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Growth, Shrinking, and Breaking of Pluronic Micelles in the Presence of Drugs and/or β-Cyclodextrin, a Study by Small-Angle Neutron Scattering and Fluorescence Spectroscopy

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The associative structures between F127 Pluronic micelles and four drugs, namely, lidocaine (LD), pentobarbital sodium salt (PB), sodium naproxen (NP), and sodium salicylate (SAL), were studied by small-angle neutron scattering (SANS). Different outcomes for the micellar aggregates are observed, which are dependent on the chemical nature of the drug and the presence of charge or otherwise: the micelles grow with LD, are hardly modified with PB, and decrease in size with both NP and SAL. The partition coefficient, determined by fluorescence spectroscopy, is directly correlated to the amount of charge, following NP ≈ SAL < PB < LD. All drugs are found to lie at the interfacial layer, with a slightly deeper localization of LD and more superficial for PB. All drugs can form inclusion complexes with heptakis(2,6-di-*O*-methyl) β-cyclodextrin (hep2,6 β-CD). Hep2,6 β-CD, as shown in previous studies (Joseph, J.; Dreiss, C. A.; Cosgrove, T. *Langmuir*, **2008**, *24*, 10005−10010; Dreiss, C. A.; Nwabunwanne, E.; Liu, R.; Brooks, N. J. *Soft Matter*, **2009**, *5*, 1888−1896), is also able to form a complex with F127, resulting in micellar breakup. In the ternary mixtures, a fine balance of forces is involved, which results in drastic micellar changes, as observed from the SANS patterns. Depending on the ratio of drug, polymer, and hep2,6 β-CD and the nature of the interactions (which is directly linked to the drug chemical structure), the presence of drug either hinders micellar breakup by β-CD (at high enough concentration of LD or PB) or leads to micellar growth (NP). These effects are mainly attributed to a preferential drug/β-CD interaction (except for PB), which, at least in the conditions studied here, explains the higher β-CD concentration needed for micellar breakup to occur.

Introduction

The poor solubility in biological fluids of nearly 50% of newly approved drugs remains the main limitation in oral administration, resulting in low gastrointestinal absorption and bioavailability.[3] Among the existing strategies, polymeric micelles are extremely attractive candidates for solubilizing hydrophobic drugs, increasing their stability against chemical degradation and metabolism by biological agents, extending circulation times in the blood,[4] and improving their bioavailability.[5−8] They present similar features to natural carriers in terms of size, structure, and transport properties and can selectively deliver drugs to specific sites of action. Their similarity to biological carriers confers them a fundamental property for pharmaceutical formulations, that is, low toxicity. Selective targeting is another key feature, allowing reduced dose, decreasing side effects and economic cost, an aspect which is particularly important in the treatment of chronic diseases. Triblock copolymers of poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) (PEO-PPO-PEO), referred to as Pluronics or Polaxamers, in particular, meet a number of essential requirements for drug delivery;[9−12] they are available commercially in large quantities and at low cost with a range of polymer architectures (block length, hydrophobic/hydrophilic ratio, molecular weight, etc.), thus providing systematic variability of key parameters and, quite critically, avoiding lengthy and costly synthesis, with formulations being readily prepared most often by simple dissolution of the drugs into the micellar core. Poly(ethylene) blocks provide protection against opsonization and uptake by the macrophages of the reticuloendothelial system (RES).[13] In addition, they have been shown to improve the treatment of tumors with the multidrug-resistant (MDR) phenotype by affecting several mechanisms of drug resistance in cancer cells,[10] which points to their selectivity. Although nondegradable, molecules in the range 10−15 000 kg·mol^{-1} are filtered by the kidney and cleared in urine[14] and have been approved by regulatory institutions (FDA) for use in pharmaceutical formulations.[6] In addition, Pluronics solutions display a sol−gel transition which can be tuned to body temperature (e.g., by addition of salts or chemical modification), making them attractive for the design of injectable implants for minimally invasive applications.

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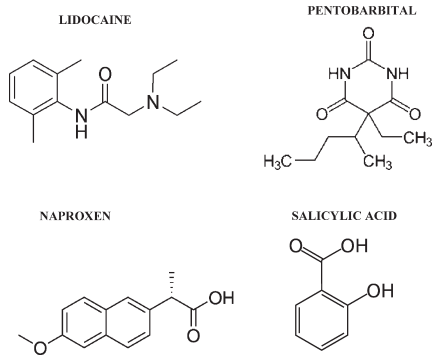
Despite the interest in polymeric micelles for delivery purposes and their already extensive use in existing formulations, there is generally little rationale behind the formulations used due to a lack of physical characterization of the nanostructures. An abundance of studies has been published on drug solubilization in Pluronics and their release characteristics.[3,6] Drug loading efficiency appears to be highly dependent on drug type and polymer structure,[12] but structure−property relationships to guide formulations have still to be established. Indeed, very few studies have focused on characterizing micellar shape and size and describing structural changes occurring upon drug encapsulation, apart from a few exceptions.[2,15−17] Small organic molecules,[18] and hence drugs,[16,19] have been shown to modify the cmc, cmt, aggregate size and shape, phase behavior (gelation boundaries), and stability of Pluronic micelles. Rationalizing these changes is fundamental, since micellar size, shape, and aggregation number are directly correlated to the locus of drug solubilization, hence drug loading capacity, release profile in vivo, circulation time, and therefore drug bioavailability.

In this contribution, we have selected four drugs, namely, the anesthetics lidocaine and pentobarbital, the NSAID naproxen (the latter two as sodium salts), and the analgesic sodium salicylate, having all related structures (Scheme 1). All these drugs are well-known and with wide clinical applications. Sodium salicylate (SAL) is the metabolite of aspirin, and both together are probably the most widely used drugs in the world, presenting a wide range of pharmacological actions, with the most known being analgesic, anti-inflammatory, and antipyretic properties, besides stroke prevention. However, the high incidence of gastrointestinal side effects is also well-known and the main cause for avoiding its prescription. Salicylic acid is used in cosmetic formulations as a denaturant, hair-conditioning agent, and skin-conditioning agent in a wide range of cosmetic products at concentrations ranging from 0.0008% to 3%. More recently, β-hydroxy acids (including sodium salicylate) have been included in cosmetics to reduce signs of skin aging. Therefore, although a very well-know drug, it is still the subject of intense research, for instance, regarding its role on increasing skin's sensitivity to ultraviolet radiation,[20] and appropriate formulations and new drug carriers are still actively sought.[21]

Naproxen (NP) is another NSAID widely used in the treatment of chronic inflammatory diseases and as a topical anti-inflammatory. As most NSAIDs, naproxen also presents a high incidence of gastrointestinal side effects. The correct formulation of NP for avoiding gastrointestinal side effects, in addition to good dissolution rate, is a wide subject of interest.[22] In addition, the photoreactivity of naproxen has been described,[23] and how it could be controlled by using appropriate drug carriers.[24]

Lidocaine (LD) is a local anesthetic used in the relief of pain, which can be used in topical or parenteral administration. Lidocaine and fentanyl are the first drugs approved by regulatory agencies for iontophoretic transdermal application (active transdermal liberation) and research to exploit this delivery system in combination

Scheme 1. Structure of Lidocaine, Pentobarbital, Naproxen, and Salicylic Acid



with other drugs, among others anti-inflammatory drugs and anesthetics,[25] is currently being carried out.

