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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · JUNE 2011

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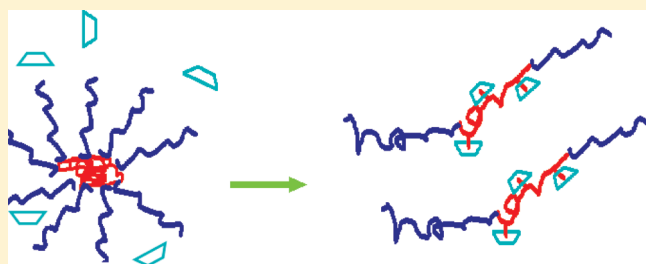
Franca Castiglione,[†] Margarita Valero,[‡] Cécile A. Dreiss,[§] and Andrea Mele^{*,†}

[†]Department of Chemistry, Materials and Chemical Engineering “G. Natta”, Politecnico di Milano, Piazza L. Da Vinci 32 I-20133, Milano, Italy

[‡]Dpto. Química Física, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, s/n, 37007 Salamanca, Spain

[§]Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, SE1 9NH London, United Kingdom

ABSTRACT: The triblock-copolymer poly(ethylene oxide)–poly(propyleneoxide)–poly(ethylene oxide) (PEO–PPO–PEO), referred to as Pluronic, is widely studied for its unique aggregation properties and its applications in drug delivery and targeting. In previous studies [Dreiss, C. A.; et al. *Soft Matter* 2009, 5, 1888–1896], we showed that the interaction of heptakis (2,6-di-O-methyl)- β cyclodextrin (DIMEB) with the triblock-copolymer Pluronic F127 in solutions above the CMC led to complete disruption of the polymeric micelles, while similar β cyclodextrins (β CD) derivatives, heptakis (2,3,6-tri-O-methyl)- β CD (TRIMEB), hydroxypropyl- β CD (HPBCD), and hydroxyethyl- β CD (HEBCD), did not induce micellar break-up. In this work, nuclear magnetic resonance spectroscopy experiments were used to elucidate the nature of the interactions leading to break-up and highlight differences between the four β CD derivatives studied, which could explain the very different outcome observed. Intermolecular nuclear Overhauser enhancements (NOEs) show that both DIMEB and TRIMEB interact selectively with the PPO methyl groups of F127 in a similar way. The interaction is mainly with the external methyl groups in the 6-position of the glucopyranose units of cyclodextrins. However, a weak but detectable interaction with the inner cyclodextrins protons is also observed. These interactions, both with the external surface and with the cavity of β CD, suggest the formation of a loose complex, rather than the widely invoked pseudorotaxane type of inclusion. In addition, these interactions seem to be necessary but not sufficient to induce micellar break-up. Diffusion measurements show decreased diffusivity of DIMEB in the presence of F127 to a larger extent than the other CD derivatives, thus confirming the unique behavior of DIMEB toward F127 polymer. From the diffusion coefficients, an average of 1 DIMEB molecule per 4.2 PO groups of F127 is determined for the highest concentration of DIMEB considered (11 wt % DIMEB dissolved in 5 wt % F127). Micellar break-up is complete at a concentration as low as 1 DIMEB molecule per 8.2 PO units.



INTRODUCTION

Molecular self-assembly, driven by noncovalent interactions, is a fascinating process that has attracted increasing interest over the past couple of decades to devise tailored supramolecular structures, often with built-in stimuli-responsiveness. Self-assembly relies on the cooperation of various interactions such as van der Waals, electrostatic, hydrophobic, and hydrogen bonding. A fascinating type of self-assembly process is the one based on “host–guest” interactions, exemplified by a particular class of materials: cyclodextrins. Cyclodextrins are cyclic oligosaccharides consisting of six, seven, or eight glucopyranose units (α , β , and γ CD) linked by 1,4- α -glycosidic bonds.^{1,2} They are all characterized by a toroidal shape with a hydrophobic cavity, which imparts them the ability to form inclusion complexes through noncovalent interactions with a variety of molecular guests that can fit into their cavity. Since the pioneering work of Harada,³ it is known that cyclodextrins can also thread onto long polymeric chains, forming so-called “(pseudo)-polyrotaxanes”. These supramolecular structures have been

intensely investigated as the basis of nanoscale molecular devices, where mobility is imparted by sliding of the cyclodextrins along the polymeric chains,⁴ leading also to applications in the biomedical field. Numerous review papers have been published on this topic.^{5–8}

The first pseudopolyrotaxane-forming polymers described by Harada were polyethylene oxide (PEO) and polypropylene oxide (PPO).^{3,9} α CD was shown to thread onto PEO, while β CD threaded onto PPO, but did not form an inclusion complex with PEO, supposedly because of its larger cavity as compared to α CD. The possibility of a “selective” geometrical fit opens up attractive perspectives with the well-known Pluronic triblock-copolymers, made up of a PPO central block flanked by two PEO blocks, widely investigated for their capacity to encapsulate drugs and their reversible thermal gelation.^{10,11} In previous studies, we

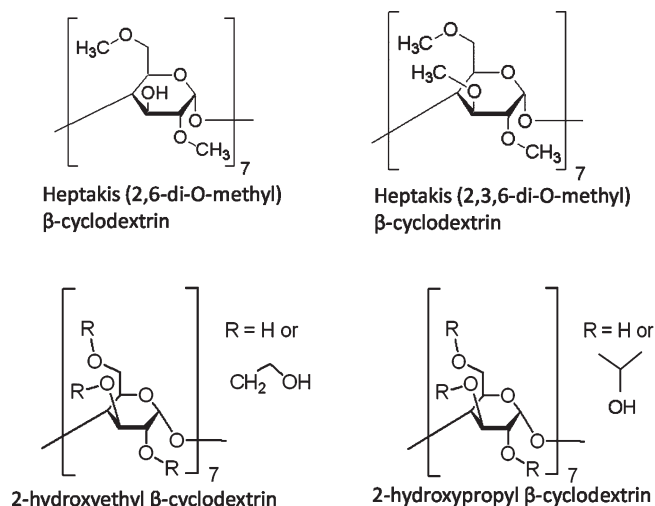
Received: April 22, 2011

Revised: June 10, 2011

Published: June 13, 2011



Chart 1. Scheme of the Chemical Structure of the Four β Cyclodextrin Derivatives Used in This Study, Showing the Repetitive Unit



