by the less stable component. A two-component film collapses when the less stable phase is squeezed out of the film. In the current case, although the mixed films are less stable than the ideal mixed film, they are still slightly more stable than the film formed by **2** alone. Again, two regions for the evolution of the data exist, $X_1 > 0.6$ and $X_1 < 0.6$.

Goodrich[14] and Pagano and Gershfeld[15] developed a thermodynamic approach to two-component films allowing the calculation of the excess free energy of mixing ($\Delta G^{\mathrm{E}}_{\mathrm{M}}$) from the equation

$$\Delta G^{\mathrm{E}}_{\mathrm{M}} = \int_0^{\Pi} \bar{A} \mathrm{d}\Pi - X_1 \int_0^{\Pi} A_1 \mathrm{d}\Pi - X_2 \int_0^{\Pi} A_2 \mathrm{d}\Pi \quad (2)$$

By fitting the experimental curves to a high-order polynomial it is possible to obtain reliable results. This thermodynamic treatment gives access to the interaction parameter (α) at various surface pressures and to the corresponding interaction energies (Δh):

$$\alpha = \frac{\Delta G^{\mathrm{E}}_{\mathrm{M}}}{RT(X_1^2 X_2 + X_1 X_2^2)} \qquad \Delta h = \frac{RT\alpha}{zL} \quad (3)$$

From the energies calculated the interaction parameter (α) and the corresponding interaction energy (Δh) were calculated for a surface pressure of 35 mN m^{-1}. They are summarized in Table 2 and in Fig. 5 are illustrated values of $\Delta G^{\mathrm{E}}_{\mathrm{M}}$at various surface pressures.

Positive values of $\Delta G^{\mathrm{E}}_{\mathrm{M}}$for the monolayers indicate that this film is less thermodynamically stable than that characterized by a complete separation of two compo-

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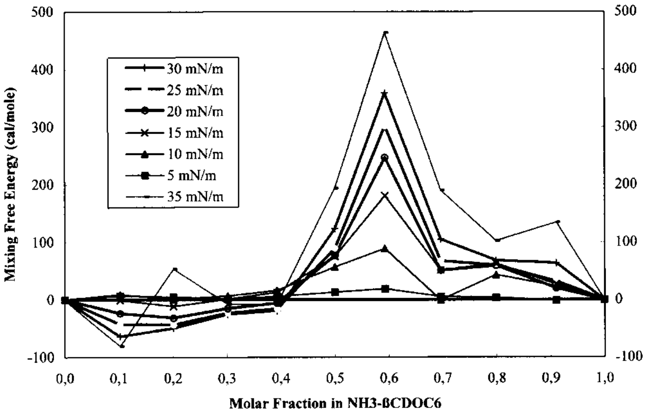

Figure 5. Free energies of mixing calculated from Eqn. 2 for various surface pressures for different compositions of the mixture of **1** and **2** as a function of the molar fraction of **1**

nents, and this suggests that partial phase separation has occurred.[16] A clear maximum is observed for all pressures at a mole fraction of 0.6 in **1**. However, the absolute values are small if compared with *RT* (586 cal mol^{-1} at 22 °C), suggesting that the interaction is not strong at low surface pressures but increases with increase in surface pressure.

To compare the results with those obtained from a different theoretical approach with another approach, the Joos approach[17] was applied to the data. The Joos method predicts the collapse pressure of a mixed monolayer (π_{cm}) with entirely miscible components based on their respective collapse pressures. The main difference between the two models lies in the fact that the additivity rule is valid for all pressures below the collapse pressure and that the Joos model is only applicable at the collapse pressure.

The Joos equation is

$$1 = X_1^s\gamma_1\left(\frac{\pi_{cm} - \pi_{c1}}{kT}A_1\right) + X_2^s\gamma_2\left(\frac{\pi_{cm} - \pi_{c2}}{kT}A_2\right) \quad (4)$$

where X_1^s and X_2^s are the mole fractions of the two components, A_1 and A_2 are the molecular areas corresponding to π_{c1} and π_{c2} respectively, γ_1 and γ_2 represent the surface activity of the two components at their collapse pressure, *k* is the Boltzmann constant and *T* is the absolute temperature.