In general, transdermal drug delivery has been shown in recent years to be a very useful route of administration, as it is non-invasive and avoids gastrointestinal absorption. This results in the avoidance of first-pass metabolism in the liver, reduced gastrointestinal side effects, and no interaction with food. Therefore, it is a very interesting alternative to oral or parenteral administration. Transdermal administration strongly depends on high drug penetration through the skin, which can be improved by the use of electric fields, iontophoretic transdermal delivery. This system is a novel drug delivery system that depends on formulation parameters such as drug content and pH,[26] and opens a wide field of study on the effect of drug carriers and additives. Overall, the recent interest in transdermal route emphasizes again the necessity of better formulations of drugs for efficient and safe delivery; in order to achieve this, the understanding of drug/carrier interactions and microstructures formed is paramount.

In this contribution, we study the structural modifications resulting from the addition of increasing amounts of each of these four drugs to F127 Pluronic micelles (5 wt %) at room temperature and under natural conditions of pH. We have used a combination of small-angle neutron scattering (SANS) and UV−vis fluorescence emission spectroscopy to examine the partitioning of the drugs inside the micelles and rationalize it in terms of the chemical structure and charge of the drug. While SANS provides direct information on the modifications of the micelles upon addition of the drug, fluorescence provides binding constants, partition coefficients, and information on changes in the drug microenvironment occurring in the presence of the polymer.

In addition to the need of a better characterization of polymer−drug complexes, a substantial improvement to the development of drug formulations is the design of triggers to control the release and delivery of a payload at selected sites of action. We have previously reported that a derivative of β-cyclodextrin, namely, heptakis(2,6-di-*O*-methyl) β-cyclodextrin (hep2,6 β-CD), is able to fully break up Pluronic micelles.[1,2] This interesting property is attributed to the specific complexation of the central block PPO with β-CD, which, by imparting hydrophilicity to the otherwise water-insoluble polymer, decreases the critical micelle concentration (cmc). We propose that such a mechanism could be developed and exploited to achieve the controlled release of an active compound encapsulated in the micellar core. Cyclodextrins, cyclic oligosaccharides with a hollow toroidal shape and a hydrophobic cavity, are well-known to form inclusion complexes through noncovalent interactions with

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a variety of molecular guests that can fit into their cavity.[27−29] By threading onto polymers, stable structures known as pseudopolyrotaxanes have been reported, following the pioneering work of Harada and co-workers.[30−33] Formation of a Pluronic−polyrotaxane would involve sliding of the β-CD along the polymer chain to preferentially localize on the PPO central block, for which there is a better geometrical fit than with PEO.[31,34] However, cyclodextrins can also form 1:1 (or 1:2) host−guest complexes with drugs; in fact, they have been widely used in the pharmaceutical industry for enhancing drug bioavailability, as they are biocompatible and well-tolerated by the body.[35,36] The driving force for the complexation is attributed to the exclusion of high energy water from the cyclodextrin cavity, hydrogen and hydrophobic binding. The complexation of drug with cyclodextrin is described by an association constant (K_{assoc}) given by eq 1:

$$[\text{drug}] + [\text{CD}] \overset{K_{assoc}}{\leftrightarrow} \frac{[\text{drug} - \text{CD}]}{[\text{drug}][\text{CD}]} \quad (1)$$

where [drug] and [CD] represent the free concentration of drug and cyclodextrin and [drug − CD] is the concentration of the complex. Complexation is a dynamic process, and the drug can be displaced if a more favorable interaction with another host is introduced in the system. Cyclodextrins have been used in combination with polymers, through either covalent links or physical association[37,38] to modify the drug release properties (e.g., by complexing the drug and either increasing its solubility or decreasing its diffusivity, acting as channelling agents, or cross-linking agents, etc); however, the mechanisms invoked are very different from the one reported in this study.

In view of exploiting β-CD-induced micellar breakup for the controlled release of drugs, we report here investigations on systems comprising polymeric micelles, drug, and β-CD. From the above, it is clear that these ternary mixtures lead to three possible types of interaction, which are in competition with each other: polymer−drug (micellar encapsulation); cyclodextrin−drug (inclusion complex); and polymer−cyclodextrin (most probably inclusion complex). Understanding the mechanisms of interaction and the nature of the complexation processes is not only necessary to improve drug loading and release profile but also fascinating from a fundamental viewpoint. In this Article, we examine the structures formed by the interaction of four drugs with Pluronics in the presence and absence of β-CD. We observe how the presence of drug affects the disruptive action of β-CD on the micelles, depending on the structure of the drug. SANS enables us to monitor structural changes in the micelles upon drug or β-CD addition, while fluorescence spectroscopy provides information on the drug microenvironment, enabling us to infer where the drug is localized and how strongly the different species interact, in binary or ternary mixtures; a combination of these two techniques leads us to propose a partial description of the interaction mechanisms observed in these complex mixtures.

Materials and Methods

Materials. Pluronic copolymer F127 comprising a central block of 65 PPO units and two side blocks of PEO (100 units each) was obtained from Sigma-Aldrich UK (M_w = 12 600 kg·mol^{-1}). Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin was obtained from Sigma-Aldrich UK (H0513, M_w = 1331.4 g·mol^{-1}) and is equally referred to in the text as β-CD or hep2,6 β-CD.

The drugs naproxen sodium salt (NP, M1275), pentobarbital sodium salt (PB, P3761), sodium salicylate (SAL, 71945), and lidocaine (LD, L7757) were purchased from Sigma. The solvents used were hexane for HPLC, dichloromethane, chloroform, methanol, acetone, and acetonitrile, all of them being analytical reagent grade, from Fisher Scientific; ethanol (EtOH, for HPLC) from Fluka; propanol (PrOH) and butanol 99% from Aldrich; and octanol 99% from Lancaster Synthesis. All materials were used as received.

Preparation of the Solutions. Drugs/Pluronics solutions were made by simple mixing. In the case of lidocaine (the only one not in salt form), the solutions were heated above the drug melting point (68 °C) to facilitate solubilization inside the micellar core. The solutions were then cooled back to room temperature. Ternary solutions containing polymer, drug, and β-CD were made by mixing the appropriate amounts of F127 and β-CD stock solutions with the drug in solid form and mixing. All solutions were prepared by weight, and "%" always refers to weight % (wt %).

SANS Measurements. Most SANS measurements were carried out on LOQ at the ISIS facility (Rutherford Appleton Laboratory, Didcot, U.K.). SANS patterns of β-CD on its own were measured on D11 at the Institut Laue Langevin (ILL) (Grenoble, France). At ISIS, the instrument uses incident wavelengths from 2.2 to 10.0 Å, sorted by time-of-flight, and a fixed sample−detector distance of 4.1 m. This provides access to scattering vectors Q from 0.009 to 0.287 Å^{-1}. On D11, three configurations were used to cover a Q range from 0.007 to 0.185 Å^{-1}, where Q is the modulus of the scattering vector. The scattering intensity was converted to the differential scattering cross section in absolute units using the standard procedures at each facility.

Solutions of F127 were 5 wt % concentrated with 0.5, 1, and 2 wt % drug salts or 0.67 wt % lidocaine. β-CD concentrations ranged from 5 to 13 wt %, corresponding to PO/βCD ratios of 6.9 to 2.6. All samples were measured in D_2O, in order to optimize the contrast and minimize the incoherent background for SANS experiments. The solutions were equilibrated for a period of at least 24 h to ensure that the complexation process was complete before performing the measurements.

Fluorescence Measurements. Measurements were performed on a Cary Eclipse fluorescence spectrophotometer (Varian, Oxford, U.K.). Two regimes of drug concentration were used: dilute (10^{-3} wt %) and concentrated (2 wt % for NP, PB, and SAL, and 0.3% for LD, because of its low aqueous solubility:[39] 5.06 mg·mL^{-1}). The aqueous solutions were prepared using Milli Q water.