have shown using SANS measurements that a methylated derivative of β CD, heptakis (2,6-di-O-methyl)- β cyclodextrin (DIMEB), is able to induce the complete rupture of Pluronic F127 micelles.^{12–14} Instead, the micelles remain intact in the presence of the same amount of three closely related β CD derivatives, heptakis(2,3,6-di-O-methyl)- β cyclodextrin (TRIMEB), 2-hydroxyethyl- β cyclodextrin (HEBCD), and 2-hydroxypropyl- β cyclodextrin (HPBCD)¹ (Chart 1). We suggested¹² that cyclodextrin's macrocycle could thread onto PEO to locate preferentially on the hydrophobic PPO group, therefore decreasing its hydrophobicity and thus the driving force for micellization. This preferential formation of an inclusion complex with the central PPO group has been described in other studies.^{15–21} However, the threading of β CD along a long PEO chain (100 units for F127) to finally settle on the PPO block seems thermodynamically quite unfavorable. Therefore, the possible establishment of nonspecific interactions between the cyclodextrin and the polymer, other than an inclusion complex, could be invoked to explain the dramatic increase in the cmc observed with DIMEB, as well as the surprising sensitivity of this process to the nature of β CD substituents. In this Article, we therefore used nuclear magnetic resonance (NMR) experiments to explore the complexation behavior of the four β CD derivatives mentioned above:¹³ DIMEB, TRIMEB, HEBCD, and HPBCD with the Pluronic F127. The aim of this work was to achieve a better insight, at atomic level, on the repertoire of interactions leading to the observed effects of DIMEB on F127 micelles, and compare the effect of β CD substituents on the polymer/CD interactions. The NMR measurements were therefore targeted at finding a rationale for the dramatically different behavior of twice-methylated β CD (DIMEB), the only one studied that is able to break-up Pluronic micelles, with respect to that of thrice-methylated β CD (TRIMEB) and randomly hydroxyethylated or hydroxypropylated β CDs (HEBCD and HPBCD), which were unable to disrupt the micelles.

Nogueiras-Nieto et al.²¹ studied and compared the interaction between HPBCD and a randomly methylated derivative of β CD, MBCD, with F127. From surface tension measurements and π -A isotherms of F127 on a subphase of CD solutions, they detected a stronger interaction with MBCD. ¹H NMR measurements showed chemical shift variations of the H₃, H₄, and H₅

protons of MBCD, which they interpreted as the inclusion of the polymer inside the β CD cavity (with HPBCD the resolution was not good enough to draw any conclusion). Finally, they also obtained TEM images of elongated rodlike structures formed in mixtures of 5 wt % F127 and 10 wt % of both CDs, strongly suggesting the presence of rotaxanes. Recently also, Tsai et al. performed an extensive NMR study of the complexation of native β CD with Pluronics of varying PO content (other than F127) in solution and in the solid state.²⁰ With 2D ROESY NMR, they observed cross-peaks between the inner cavity H₅ and H₃ protons of β CD and the methyl protons of PPO. Using ¹³C CP/MAS NMR spectroscopy on dehydrated β CD/polymer mixtures, they were able to infer the presence of inclusion complexes.

In this contribution, we use the 2D NOESY technique in combination with pulse field gradient spin-echo (PGSE) diffusion experiments. Our results reveal the existence of the same type of interactions between F127 and both DIMEB and TRIMEB: between the PPO methyl group and both the outer surface and the cavity of the β CDs. We show that these interactions do not exclude rotaxane formation, but are in better agreement with a looser type of aggregate, not necessarily involving threading of β CD. Diffusion experiments also detect an association between the polymer and all types of β CDs, however, with a substantially larger fraction of DIMEB involved in the complexation.

EXPERIMENTAL METHODS

Materials. PPO polymer (M_w 2000) and Pluronic triblock copolymer F127 (M_w 12 600) comprising a central block of 65 PPO units and two side-blocks of PEO (100 units) were obtained from Sigma Aldrich UK. The β cyclodextrin derivatives were obtained from Sigma Aldrich UK: heptakis (2,6-di-O-methyl)- β cyclodextrin (DIMEB), heptakis (2,3,6-di-O-methyl)- β cyclodextrin (TRIMEB), 2-hydroxyethyl- β cyclodextrin (HEBCD), and 2-hydroxypropyl- β cyclodextrin (HPBCD). The molecular structure of the four β CD derivatives is reported in Chart 1. The Pluronic/ β CD solutions were prepared by weighing the appropriate amounts of polymer, β CD, and deuterated water (D₂O), to obtain the target concentration of 5 wt % of F127 polymer and 3–11 wt % of β CD derivatives. The samples were stored in the refrigerator to aid solubilization.

NMR Measurements. The ¹H 1D and 2D NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500 MHz proton frequency. The {¹H–¹H} NOESY experiments were acquired with mixing times of 100, 300, and 500 ms; 512 experiments were performed in the F1 dimension with 16 scans for each of the t_1 increments and sweep width of 6.6 ppm. Selective ¹H NOESY experiments were carried out by using soft Gaussian pulses and library pulse sequence. Self-diffusion coefficients were measured by PFG experiments. A pulsed gradient unit capable of producing magnetic field pulse gradients in the z-direction of 53 G cm^{–1} was used. All of the experiments were performed using the bipolar pulse longitudinal eddy current delay (BPPLIED) pulse sequence. The duration of the magnetic field pulse gradients (δ) and the diffusion times (Δ) were optimized for each sample to obtain complete dephasing of the signals with the maximum gradient strength. In each DOSY experiment, a series of 64 spectra with 32K points were collected. For the investigated samples, δ values were in the 2–3 ms, while the Δ values were in the range 0.1–0.5 s. The pulse gradients were incremented from 2% to 95% of the maximum gradient