Table 3. Interaction parameters (ξ) and interaction energies (ΔE) as calcluated from the Joos equation

Molar ratio X_1	ξ	ΔE
0.10	5.3	518
0.20	2.5	247
0.30	0.7	70
0.39	0.7	72
0.49	−0.1	−14
0.59	−2.0	−191
0.70	−1.0	−102
0.80	0.0	0
0.91	−0.6	−62

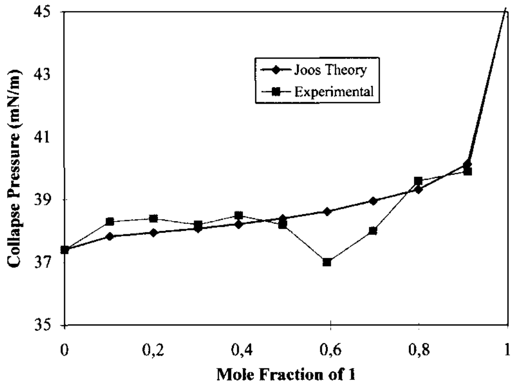

Figure 6. Experimental collapse pressure of mixtures of **1** and **2** as a mole fraction of **1** versus the theoretical collapse pressure calculated according the Joos theory

In all cases where no interactions between the two components occur, the surface activity coefficients are presumed to be equal to unity ($\gamma_1 = \gamma_2 = 1$), and the theoretical curve corresponding to this hypothesis should fit the experimental data. However, in case of the non-ideal mixing of the two constituents the activity coefficient is no longer equal to unity and is now dependent on the interaction parameter ξ according to the relation

$$\begin{aligned}\gamma_1 &= \exp[\xi(X_2^s)^2]\\ \gamma_2 &= \exp[\xi(X_1^s)^2]\end{aligned} \quad (5)$$

Hence, for a regular mixture the two activity parameters obeys the Gibbs–Duhem equation as

$$X_1^s \mathrm{d}\ln\gamma_1 + X_2^s \mathrm{d}\ln\gamma_2 \quad (6)$$

The knowledge of the interaction parameter allows us, again, to calculate the interaction energy ΔE:

$$\Delta E = \frac{\xi RT}{zL} \quad (7)$$

where *zL* is the number of neighbours of each molecule. Considering a closed-packed hexagonal lattice, if the molecular areas are not too different (which is our case), we can then assume $zL = 6$. In Table 3 are given the calculated ΔE and ξ values.

Fig. 6 shows the experimental curve along with the theoretical curve according to the Joos approach. This approach shows good agreement between theory and experimental except for the region $0.5 < X_1 < 0.7$. All attempts to incorporate in the Eqn. 4 varying interaction parameters to take this region into account gave no better

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results. This can be ascribed to the particular nature of the interactions which occur in this region and which are apparently very different from those occurring in all other areas of the miscibility measurements.

The expansion of the film and the aspect of the curves with a pivot point promotes the idea that some regions in the film have a definite composition; effectively there exists a eutectic and for this eutectic specific ordering in the monolayer exists. The eutectic represents the formation of a 3:2 complex between **1** and **2**. The stabilization of such a complex must arise from modification of the H-bonding and hydration networks around the polar head groups; the exact nature of the complex is uncertain and we are currently trying to obtain crystals of the 3:2 complex for the analogous non-amphiphilic molecules. The possibility of a simpler 2:1 complex is ruled out by the lack of any skew around the maximum at a mole fraction of 0.6; in the case of such a 2:1 complex it would be expected that the values observed at a mole fraction of 0.7 would be greater than those at a mole fraction of 0.5. This not being the case, we favour the 3:2 model.