The following setup conditions were used for the drugs. Lidocaine: λ_{exc} = 262 nm, slits = 5−10 nm, PMT = 800 V. Pentobarbital: λ_{exc} = 240 nm, slits = 5−10 nm, PMT = 800 V. Naproxen sodium salt: λ_{exc} = 317 nm, slits = 5−5 nm, PMT = 400 V. Sodium salicylate: λ_{exc} = 296 nm, slits = 5−5 nm, PMT = 500 V.

The partition coefficients were determined using the method proposed by de la Guardia et al.[40]

$$\left(\frac{I}{I_0} - 1\right)^{-1} = \left(\frac{I_M}{I_0} - 1\right)^{-1}\left(1 + \frac{1}{\gamma K C_M}\right) \quad (2)$$

where C_M is the micellar concentration with $C_M = (C_S - \text{cmc})$, C_S being the total surfactant concentration. K is the binding constant

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of the drug to the micelle and γ is the ratio of molar absortivities of the drug in both media (it is 1 in all cases). The cmc value used is 0.26 ± 0.03 wt %.[16]

The partition coefficients can be calculated from the binding constant following eq 3.[41]

$$K = (P_{MW} - 1)\bar{V} \quad (3)$$

where $\bar{V}$ is the partial molar volume of the polymeric surfactant (a value of 1.24 was used).

The binding constant of each drug to cyclodextrin, K_B, was determined using the following expression:

$$F = \frac{(F_0 + F_\infty K_B[CD])}{1 + K_B[CD]} \quad (4)$$

where F is the measured fluorescence intensity, and F_0 and F_∞ are the fluorescence intensity when all the drug is free and complexed, respectively (both are experimental parameters). K_B is the binding constant, obtained by fitting the experimental data. [CD] is the concentration of free β-cyclodextrin, which, in dilute systems, corresponds to the analytical concentration, since [CD] $\gg$ [drug]. In concentrated solutions instead, the free β-CD concentration was determined by means of a Taylor's series expansion of the equation that relates the total concentration to the binding constant and free cyclodextrin concentration as described in the literature.[42] A nonlinear least-squares method was used to fit the experimental results to eq 4.

Results and Discussion

1. Micellar Structural Changes in Binary and Ternary Systems Monitored by SANS. *1.1. Effect of Drugs on F127 micelles. Lidocaine.* Previous measurements[2] on concentrated F127 micellar solutions (15 wt %) have shown an increase in the aggregation number and micellar size when incorporating lidocaine up to 2 wt %. In addition, the presence of drug drove F127 micelle organization into a macrolattice, leading to gelation at a lower polymer concentration than in the absence of drug.[2] Measurements performed in more dilute conditions, 5 wt % micellar solutions in the presence of 0.67 wt % lidocaine (near saturation), are shown in Figure 1. The scattering of the drug dissolved as a molecular species is negligible, and hence, changes in the SANS patterns arise solely from changes in micellar structure (this applies to all the drugs studied here). The incorporation of lidocaine leads to a clear shift of the micellar scattering to lower Q values, accompanied by an overall increase in the intensity; the combination of these two features reflects an increase in micellar size. This is in agreement with the results of Sharma et al.[16] who showed an increase in core size from 43 to 58 Å and corona from 66 to 71 Å upon incorporation of lidocaine to saturation. Our data also indicate that lidocaine is encapsulated into the micelles, leading to a net increase in their size. This is in agreement with the high selectivity of Pluronics for aromatics (over aliphatics)[43] and previously reported formulations of lidocaine with Pluronics.[15,16,19,44,45]

Pentobarbital Sodium Salt. In comparison to lidocaine, the effect of pentobarbital on F127 micellar structure is minimal (Figure 1). Addition of 2 wt % leads to a slight increase in intensity, but no shift to lower Q values (hence no change in micellar size), accompanied by a slight decrease of the signal at lower Q,

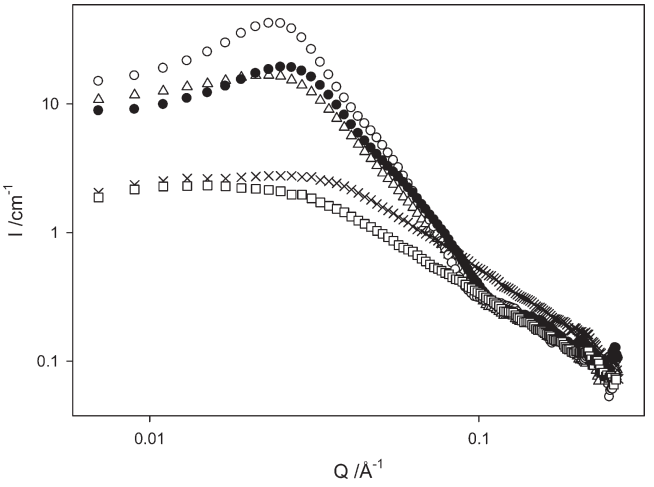

Figure 1. Small-angle neutron scattering patterns from solutions of 5% F127 in D_2O (△) and in the presence of 0.67% lidocaine (○), 2% pentobarbital (●), 2% naproxen (×), or 2% sodium salicylate (□), showing different effects on the micellar aggregates, depending on the drug added.

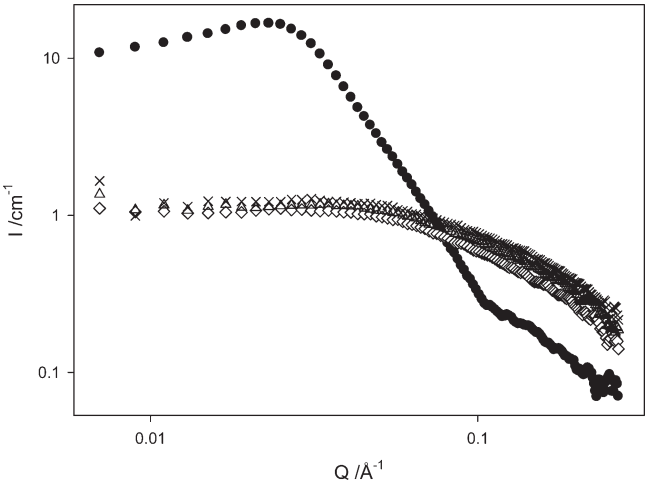

Figure 2. SANS patterns from 5% F127 micellar solutions alone (●) and in the presence of 7% (◇), 9% (△), and 11% (×) hep2,6 β-CD, where full demicellization occurs.

reflecting an enhancement of intermicellar interactions, probably arising from increased charge repulsions. This effect is gradual when adding 0.5−2 wt % PB (only the highest concentration is shown here; 1 wt % PB is shown in Figure 3). It is possible that most PB stays solubilized in water, therefore not disturbing very much the structure of the micelles. Some interaction however with F127 micelles could occur with the outer layer, explaining the stronger micellar repulsions observed and the slight increase in intensity. No published data have been found on the interaction of pentobarbital with Pluronic micelles.