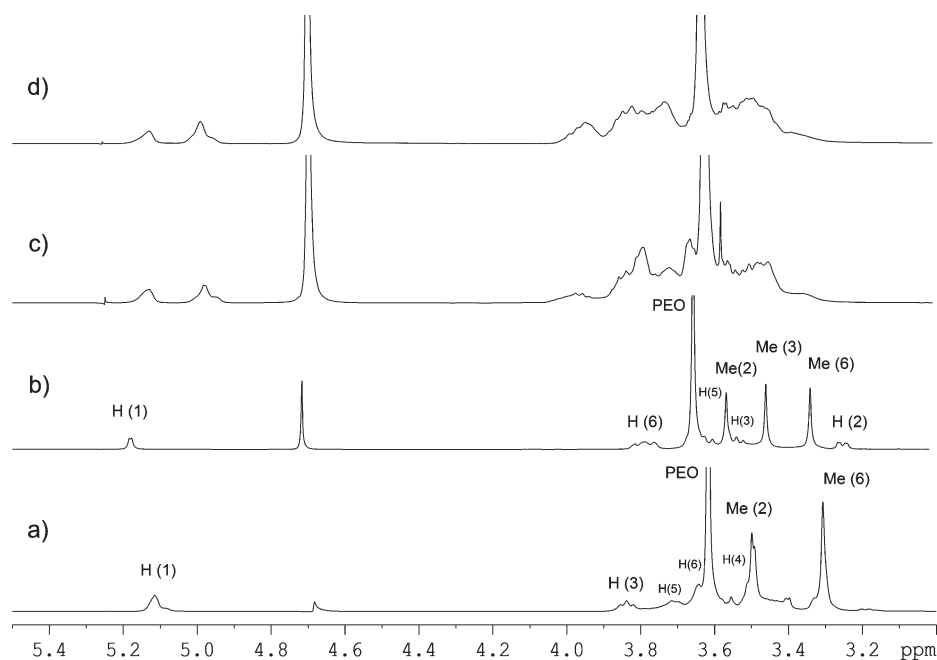


Figure 1. ^1H NMR spectra of DIMEB (a), TRIMEB (b), HEBCD (c), and HPBCD (d) at 6 wt % in D_2O .

strength in a linear ramp. The temperature was set and controlled at 298 K with an air flow of 535 L h^{-1} to avoid any temperature fluctuations due to sample heating during the magnetic field pulse gradients. The precision of the measured diffusion coefficient is within 10%.

Optical Rotation. The measurements were performed on a JASCO-600 instrument between 600 and 200 nm in a 1 cm path length cell on 10 wt % DIMEB solutions at increasing F127 concentration (1–10 wt %).

RESULTS AND DISCUSSION

^1H NMR Spectroscopy. The interaction between βCD derivatives and Pluronic micelles in solution was studied by ^1H NMR diffusion spectroscopy and NOE experiments. The ^1H NMR spectra (6 wt % samples) of DIMEB, TRIMEB, HEBCD, and HPBCD dissolved in 5 wt % F127/ D_2O are reported in Figure 1. Solutions containing DIMEB and TRIMEB gave a reasonable spectral resolution, while the other βCD derivatives (HEBCD and HPBCD) were less well resolved. In particular, they had similar broad lines, and both showed two separate signals for the anomeric proton, because these CDs consist of a mixture of closely related derivatives with different degrees of substitution and isomeric forms.²² Therefore, the spectra of these two CD derivatives are not discussed further in this section. Figure 2 presents the ^1H spectra of DIMEB (6 wt %) dissolved in pure D_2O , F127/ D_2O (5 wt %), and PPO/ D_2O (0.5 wt %), respectively. The reference spectrum (DIMEB in D_2O) and the DIMEB solution in PPO/ D_2O show similar spectral resolution and peak positions, while a clear broadening of all peaks is observable in the F127/ D_2O solution. In addition, the chemical shift of protons 3 and 6 is shifted upfield of 16 Hz, while protons in position 5 undergo a chemical shift change of 7 Hz in the D_2O /F127 solution, thus indicating either some association between the CD moieties and the polymer chains in this latter case or a viscosity effect. It has been shown, however, from turbidity measurements

that 0.5 wt % PPO of M_w 2000 is fully solubilized by 2.5 wt % DIMEB;¹³ therefore, some interaction must be present. However, our results show that the interaction with PPO is not strong enough to give detectable changes in the NMR spectrum, suggesting that the interaction is either weaker or of a different nature than the interaction with F127. Moreover, the variation of the DIMEB concentration (3–11%) slightly influences the peaks position by shifting the whole spectrum 25 Hz upfield.

Somewhat different results were obtained from TRIMEB solution (Figure 3). In this case, no relevant chemical shift changes are observed between the reference spectrum (TRIMEB in D_2O) and that in the presence of F127 (trace 2). The variation of the TRIMEB concentration (3–11%) slightly influences the peaks position by shifting the whole spectrum of 38 Hz upfield and unselectively. Only little chemical shift variations in the range of 10–38 Hz downfield are observed for all of the TRIMEB protons in the PPO/ D_2O solution (trace 3). This is somehow surprising because full solubilization of PPO by TRIMEB is not achieved in these conditions,¹³ although a partial decrease in the turbidity was obtained, suggesting that some polymer/CD interaction occurs.

NOE Experiments. Information on the structure of Pluronic/ βCD aggregates and the mechanism of interaction can be obtained by intermolecular NOEs. According to the theory, the cross-relaxation is dominated by rotational tumbling of the molecule with rotational correlation time τ_c , leading to the r^{-6} distance dependency of the NOE intensity (r being the internuclear distance of the cross-relaxing nuclei). Therefore, only short-range and specific interactions are expected to give rise to NOE, and a threshold of $r = 4 \text{ \AA}$ is commonly accepted for vanishing NOEs. This is strictly true for intramolecular NOE, but it may be extended to the intermolecular case by assuming that the molecular assembly shows a single correlation time or, in other words, that the association is sufficiently stable to develop NOE as a single ensemble, thus converting the intermolecular NOE into an intramolecular one within a long lasting aggregate. Assuming that