In addition to the above thermodynamic treatment of the mixtures, Brewster angle microscopy[18] was used to obtain further information on the film structure. This *in situ* microscopy technique possesses the advantage over fluorescence microscopy of observing the phase behaviour at the air water interface without the perturbation of the film caused by incorporation of a fluorescent probe.

The behaviour of the two molecules alone has already been described.[19] For ease of comparison we can note the NH_3-βCDOC$_6$ (**1**), displays an apparently simple behaviour. At low pressure a film of very low reflectivity exists which undergoes a nucleation phenomenon beginning at a pressure lower than the collapse pressure. There is no further change in the aspect of this film under further compression. During decompression, all the processes seen during the compression are shown to be reversible. No fracturing of the film occurs and at low pressure a film of very low reflectivity is again observed.

For **2**, at high molecular areas (700–600 Å^2 per molecule) the film presents the appearance of a two-dimensional foam. Above zero pressure the behaviour of the film is closely similar to that of **1**, that is, first the appearance of a homogeneous film of low reflectivity and second at higher pressure a nucleation phenomenon. The main difference between the two molecules is that for **2** during expansion of the film there is no strict reversiblity. Under decompression at intermediate pressures structures typical of an LC–LE transition exist.

Given the formation of the 3:2 complex, it was of interest to observe the behaviour of mixed films. A series of experiments were carried out at various mole fractions of **1**. It was observed that for mole fractions >0.2 in **1** there is no apparent difference in the images between those of the mixture and those of **1** itself. All images shown are 850×850 μm. The images are recorded in real time as a video recording and the figures given are representative of the structures observed.

It is in the region of mole fractions <0.2 in **1** that a number of interesting phenomena are observed. For a 0.1 mole fraction in **1** during the compression the film behaves in the same way as a film of pure **2**. At low pressure and high molecular areas there exists a foam [Fig. S1(a) and in a more irregular form Fig. S1(b)] which disappears when the pressures rises to form a homogeneous film of low reflectivity [Fig. S1(c)] which tends then to nucleate [Fig. S1(d)]. (Figures S1–S3 are available from the epoc website at http://www.wiley.com/epoc.)

The main features of interest arise during decompression. Unexpectedly and in contrast to the two pure compounds, the mixture separates at relatively high surface pressure (30 mN m^{-1}) into two distinct phases [Fig. S2(a)] forming a network of two-dimensional cages of high reflectivity surrounding zones of lower reflectivity [Fig. S2(b)]. At this mole ratio the two-dimensional foam structures are highly irregular. This microscopic patterning at the surface seems to be governed by the formation of a 3:2 complex between **1** and **2**; in this case excess of **2** will be present within the cage structures. That this occurs is demonstrated by the behaviour of the phase present within the structures; at high areas and zero surface pressure the phenomenon of two-dimensional foaming typical of the decompression of **2** but absent for **1** is observed [Fig. S2(c) and (d)]. This last process is strictly limited within the areas delimited by the cages. In the course of the decompression the rigid nature of the walls formed by the complex is shown by the break-up of the cage structures during which fragments of the walls remain present; this is particularly evident in Fig. S2(c).

Similar processes are observed for the film composed of a mole fraction of 0.2 in **1**; there is again phase separation into cage structures [Fig. S3(b) and (c)]. However, there is a much higher degree of organization present in the system and a pseudo-hexagonal array is present which elongates slowly along the decompression axis (Fig. S3(d)]. Phase changes arising from pure **2** are again observed within the cages.

CONCLUSION

We have demonstrated that in mixed films of two amphiphilic cyclodextrins there exists a discrete complex of apparent composition 3:2 for **1** and **2**. The formation of this complex leads to microscopic phase separation which can be observed by Brewster angle microscopy; the apparently rigid phase formed by the complex self-organizes into cage structures at the air water interface which act as *in situ* micro-Langmuir troughs in which phase changes of the encapsulated excess amphiphilic cyclodextrin can be observed.

We are currently investigating the possibility of using these mesoscopically two-dimensional phase-separated foams to encapsulate other amphiphilic molecules.

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