Naproxen Sodium Salt. Formulations in F127 micelles have been shown to significantly improve naproxen solubility and half-life.[46,47] Figure 1 shows the effect of adding 2 wt % naproxen salt to 5 wt % micellar solutions of F127. The intensity of the scattering pattern decreases significantly, together with a shift of the main scattering peak (0.025 $Å^{-1}$) to a higher Q value, while micellar features are all smoothed out. This reflects a shrinking of the

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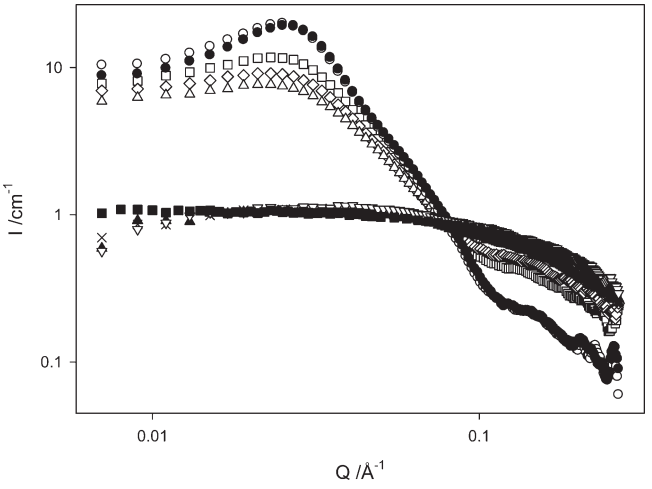

Figure 3. SANS patterns from 5% F127 micellar solutions with the drug pentobarbital at two concentrations: 1% (○) and 2% (●), and varying amounts of hep2,6 β-CD. F127 solutions with 1% PB and 9% (▲), 11% (×), and 13% (▽) hep2,6 β-CD show full breakup of the micelles. Instead, F127 with a higher amount of drug (2% PB) and 5% (□), 7% (◇), and 9% (△) hep2,6 β-CD shows only a slight decrease in the size of the micelles. The scattering from solutions of 11% hep2,6 β-CD alone (■) is also shown for comparison (this last set was measured at ILL, hence on a different Q range, and scaled down from a solution containing 13% hep2,6 β-CD).

micelles (which is gradual upon addition of 0.5 to 2 wt % NP) and a weakening of intermicellar interactions. This suggests a mechanism of interaction whereby the charged drug interacts strongly with the micelles, inserting itself in the aggregate (possibly the palisade layer, as the drug is largely soluble in water), introducing electrostatic repulsions and therefore reducing the packing of the chains; as a consequence, the micellar aggregation number decreases. This result is in agreement with the data published by Sharma and Bhatia[15] who reported a decrease in the aggregation number in 2 wt % F127 micelles from 88.6 to 51.9 upon addition of naproxen.

Sodium Salicylate. The effect of 2 wt % SAL on the Pluronic micelles is very similar to that of naproxen (Figure 1). With the addition of 2 wt %, a significant reduction in micellar size occurs, shown by the collapse of the intensity and disappearance of micellar structural features. This suggests again a significant interaction of the salt with the micellar aggregates. The similarity with NP is not surprising, as NP and SAL structures are fairly related, with NP bearing an additional aromatic ring. The effect of sodium salicylate on Pluronic micelles, to our knowledge, has not been reported elsewhere.

1.2. Effect of β-CD on Drug-Containing Micelles. We have recently shown that the gradual addition of hep2,6 β-CD to F127 Pluronic micelles leads to their gradual disruption;[1,2] the effect is highly dependent on temperature. At 25 °C, the addition of 7−11 wt % hep β-CD to 5 wt % F127 micellar solutions leads to complete disruption of the micelles to their unimers as shown in Figure 2 (the micellar disruption observed at 37 °C is more gradual[2]).

Lidocaine. We have also previously shown that the disruptive effect of β-CD on Pluronic micelles is weakened in the presence of the drug lidocaine.[2] This can be explained by two competitive processes of interaction, or a combination of both: (1) the hydrophobic drive of the drug stabilizes the micelles against the disruptive action of β-CD; (2) the formation of a drug/β-CD inclusion complex reduces the number of β-CD molecules available to interact with PPO and hence reduces micellar breakup. These two hypothese are later discussed in the text for each drug and referred to by hypothesis (1) and (2).

Pentobarbital Sodium Salt (*NaPB*). The addition of 9−13 wt % β-CD (equivalent to PO units/β-CD ratios of 3.8 to 2.6) to 5 wt % F127 micellar solutions in the presence of 1% PB (Figure 3) leads to a complete disruption of the micelles. The patterns obtained are almost identical to the ones obtained for pure micelles of F127 in the absence of drug (shown in Figure 2) and to the scattering pattern of β-CD alone in solution (shown in the inset of Figure 3). This confirms the absence of any residual micellar scattering, and hence, the polymer has been totally reduced to its unimeric form. The breakup is achieved with the lowest amount of β-CD studied (9%) and does not change with further addition of β-CD, showing that even if further β-CD/polymer complexation occurs, it does not alter the structures formed (however, it is possible that the onset of micellar breakup occurs below 9% β-CD, not studied here). In contrast, the same complexes at a higher PB concentration of 2 wt % (also shown in Figure 3) lead to a completely different picture: in this case, the presence of PB clearly weakens the disruptive action of β-CD on the micelles, as shown by the very gradual smoothing out of micellar scattering, instead of its collapse. At the highest β-CD concentration studied (in this case 9%), the scattering pattern is still very structured and originates from micelles, instead of unimers. Comparing 9% β-CD addition to the micellar solutions containing either 1 or 2 wt % PB, it is very clear that the drug is able to reduce the disruptive action of β-CD on the micelles, provided it is above a critical concentration, which may be linked to either a critical PB/F127 or PB/ β-CD ratio, or a combination, depending on the mechanism of action. Interestingly, the impact of PB on F127 micelles was found to be moderate (Figure 1), suggesting weak drug/polymer interactions, and therefore pointing to hypothesis (2), namely, a favorable interaction between β-CD and PB (hypothesis (2)), which would reduce the availability of β-CD to complex the polymer. The fluorescence data presented below give additional evidence to discuss this hypothesis in more quantitative terms.

Naproxen Sodium Salt. The effect of β-CD (9−13 wt %) on F127 micelles in the presence of 1 wt % naproxen is very similar to that of PB: the micelles are fully broken up, as shown by the collapse of the scattering pattern (Figure 4), and all curves superimpose with the ones shown for 1% PB data in the presence of β-CD. This is somehow surprising as the interaction of F127 micelles with NP seemed stronger than with PB (Figure 1). However, the resulting structures may be identical (namely, unimers), but the mechanism of interaction different. At a higher concentration of NP (2 wt %), a very interesting and unexpected effect is observed (Figure 4): the addition of 7 and 9 wt % β-CD leads to an increase in micellar size, therefore offsetting the disruptive effect that NP had on the micelles (Figure 1). At higher concentrations of β-CD, however (11 and 13 wt %), this effect is no longer seen and Pluronics are reduced to unimers, as in the case of the lower drug concentration (1%). These intriguing results suggest that at low concentration β-CD could be complexing preferentially with NP, therefore decreasing drug/polymer interaction. As a result of this preferential β-CD/drug interaction, the micelles would grow (since charge repulsions induced by the drug are reduced). At higher concentrations, however, possibly after full β-CD/drug complexation, β-CD could interact with the polymer by breaking up the micelles, resulting in a pattern similar to the one observed in the absence of drug and at a lower drug concentration (1%). Further studies on a range of β-CD and drug ratios would need to be carried out to pinpoint the occurrence