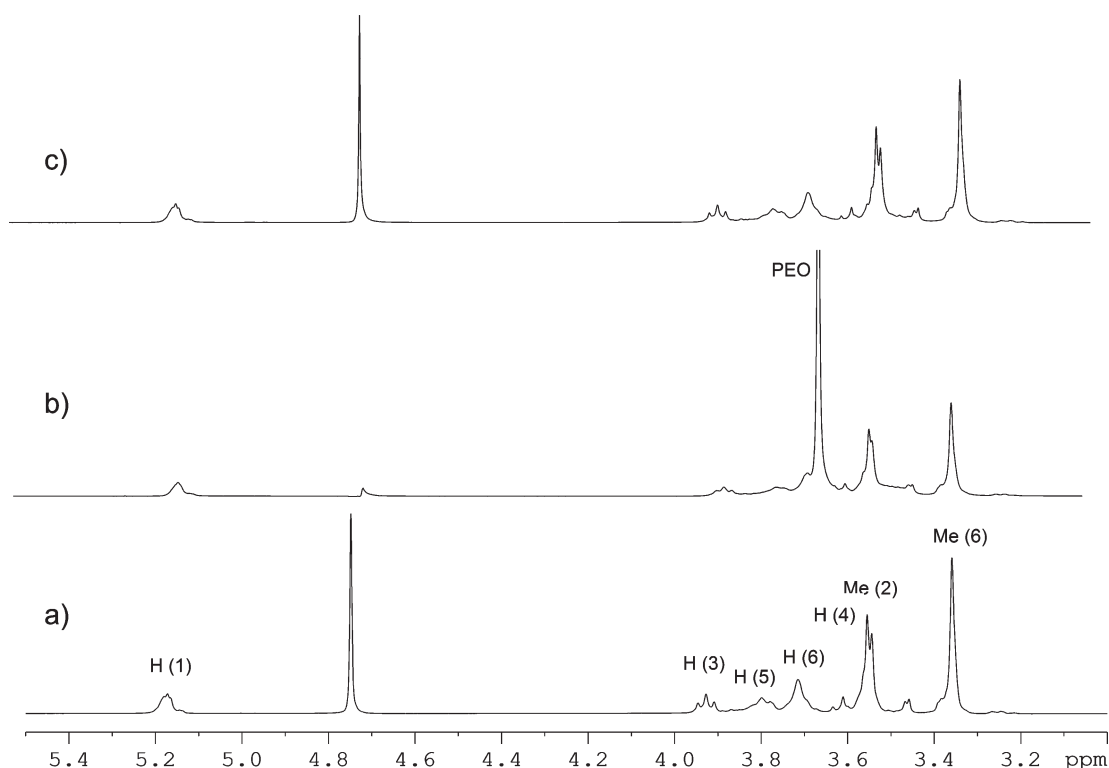


Figure 2. ^1H NMR spectra of 6 wt % DIMEB dissolved in D_2O (a), 5 wt % F127 Pluronic (b), and 0.5 wt % PPO (c).

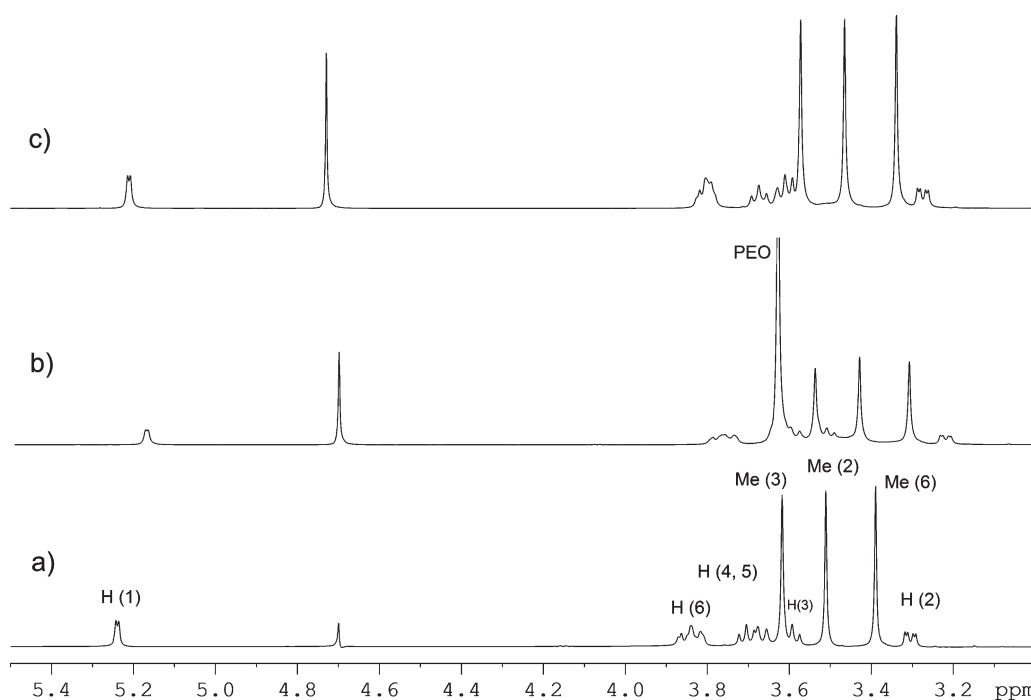


Figure 3. ^1H NMR spectra of 6 wt % TRIMEB dissolved in D_2O (a), 5 wt % F127 Pluronic (b), and 0.5 wt % PPO (c).

micelles fall in this case, $\{^1\text{H}-^1\text{H}\}$ NOE data can potentially provide details of βCD organization in the Pluronic micelles.^{23–26}

$\{^1\text{H}-^1\text{H}\}$ NOESY spectra were acquired at several mixing times for the solutions of DIMEB and TRIMEB dissolved in 5% Pluronic and 0.5% PPO/ D_2O solutions. In all of the experiments, the NOESY cross peaks were in phase with respect to the

diagonal, showing that the system was in the slowly tumbling regime ($\omega\tau_c \gg 1$). This may be due to the presence of association between cyclodextrin and polymer, as well as to viscosity effects. For the purpose of NOE assignment, it is worth noting that both DIMEB and TRIMEB showed a well-resolved and isolated methyl resonance of the PPO block at 1.08 ppm,

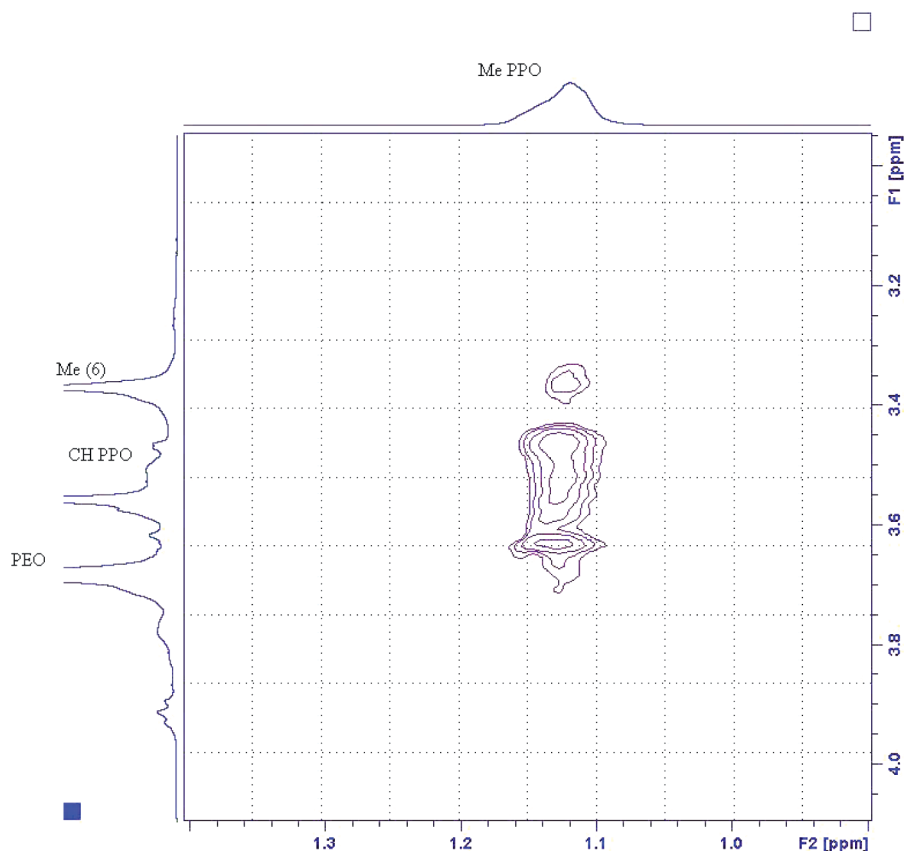


Figure 4. ^1H NOESY contour plot (expansion) of DIMEB (11 wt % DIMEB dissolved in 5 wt % F127 in D_2O ; mixing time: 300 ms).

whereas the PEO resonances resulted partially overlapped with the cyclodextrins peaks. Moreover, the two-dimensional spectrum of DIMEB (Figure 4) showed a cross-peak between the methyl groups of PPO and the methyl groups in position 6 of DIMEB, which are surely localized outside the cyclodextrin's cavity. It is interesting to note that such close PPO/DIMEB contact has not been reported before. We expected this interaction to be a specificity of DIMEB, possibly explaining the difference from the other β CD derivatives in its interaction with the Pluronic. However, a similar result was observed for the thrice-methylated derivative TRIMEB (Figure 5). Therefore, this methyl–methyl interaction could contribute to the different behavior observed between F127, HEBCD, and HPBCD, on one hand, as compared to F127 and DIMEB, on the other hand; however, it does not justify the different behavior observed between DIMEB and TRIMEB with F127 observed by SANS.¹³ It is worth highlighting, however, that, as compared to HEBCD and HPBCD, some interaction seems to exist between TRIMEB and F127 micelles, as revealed by the gradual, very slight alteration of the micellar SANS pattern (as well as the gradual, very slight solubilization of PPO mentioned above).¹³ Therefore, this methyl–methyl interaction may be necessary but not sufficient to induce micellar break-up, and some other mechanism must be contributing to the rupture. To determine whether PEO interacts with the cyclodextrin and to obtain more specific information on the PPO/CD interaction, selective one-dimensional NOESY experiments were performed. The selective irradiation of the inner cavity protons, H_3 and H_5 , of DIMEB gave a weak but detectable NOE with the methyl groups of PPO,

giving evidence of the inclusion of the methyl group of PPO inside the DIMEB cavity. Nogueiras et al.²¹ also observed a chemical shift variation of the inner protons of randomly methylated β CD (MBCD) due to the interaction with F127, as well as Tsai²⁰ with β CD and other Pluronic derivatives. Conversely, the selective irradiation of the same protons, in the same conditions, did not afford any detectable NOE with the CH_2 protons of PEO, therefore clearly indicating that no interaction exists between the inner protons of DIMEB and the PEO groups of the F127. This finding therefore confirms the preferential location of the cyclodextrins on the inner hydrophobic PPO part of the triblock copolymer, far from the PEO groups. However, this finding alone is not sufficient evidence for the formation of polyrotaxanes, with β CD threading along PEO to preferentially locate on the PPO block, but it does not rule it out either. A similar two-dimensional NOESY map was also obtained for TRIMEB (Figure 5), showing the same type of interaction between TRIMEB inner protons with the methyl of PPO from the polymer. DIMEB and TRIMEB share this interaction, which therefore does not seem to be the discriminating factor for the micellar break-up observed in the presence of DIMEB only. Interestingly, previous IR measurements¹³ had also failed to show a different pattern for mixtures of DIMEB and F127 as compared to TRIMEB with F127.

In summary, NOE data pointed out that both the inner protons and the external methyl groups of methylated CDs are in close spatial proximity with PPO methyl groups. At the same time, no detectable NOE contact between CD and PEO was observed. The observed NOEs are consistent with the inclusion