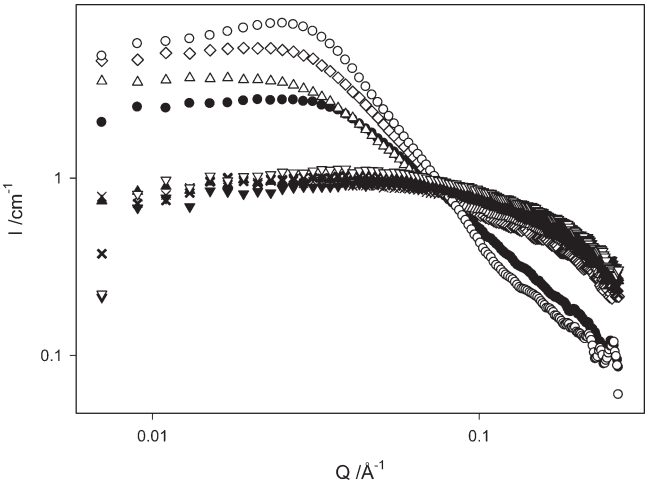

Figure 4. SANS patterns from 5% F127 micellar solutions with the drug naproxen at two concentrations: 1% (×) and 2% (●), and varying amounts of hep2,6 β-CD. F127 solutions with 1% naproxen and 9% (▲), 11% (×), and 13% (▽) hep2,6 β-CD show full breakup of the micelles. Instead, solutions of F127 with a higher amount of drug (2% NP) and 7% (◇) and 9% (△) hep2,6 β-CD show a growth of the micelles (as compared to F127 with 2% NP (●)), while full breakup is observed with 11% (×) and 13% (▼) hep2,6 β-CD.

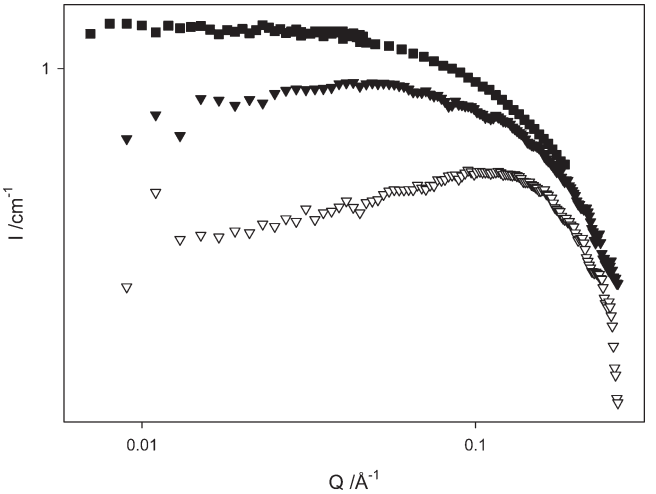

Figure 5. Comparison of the SANS patterns obtained from solutions of 13% hep2,6 β-CD alone (■) and the complexes formed by (i) 5% F127, 2% naproxen and 13% hep2,6 β-CD (▼); (ii) 2% naproxen and 13% hep2,6 β-CD (▽).

of these phase transitions. However, these results highlight the delicate balance of forces involved and show how this balance can be reversed by small changes in species ratio. The results also provide strong evidence for a preferential interaction of β-CD with the drug and open up an interesting perspective for delivery, where β-CD can cause a displacement of the drug from the interior of the micelles into β-CD inclusion complexes. These hypotheses are examined in more detail with fluorescence spectroscopy.

In order to better characterize the nature of the complexes, the scattering of naproxen (2%) in the presence of β-CD (13%) is shown in Figure 5, together with the scattering from β-CD alone at the same concentration and the three-component mixture (with 5% F127). The scattering patterns obtained from both the binary and ternary mixtures are lower than the scattering from β-CD on its own, confirming therefore that total breakup of the micelles (in the ternary mixture) and full complexation (in both binary and ternary mixtures) have occurred. As the scattering from the drug alone is negligible, the modified scattering pattern obtained from the drug/β-CD mixture compared to β-CD alone confirms the formation of a complex. The decrease in intensity at low Q in both mixtures reflects the occurrence of strong interactions, attributed to charge repulsion coming from the drug included in the complexes.

2. Complexation Behavior from Fluorescence Measurements. Fluorescence is a very sensitive technique to study changes in the microenvironment of a given compound. Fluorescence requires low absorbance at the excitation wavelength; for this reason, it is usually carried out in dilute systems and the results are extrapolated to concentrated conditions. However, the purpose of drug formulations is usually to encapsulate high loadings of drug, and therefore, rather concentrated systems are the norm. In addition, simple extrapolation of the results obtained in dilute solutions is not always a true representation of the systems at high concentration. Finally, SANS measurements require relatively high concentrations to be able to detect structural changes, and we therefore needed to reproduce the same conditions for the fluorescence measurements. For these reasons, the fluorescence measurements were performed on two sets of samples: dilute (10^{-3}%) and concentrated (2%, except 0.3% for LD). The study was carried out in three stages: (i) determination of the partition coefficient of the drugs into F127; (ii) determination of the binding constant of the drugs to hep2,6 β-CD; (iii) determination of the binding of the drugs to hep2,6 β-CD in the presence of F127.

2.1. Interaction of the Drugs with F217: Partition Coefficient. Lidocaine. In water, the drug displays three bands centered around 290, 350, and 410 nm (Figure 6A(1)), which are not always present, depending on factors which are not well understood. It is clear however that several species exist in equilibrium, whose proportion varies with properties of the environment. Addition of F127 produces a hyperchromic effect and a change in the shape of the spectrum at both drug concentrations (Figure 6A(2) and (3)). In addition, a red shift in the lower wavelength band (from 290 to 304 nm) is also observed at high LD concentrations. Together, all these changes show that LD interacts with the polymer and promotes structural changes in the aggregate.

The binding constant of LD to F127 and its partition coefficient determined by fitting the fluorescence intensity at the maxima of the spectrum (cf. Materials and Methods) are presented in Table 1. They show that the presence of increasing amounts of drug promotes its partition into the micelles, as P_{MW} increases from 2.21 in dilute conditions to 28.8 in concentrated solutions. This latter value is in better agreement with the value of 20 reported for this system at saturation obtained from UV spectroscopy.[16]

In order to bring additional insight into the microenvironment of the drug inside the micelles, we next attempted to examine the bands of the fluorescence spectra in more detail. We performed pH variation studies of aqueous solutions of LD, which show that the emission band centered around 290 nm is present at all pH, while the species emitting at 410 appears at high pH (cf. Supporting Information). This suggests that inclusion of LD inside F127 micelles at high drug concentration promotes the appearance of species that are formed in basic media (since emission at 410 nm is enhanced). The enhanced dissociation of weak acid compounds by polymer addition has been reported before.[48] This effect depends on the solvation of the acid in the presence of polymer.[48] In order to establish whether the drug inside the micelle presents a change in solvation, the effect of solvent on LD emissive behavior was studied. As expected, an important solvatochromism is observed (cf. Supporting Information). In these homogeneous media, LD

(48) Sekhon, S. S.; Arora, N.; Singh, H. P. *Solid State Ionics* **2003**, *160*, 301–307.