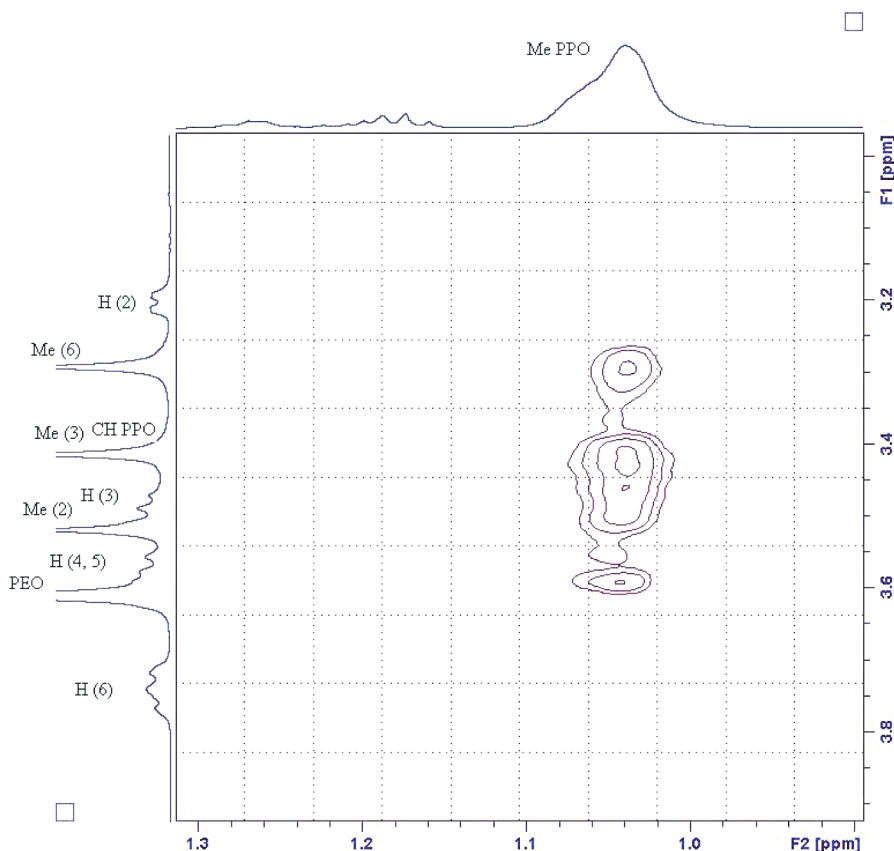


Figure 5. ^1H NOESY contour plot (expansion) of TRIMEB (11 wt % TRIMEB dissolved in 5 wt % F127 in D_2O ; mixing time: 300 ms).

of the CH_3 groups of PPO within the cyclodextrin's void cavity. However, the NOE intensity is quite low, thus suggesting the formation of "loose" inclusion complexes. These results do not fit well with threading of βCD onto the polymer chain as the only interaction mechanism. Therefore, in the range of concentrations and conditions used in this work, relevant contribution to the polymer/cyclodextrin contacts arising from interaction mechanisms different from polyrotaxane formation should be accounted for.

The ^1H selective NOE experiments performed in the PPO/ D_2O solutions for DIMEB and TRIMEB gave similar results but clearly of lower intensity (data not shown), in particular for the interaction with H_3 and H_5 . This weaker interaction implies that the groups involved in the interaction are further apart and supports the picture of loose inclusion of the PPO methyl group into the apolar cavity of cyclodextrin as just one of the possible interaction mechanisms. Therefore, NOE data seem to indicate that, in the case of PPO, DIMEB and TRIMEB act as solvating agents, rather than host molecules with selective binding sites, able to induce PPO polymer solubilization.¹³ Additionally, it also suggests that the CD/F127 aggregation behavior is modulated by the presence of PEO: the PEO blocks must play a role, possibly by driving the conformation of the polymer in such a way that the PPO blocks are suitably exposed to the cyclodextrins to let the polymer/CD interaction occur.

Sample containing the randomly substituted βCD s HEB CD and HPBCD gave too broad lines to be assigned and interpreted in the NOESY maps and therefore are not discussed.

Diffusion Coefficients. The diffusion experiments provide information on the "dynamic" rather than the "structural"

properties. The diffusion is related to the molecular weight of the species (single molecules or aggregates) subjected to Brownian motion and to the viscosity of the solution. The self-diffusion coefficients of all cyclodextrin derivatives were measured by pulsed field gradient spin-echo NMR spectroscopy (PFGSE-NMR) in the ^1H frequency domain. The observed echo intensity I is related to the experimental parameters by the Stejskal–Tanner equation:

$$I = I_0 \exp \left[-(\gamma g \delta)^2 D \left(\Delta - \frac{\delta}{3} \right) \right] \quad (1)$$

where I_0 is the echo intensity without field gradient, γ is the gyromagnetic ratio of the observed nucleus, g is the magnetic field gradient strength, δ is the duration of the field gradient with magnitude g , Δ is the interval between the two gradient pulses, and D is the diffusion constant. The latter is extracted from the slope of a linear fit of $\ln(I/I_0)$ versus g^2 . The resulting self-diffusion coefficients D (m^2/s) for the βCD derivatives dissolved in pure D_2O , in PPO/ D_2O , and in F127/ D_2O at a fixed CD concentration of 6 wt % are reported in Table 1.

The first column lists the reference data of the βCD derivatives diffusion coefficients in D_2O . The data reported in the second column show that the presence of PPO does not affect the diffusivity of cyclodextrins, thus indicating that PPO and cyclodextrins show independent and uncorrelated motions, in good agreement with a solvent–solute type of interaction. Conversely, the diffusion coefficients obtained in the presence of F127 are significantly lower than the corresponding values in D_2O and in the presence of PPO. This finding is consistent with a certain

Table 1. Diffusion Coefficients (m^2/s) of CD Derivatives^a

| | D in D_2O | D in $\text{PPO}/\text{D}_2\text{O}$ | D in $\text{F127}/\text{D}_2\text{O}$ |
|--------|-----------------------------|--|---|
| DIMB | 1.8×10^{-10} | 1.8×10^{-10} | 0.9×10^{-10} |
| TRIMEB | 1.8×10^{-10} | 1.9×10^{-10} | 1.1×10^{-10} |
| HEBCD | 1.9×10^{-10} | 2.2×10^{-10} | 1.4×10^{-10} |
| HPBCD | 2.0×10^{-10} | 2.1×10^{-10} | 1.3×10^{-10} |

^a Concentrations: 6 wt % cyclodextrins dissolved in D_2O , $\text{PPO}/\text{D}_2\text{O}$ (0.5 wt %), and $\text{F127}/\text{D}_2\text{O}$ (5 wt %).

Table 2. Diffusion Coefficients (m^2/s) of CD Derivatives Dissolved in 5% F127/ D_2O at Different CD Concentrations

| | D (3% CD) | D (6% CD) | D (9% CD) | D (11% CD) |
|--------|------------------------|------------------------|------------------------|------------------------|
| DIMB | 1.20×10^{-10} | 0.90×10^{-10} | 0.67×10^{-10} | 0.59×10^{-10} |
| TRIMEB | 1.30×10^{-10} | 1.15×10^{-10} | 1.08×10^{-10} | 0.96×10^{-10} |
| HEBCD | 1.60×10^{-10} | 1.44×10^{-10} | 1.36×10^{-10} | 1.19×10^{-10} |
| HPBCD | 1.42×10^{-10} | 1.25×10^{-10} | 1.20×10^{-10} | 0.98×10^{-10} |

degree of association of all of the cyclodextrins with the Pluronic copolymer. In addition, DIMB seems to be the most interacting (50% decrease of D), followed by TRIMEB (39% decrease), as compared to the hydroxylated CDs for which the effect on D is of lower magnitude. Therefore, specific interactions of F127 with DIMB and TRIMEB are confirmed, in good agreement with the methyl–methyl PPO/methylated-CDs interaction observed.