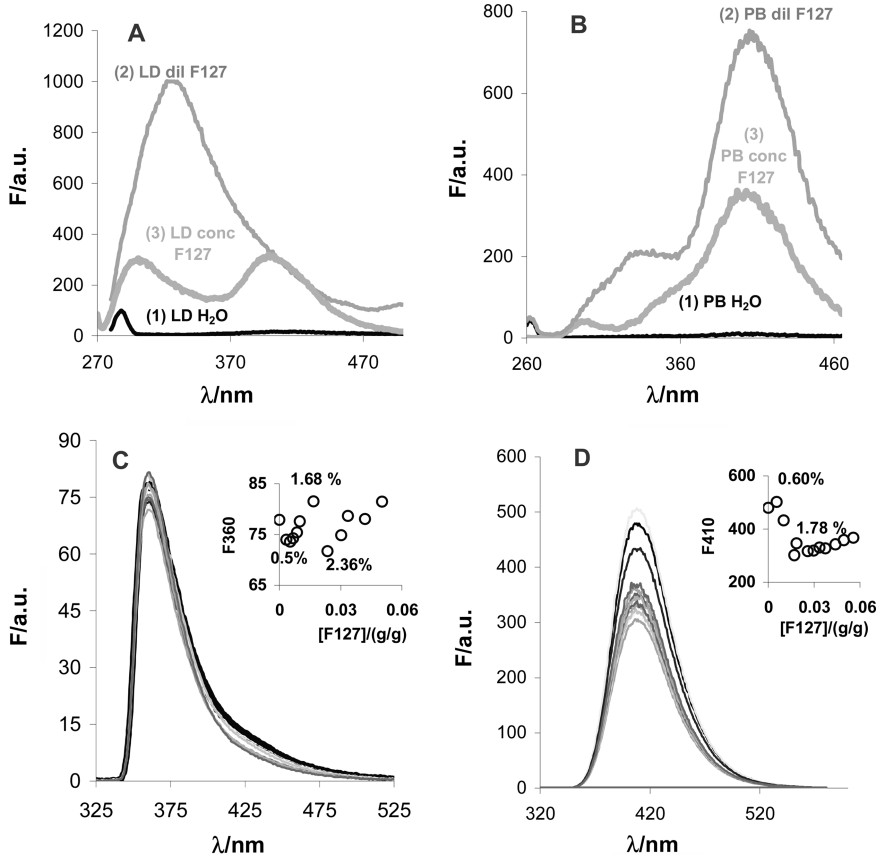

Figure 6. Fluorescence spectra of the drugs in different media. (A) (1) LD in H_2O, presenting three bands centered at λ_{max} = 290, 350, and 410 nm; (2) LD 10^{-3}% and F127 5%, presenting a new band at λ_{max} = 330 nm (corresponding to the emission of LD in a very high HBD environment) and a shoulder around 410 nm (corresponding to the emission of LD in a low HBD environment); (3) LD 0.3% and F127 5%, presenting two bands, one at 304 nm (corresponding to species emitting at 290 nm in water, but red-shifted by F127 addition due to the partition of LD into the apolar micelle environment) and a second band at 410 nm (corresponding to the emission of LD in a low HBD environment). (B) (1) PB in H_2O. The spectrum presents three bands centered at λ_{max} = 260, 350, and 410 nm; (2) PB 10^{-3}% and F127 5%, λ_{max} = 260 nm, 335 nm (corresponding to the emission of PB in a very high HBD environment) and λ_{max} = 410 nm (corresponding to the emission of PB in a low HBD environment); (3) PB 2% and F127 5%, λ_{max} = 298 nm, 410 nm, and a shoulder around 350 nm (corresponding to the emission of PB in a high HBD environment); the changes observed are related to the inclusion of PB in the micelle and show that only the region of the micelle with acidic character is affected. (C) NP 2% in H_2O and in the presence of 0−5% Pluronic F127. Inset: Variation of the fluorescence intensity with F127 concentration at the emission maximum of 2% NP. The increase in fluorescence which follows premicellar and micellar aggregate formation shows that NP is in contact with water molecules at the micelle surface. (D) SAL 2% in H_2O and in the presence of 0−5% of Pluronic F127. Inset: Variation of the fluorescence intensity with F127 concentration at the emission maximum of 2% SAL. Quenching occurring in the region of the cmc indicates hydrogen bond formation with the water shell of the micelle.

presents one or two bands centered at different wavelengths, which vary with the properties of the solvent. The main observations can be summarized as follows: (i) the band emitting at low wavelength is red-shifted when the polarity decreases (290 nm in water and 300 nm in hexane or DCM); (ii) the emission of the drug in apolar solvents strongly increases; (iii) the band appearing at high wavelength, when present, is linearly red-shifted as the hydrogen bond donor (HDB) ability decreases, in good agreement with the pH data. On this basis, the increase in intensity (at both drug concentrations) and the red shift of the 290 nm band (at high drug concentration) observed with F127 addition can be interpreted as LD being displaced into the lower polarity micellar environment. The position of the higher wavelength bands, related to the HBD ability of the micelle, shows that at low concentration LD is in contact with two different environments, one with high HBD ability (higher even than that of water, with a band at 335 nm) and another one with a very low HBD environment (shoulder centered at 410 nm). Therefore, LD is inside a micellar region with this dual nature, which could be the micelle interface. Considering the structure of lidocaine (Scheme 1), the LD benzene group is most probably inside the apolar region of the micelle with the substituents protruding into the water shell. Previous studies using UV spectroscopy have indeed reported limited partitioning of LD inside the F127 micellar core,[16] and FTIR studies have shown that uncharged local anesthetics are mainly located in the membrane/water interface, competing with the polar heads of phospholipids in binding with water molecules.[49,50]

At high drug concentration instead, the enhancement of the 410 nm band points to a change in the microenvironment around the drug. LD is less in contact with the protonating zone of the micelle, that is, the OH terminal groups of PEO or the water molecules. On this basis, it is possible to speculate that as the drug concentration increases, the micelles become tighter, changing the solvation of the micelle. Overall, the good partitioning of LD inside F127 micelles concurs with the net increase in aggregate size detected by SANS (Figure 1 and ref 2).

Sodium Pentobarbital. The fluorescence spectrum of PB in water presents three main bands: 260, 350, and 410 nm

(49) Ueda, I.; Chiou, J. S.; Krishna, P. R.; Kamaya, H. *Biochim. Biophys. Acta* **1994**, *1190*, 421–429.
(50) Fraceto, L. F.; de Paula, E. *Rev. Ciênc. Farm. Basic Apl.* **2006**, *27*, 27–35.

Table 1. Partition Coefficients of the Drugs in 5% Pluronic F127 (in Water)

drug	K/(g/g)	P_{MW}
lidocaine	**dilute**	**dilute**
	1.50 ± 0.13 (F_{330})	2.21 ± 0.15(F_{330})
	2.20 ± 0.09 (F_{290})	2.77 ± 0.12 (F_{290})
	concentrated	**concentrated**
	34.5 ± 0.9 (F_{293})	28.8 (F_{293})
pentobarbital	**dilute**	**dilute**
	8.83 ± 0.99 (F_{335})	6.59 ± 1.00 (F_{335})
	concentrated	**concentrated**
	7.79 ± 2.0 (F_{298})	7.28 ± 2.32 (F_{298})
	8.18 ± 1.69 (F_{350})	7.60 ± 1.90 (F_{350})
salicylate	**concentrated**	**concentrated**
	4.55 ± 0.32	4.67 ± 0.37
naproxen	**concentrated**	**concentrated**
	3.18 ± 0.90	3.56 ± 0.95

(Figure 6B(1)). The addition of F127 to aqueous solutions of PB (at 10^{-3}% and 2%) produces a strong hyperchromic effect, reflecting drug/micelle interaction (Figure 6B(2) and (3)). The shape of the spectrum is similar at both drug concentrations, but, as observed for LD, the maxima positions are different.

The partition coefficients determined at both drug concentrations are the same (Table 1), and they are also identical at both maxima (298 and 350 nm) appearing in the concentrated system. This suggests that the presence of PB does not change the properties of the micelle, which is in very good agreement with the SANS data, where very little change was detected in the binary systems (Figure 1). It is much lower than LD (at high concentration), in good agreement with the better ability of aromatic compounds to be solubilized inside Pluronic micelles.[43] In addition, the presence of heteroatoms and electron pairs in the PB structure, added to the presence of charge, is likely to contribute to the lower partition of PB inside the micelles, compared to LD. These results certainly corroborate the substantial micellar growth seen with lidocaine, compared to the very weak effect of PB on the micellar structure (Figure 1).

The effect of pH and solvent was studied next to obtain more insight on the nature of the interactions. The bands centered at 298 and 350 nm appear at acid pH (below pH = 3), whereas a strong band around 410 nm appears at pH > 12 (cf. Supporting Information). The fluorescent behavior of PB in homogeneous solvents shows a strong solvatochromic effect (cf. Supporting Information). Overall, we observed that (i) the band centered at 298 nm clearly appears in apolar solvents (but it is also present at low pH); (ii) the band centered at 355 nm appears in solvents with high HBD, in good agreement with the bands present at low pH; (iii) the band centered at 410 nm appears exclusively in acetone (very low HBD) as a sole band; (iv) PB is strongly fluorescent in propanol, butanol, and octanol, and all these solvents have in common a high viscosity. Quite remarkably, in propanol, all the bands appear clearly (300, 350, and 410 nm). Taken together, these results point to the inclusion of PB inside the micelles in an apolar ($\varepsilon \sim 20$) environment similar to propanol and with double acid-basic character, therefore at the interface, as observed for LD, most probably in the PEO headgroups and in contact with the water shell. Therefore, PB must be included in the micelle through its hydrophobic substituents, with the polar ring in contact with the water shell, as observed for LD. However, there are clear differences between the spectra of PB and LD in F127. The low wavelength band maxima positions show that, as for LD, the acid character of the micelle decreases upon PB addition. However, and in contrast to LD, the basic character of the aggregate is not modified by PB (as the 410 nm band does not change). This behavior seems to indicate that PB only modifies one of the two regions of the micelle which it is in contact with. By considering the different volumes of the hydrophobic (large in LD) and hydrophilic (large in PB) regions of LD and PB, together with the fluorescence data, it is possible to surmise that PB is "anchored" to the surface of the micelles through the lateral chains, whereas LD is inside the micelle at the core/shell interface.

Localization of most of the PB molecules at the surface of the micelles, together with the presence of charge, concurs with the results from the SANS analysis, which showed that PB may affect intermicellar interactions but only marginally the micellar structure, whereas LD, partitioned also at the interface but with most of the molecules deeper inside the micelles, significantly affects the structures.

Sodium Salicylate and Naproxen Sodium Salt. Addition of F127 to a dilute drug solution, in both cases, produces no changes in the fluorescence intensity, indicating no partition. At high drug concentration, F127 addition changes the emission intensity of NP and SAL, showing the occurrence of interaction with F127 (Figure 6C and D).

Further analysis of the fluorescence behavior highlights some differences between the two drugs. In the case of salicylate, a strong quenching by F127 addition is produced (Figure 6D, inset). Salicylate has been reported to be quenched by intra- and intermolecular hydrogen bond formation in aqueous solutions,[51] whereas it does not interact with alcohols.[52] Therefore, this sharp quenching occurring in the region of the cmc clearly points to the inclusion of the drug inside the micelles and the formation of hydrogen bonds with the water shell of the micelle. Naproxen, in contrast, is quenched by intermolecular hydrogen bonds formed with alcohols, but not in water.[53] On this basis, the increase in fluorescence (Figure 6C, inset) which follows premicellar and micellar aggregate formation shows that in these aggregates, NP, like SAL, is in contact with water molecules at the micelle surface.

The partition coefficients (Table 1) are of the same order of magnitude for both (3.56 and 4.67 for NP and SAL, respectively), and lower than those for the other two drugs (LD and PB). The similarity in partition coefficient for both compounds concurs with the results from SANS, which showed an almost identical effect of both drugs on the micelles (Figure 1). It is known that the partition in Pluronics is favored for aromatic compounds compared to nonaromatics.[43] Salicylate, naproxen, and lidocaine are aromatic compounds, LD and SAL have quite a similar aromatic structure, and the main difference between these two drugs is the presence of charge in the former. Moreover, NP presents a more hydrophobic aromatic ring than SAL, but the same charge. The presence of charge clearly decreases the binding constant, following the trend: NP $\approx$ SAL < PB < LD. Therefore, it is possible to speculate that the main cause of poor partitioning inside the micelles arises from charge repulsion between the drug molecules; for comparison, the partition coefficient reported for molecular naproxen is 355 ± 64.[16]

Stability of the micelles, and therefore cmc and aggregation number (N_{agg}), is known to be the result of a repulsive-attractive balance of forces between the hydrophobic chains and the hydrophilic headgroups of the surfactant, respectively, acting mainly on

(51) Bisht, P. B.; Tripathi, H. B.; Pant, D. D. *J. Photochem. Photobiol., A* **1995**, *90*, 103–108.
(52) Escándar, G. M. *Spectrochim. Acta, Part A* **1999**, *55*, 1743–1752.
(53) Velázquez, M. M.; Valero, M.; Rodríguez, L. J.; Costa, S. M. B.; Santos, M. A. *J. Photochem. Photobiol., B* **1995**, *29*, 23–31.

the interfacial region of the aggregates.[54] Our results show how the presence of additives contributes to modify this balance of forces in the aggregate, resulting in micellar size changes that limit the partition, owing to the stability of the loaded micelle. In summary, and combining the fluorescence data with the SANS results, an increase in the attractive forces between the hydrophobic chains increases micellar size and consequently N_{agg} (LD, high partition); in contrast, an increase in repulsive forces between the hydrophilic headgroups decreases micellar size (NP and SAL, low partition). In the case of PB, SANS data show that addition of the drug only affects very marginally the micellar structure, with a slight increase in intermicellar repulsions, while the fluorescence data show that all the drugs are localized at the micelle interface. Hence, for PB, the large polar head allows the ionizable group to stay in water far from the hydrocarbon lateral chains, which are likely to be included in the micelle; therefore, the presence of PB does not contribute to the overall repulsive forces in the core−shell region of the aggregate, which effectively leads to minor micellar structural changes, but to a slight increase in surface repulsions.

2.2. Binding Constant of the Drugs to β-CD, in the Absence and Presence of Pluronics. In the following section, we examine the interactions in the ternary systems composed of drugs, Pluronics and heptakis(2,6-di-*O*-methyl)-*β*-cyclodextrin. Prior to studying the ternary mixtures, the complexation of the drugs with hep2,6 *β*-CD is studied first for each drug, and a binding constant determined, both at low and high drug concentration. The effect of the presence of polymer (5%) on the complexation behavior of the drug is then studied.

Lidocaine. The presence of hep2,6 *β*-CD produces an increase in LD emission intensity and a red shift of the 290 nm band, showing the inclusion of LD inside the *β*-CD cavity (cf. section 2.1). At both drug concentrations, the intensity variation fits well to a 1:1 complex model (Figure 7). The binding constant determined at low LD concentration (Table 2) is very low 3.80 ± 0.1 (g/g), and it is increased to 13.01 ± 0.84 (g/g) (Table 2) at high drug concentration.