To investigate the intensity and the selectivity of the interaction of the different cyclodextrins with Pluronic copolymer, the diffusion coefficients were measured at several CD concentrations, keeping the Pluronic polymer concentration constant at 5 wt %. The results are reported in Table 2. A modification of the solution composition is expected to induce changes in the viscosity, but these changes are supposed to affect all compounds in a similar way and are therefore not considered. The results show a gradual decrease of the diffusion coefficient of all β CDs with increasing concentration.

The diffusion coefficient of F127 (5 wt %) in D_2O is $1.7 \times 10^{-11} \text{ m}^2/\text{s}$. In the presence of all β CDs over the range of concentrations shown in Table 2, it does not change, showing that the diffusion behavior of the polymer is not affected by the addition of CD, probably due to the high molecular weight of the polymer as compared to that of CD. Moreover, for all of the measurements, two separate diffusion coefficients corresponding to the Pluronic and the CD resonances, respectively, were observed, indicating that the components are in fast exchange on the NMR time scale. In the case of formation of polyrotaxanes, the high kinetic stability (i.e., a low dissociation rate constant) has been taken as a criterion for threading, and NMR techniques were used to monitor the threading kinetics, indicating that the rotaxane formation is slow on the NMR time scale.^{27,28} In the case of F127/DIMB interactions, the fast exchange regime observed further supports the minor contribution of a polyrotaxane-type complex to the interaction mechanisms between the components. Under such conditions, the observed diffusion coefficient is the weighted average of the D values in the two environments. The time average diffusion coefficient D_j for the j th species is:

$$D_j = p_j D_j^{\text{bound}} + (1 - p_j) D_j^{\text{free}} \quad (2)$$

where p_j is the molar fraction of the bound j th component, and D_j^{bound} and D_j^{free} are the tracer diffusion coefficients for bound and

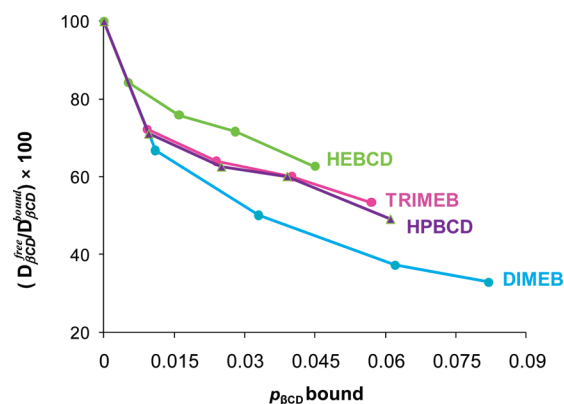


Figure 6. Relative decrease (%) of the self-diffusion coefficient of DIMB, TRIMEB, HEBCD, and HPBCD dissolved in Pluronic F127/ D_2O (5%) as a function of the bound fraction of cyclodextrin, $p_{\beta\text{CD}}$ (calculated from eq 3; see text).

free molecules, respectively. It is clear that $D_j^{\text{bound}} < D_j^{\text{free}}$ only for cyclodextrins, while $D_j^{\text{bound}} \approx D_j^{\text{free}}$ for the polymeric component. The decrease of the diffusion coefficient indicates an increasing value of bound β CD fraction (p_j) at increasing β CD concentration, in good agreement with mass action on the supramolecular aggregation equilibrium.

As reported by Wimmer et al.,²⁹ from eq 2 the fraction of bound cyclodextrin can be determined by:

$$p_{\beta\text{CD}} = (D_{\beta\text{CD}} - D_{\beta\text{CD}}^{\text{free}}) / (D_{\beta\text{CD}}^{\text{bound}} - D_{\beta\text{CD}}^{\text{free}}) \quad (3)$$

where $D_{\beta\text{CD}}^{\text{bound}}$ is the diffusion coefficient of the CD when it is complexed to the polymer. Considering that CD is small as compared to the polymer and that the diffusion coefficient of F127 remains constant upon addition of CD, it can be assumed that $D_{\beta\text{CD}}^{\text{bound}} = D_{\text{polymer}}^{\text{free}}$.

Also, $p_{\beta\text{CD}}$ is expressed by:

$$p_{\beta\text{CD}} = [\beta\text{CD:F127}] / ([\beta\text{CD}] + [\beta\text{CD:F127}]) \quad (4)$$

where $[\beta\text{CD:F127}]$ and $[\beta\text{CD}]$ are the concentrations of the complexed and free β CD, respectively. Figure 6 shows the relative decrease (%) of the cyclodextrins diffusion coefficients, $D_{\beta\text{CD}}^{\text{free}}/D_{\beta\text{CD}}^{\text{bound}}$, as a function of the molar fraction of complexed cyclodextrin ($p_{\beta\text{CD}}$, eq 4) in the presence of 5 wt % F127. The quantity ($p_{\beta\text{CD}}$ bound) is calculated using eq 3. It is known from the literature that the formation of supramolecular assemblies leads to a reduction of the molecular motion of one (or both) components of the aggregate in solution.^{30,31} In the present study, diffusion data suggest that the effective size of all CDs increases monotonically with increasing concentration of CD (F127 kept at constant concentration; see Table 2), thus resulting in a decrease in its diffusion coefficient. This behavior indicates the presence of intermolecular interactions that promote the aggregation of cyclodextrin with the Pluronic copolymer. We note that the self-association of cyclodextrins is not expected to provide a major contribution in the case of substituted cyclodextrins.^{32,33} The formation of a supramolecular assembly results in an effective increase in the size of all of the cyclodextrins and a decrease in the mobility correlated to that of the larger component of the association, that is, the polymer. In addition, the plots in Figure 6 show that the DIMB behavior is appreciably different from that obtained for the other β CD derivatives. Indeed, DIMB shows the steepest decrease of the

relative diffusivity with increasing complexation. This trend correlates well with the specific effect of DIMEB on F127 micellar rupture¹³ as compared to the other substituted CDs.

Previous work¹⁴ showed that micellar break-up is complete at 7 wt % DIMEB. From the diffusion measurement results in Tables 1 and 2, the fraction of β CD bound $p_{\beta\text{CD}}$ at this concentration can be determined: $p_{\beta\text{CD}} = 0.60$ (by extrapolation we estimated $D_{\beta\text{CD}} = 0.82 \times 10^{-10} \text{ m}^2/\text{s}$ at 7 wt %), showing that the micelles are fully ruptured at a CD concentration as low as 8.2 PO units per CD molecule. At the highest β CD concentration studied, 11 wt %, the PO:CD ratios are 4.2 for DIMEB, 6.5 for TRIMEB, 8.2 for HEB CD, and 5.8 for HPBCD; therefore, a much more extensive association is observed with DIMEB (as expected from the lower diffusion coefficients). These numbers are higher than the 3:2²⁰ or 3.2:1¹⁹ PO:CD ratios reported by other authors. This fact could suggest that polypseudorotaxane formation may take place at a higher number of CD per PO units. We also hypothesize that threading may only be possible when the polymer chains are in the unimeric state, either below the CMC²⁰ or after disruption of the micelles by cyclodextrins and high enough CD:PO ratio.

Finally, further experimental clues for the minor contribution of polyrotaxane formation in the F127/DIMEB interaction were provided by optical rotation (OR) experiments. The results of OR experiments are shown in SI. OR has been used as a technique to detect inclusion complex formation.⁹ No spectral changes were detected upon adding increasing amounts of F127 to DIMEB solutions, which again favors the hypothesis of the absence of polyrotaxane formation.

CONCLUSIONS

In this work, we investigated the complexation behavior of four β CD derivatives with micelles of Pluronic F127 (PEO₁₀₀–PPO₆₅–PEO₁₀₀), DIMEB, TRIMEB, HEB CD, and HPBCD, to elucidate the selective micellar rupture obtained with DIMEB only reported in previous studies. The results obtained from the NOESY experiments showed that the methyl groups in position 6 of the glucopyranose rings of the two methylated cyclodextrins, DIMEB and TRIMEB, interact with the methyl of the PPO polymer chains. This indicates that the polymer chains are able to interact with the substituents of the cyclodextrin derivatives located outside the lipophilic cavity. In addition, a weak NOE contact between the methyl groups of PPO and the inner H atoms of these two CDs was also observed, suggesting inclusion of the PPO methyl groups inside the CDs' cavity. In the case of HEB CD and HPBCD, the impossibility of obtaining well-resolved spectra did not enable us to collect meaningful NOEs and to speculate on the existence of a similar interaction with the PPO group. Our NOE data therefore give evidence of selective interaction of the PPO portion of Pluronic with both the outer CH₃ substituents and the inner H₃/H₅ protons of the examined CD derivatives. Nevertheless, these data are not consistent with the often invoked polyrotaxane formation as the only mechanism of association between these components. The absence of any change in the OR spectra of DIMEB upon addition of F127 and the weak interaction of PPO methyl with both the outer surface and the cavity of DIMEB and TRIMEB enable us to propose the formation of another type of softer association with respect to threading, where β CD and polymer are more loosely bound.

The PFGSE-NMR experiments showed that the diffusion coefficient of all β CD derivatives measured in D₂O, PPO/D₂O, and F127/D₂O at a fixed β CD concentration follows the order $D_{\text{F127/D2O}} < D_{\text{D2O}} \approx D_{\text{PPO/D2O}}$, indicating a decrease of

diffusivity of the β CD derivatives in the presence of Pluronic, hence the occurrence of association phenomena with all of the tested cyclodextrins. However, DIMEB was found to undergo a more marked decrease of diffusivity as compared to the other β CD derivatives over the same concentration range, thus suggesting that a higher fraction of DIMEB is involved in complex formation with the polymer. In all systems at all concentrations studied, two distinct diffusion coefficients, corresponding to the Pluronic and the CD resonances, were observed. This fact demonstrates that the associated and free components are in fast exchange on the NMR time scale. This point is consistent with the hypothesis of a loose complex formation rather than a pseudopolyrotaxane type. In addition, diffusion coefficients enabled us to extract a ratio of PO/CD_{bound} at 11 wt % CD of 4.2:1 for DIMEB, 6.5:1 for TRIMEB, 8.2:1 for HEB CD, and 5.8:1 for HPBCD.

Overall, ¹H NMR NOESY did not reveal any major difference between the interaction of DIMEB with F127, on one hand, and TRIMEB with F127, on the other, which is surprising, given the fact that DIMEB breaks up the micelles completely, while TRIMEB affects them only marginally. The combined use of diffusion measurements and NOE data, however, highlighted a much stronger association of DIMEB with F127, as compared to the other CD derivatives, in good agreement with the previously reported micellar rupture observed by SANS for DIMEB only. Therefore, the interaction between the PPO methyl groups and both DIMEB and TRIMEB detected by NMR is a necessary but not sufficient condition to induce micellar break-up. The explanation must lie somewhere else. The free hydroxyl group of DIMEB is likely to play a role, possibly by hydrogen-bonding with PPO, therefore contributing to its solubilization. The overall picture emerging from the experimental data is that DIMEB only has the correct combination of steric hindrance, inclusion ability, lipophilic side chains (the CH₃ groups), and hydrogen-bond donor sites that make DIMEB the selective agent for F127 micelles disruption. Fully methylated TRIMEB is able to associate with PPO in a similar way, as the present work has shown, but the absence of hydrogen bonding is likely to prevent the solubilization of PPO units, which is necessary to reduce the CMC. For HEB CD and HPBCD, some association with the polymer seems to occur (as detected from the diffusion data), but it is not sufficient to induce the micellar rupture obtained with DIMEB. In this case, the steric hindrance of HEB CD and HPBCD is expected to hamper the hydrogen-bond formation with PPO.

AUTHOR INFORMATION

Corresponding Author

*E-mail: andrea.mele@polimi.it.

ACKNOWLEDGMENT

M.V. wishes to thank the Erasmus program for supporting her stay in Milan. Alex Drake is acknowledged for his help with the OR measurements.

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