The same studies were then performed in the presence of F127. At low drug concentration in 5% F127, *β*-CD addition increases the emission intensity, and the maximum is blue-shifted. These changes show that LD/*β*-CD inclusion also occurs in the presence of Pluronic micelles, despite the low binding constant measured in the absence of polymer. In the concentrated LD systems in the presence of 5% F127, addition of *β*-CD leads to an irregular pattern in the emission intensity of the drug. A break in the fluorescence trend is observed around 3% of *β*-CD, reflecting a modification in the behavior of the system, probably related to micellar breakup. These changes show that the competition between LD and Pluronic is still present at these high concentrations. These results suggest that drug/CD complexation lead to a weakening of the breaking up of the micelles by *β*-CD, as reported earlier,[2] because of the reduced availability of *β*-CD molecules, therefore bringing support to hypothesis (2) (we cannot, however, discard hypothesis (1)).

Sodium Pentobarbital. The addition of *β*-CD to dilute aqueous solutions of the drug produces a change in fluorescence intensity and a strong blue shift (from 358 to 338 nm), showing the inclusion of the drug inside the hydrophobic cavity of hep2,6 *β*-CD. The fluorescence intensity data fit well to a 1:1 stoichiometry (Figure 7A). The binding constant obtained (Table 2) is higher than that for LD. Addition of hep2,6 *β*-CD to aqueous solutions of PB and F127 produces the same changes as in the absence of polymer, showing that hep 2,6 *β*-CD/PB complexation occurs all the same. This confirms again a competition between drug and Pluronic for complexing with the cyclodextrin.

The addition of increasing amounts of hep2,6 *β*-CD to 2% PB aqueous solutions slightly increases the fluorescence intensity up

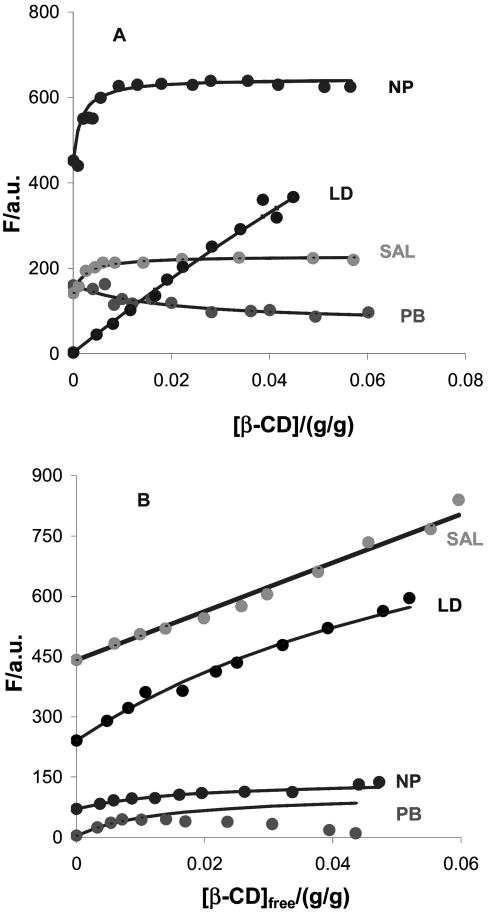

Figure 7. Experimental fluorescence data (dots) and fitting (solid line) for the determination of drug/*β*-CD binding constant. (A) Dilute drug systems (10^{-3}%). (B) Concentrated drug systems (0.3% for LD and 2% for the other drugs).

Table 2. Binding Constants (g/g) of 1:1 Drug/hep2,6 *β*-CD at 25°C (in Water)

drug	without F127	with F127
lidocaine	**dilute**	**dilute**
	3.80 ± 0.1 (F_{290})	–
	concentrated	**concentrated**
	13.01 ± 0.84 (F_{290})	–
pentobarbital	**dilute**	**dilute**
	48.92 ± 1.9 (F_{352})	–
	51.21 ± 8.8 (F_{262})	
	concentrated	**concentrated**
	72.2 (F_{299})	–
	197.9 (F_{355})	
salicylate	**dilute**	**dilute**
	440.9 ± 30.1	277.5 ± 47.5
	concentrated	**concentrated**
	0.025 ± 0.001	4.50 ± 0.46
naproxen	**dilute**	**dilute**
	625 ± 25	211.6 ± 58.4
	concentrated	**concentrated**
	56.55 ± 20.6	123.4 ± 25.89

(54) Israelachvilli, J. N. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: San Diego, 1992; p 367.

to 2% β-CD (Figure.7B), in good agreement with a 1:1 complex. Further addition of cyclodextrin produces a decrease in the emission intensity. These changes point out to a PB/hep2,6 β-CD complex formation. However, the interaction seems to be different at low and high β-CD concentration, probably reflecting the formation of two types of complexes, as described for barbiturates with hydroxypropyl β-CD.[55] From these data, it is not possible to obtain a good fit; however, for indicative purposes only, we determined a binding constant using the fluorescence variation of the different bands appearing in the spectra: its value varies between 72.2 and 198 (g/g).

The addition of hep2,6 β-CD to aqueous PB solutions in the presence of 5% F127 decreases the fluorescence intensity. All these changes clearly show that the drug is being displaced from F127 micelles into the β-CD cavity. The spectrum is similar to the one of PB at low β-CD concentration in the absence of F127, indicating that only one type of complex is now formed, in good agreement with a competition between the micelle and the cyclodextrin for binding the drug. It is also clear that the presence of PB decreases the free avalaible CD for Pluronic complexation, but as observed for LD the existence of other contributions cannot be ruled out.

Therefore, three equilibria are present: PB/CD, PB/micelle, and probably F127/CD, as reflected by the binding, partition, and breaking of the Pluronic micelle, respectively. If we suppose that the presence of F127 does not modify the PB/β-CD binding constant (K = 51.21 (g/g)) and if the drug partitioning is not competing with its complexation with β-CD, at 1% drug, the available (free) β-CD concentration would be 6.2 and 8.2% for 7 and 9% total β-CD, respectively. On the opposite, if we consider that the partitioning is 100% competitive for the complexation (in other words, that all the drug inside the micelles is not available for β-CD complexation), at 1% PB and 7−9% β-CD, the free β-CD would be 6.7−8.7%. In real conditions, the effective free β-CD must be between these two extreme scenarios, that is, 6.2−6.7% for 7% total and 8.2−8.7% for 9% total. At 9% total β-CD (8.2−8.7% effective), the micelles break (Figure 3), as it would be expected since they break with 7% β-CD in the absence of drug (Figure 2). With 2% PB instead, the free β-CD determined for the same scenarios is between 5.5 and 6.3% and 7.4−8.3% for 7 and 9% total β-CD, respectively. For 9% total β-CD, however (i.e., above 7% effective), the micelles are not fully broken (Figure 3). Therefore, in this case, the drug competition for β-CD, even including the partitioning of the drug inside the micelles, cannot be the only factor involved in weakening the micellar breaking process, and thus, hypothesis (1) must be considered in addition to hypothesis (2).

Sodium Salicylate and Sodium Naproxen. The addition of hep2,6 β-CD to both dilute and concentrated solutions of salicylate produces an increase in fluorescence intensity (Figure 7). However, neither the emission maxima position nor the shape of the spectrum is modified.

Addition of hep2,6 β-CD to solutions of NP also produces changes in the emission spectrum, but the effect is dependent on drug concentration. In the dilute system, an increase in fluorescence is observed, besides the appearance of an iso-emissive point around 335 nm and a blue shift from 355 to 350 nm. A blue shift has been reported when the drug is in apolar solvents.[53] In the concentrated drug systems, addition of hep2,6 β-CD leads to an increase in the monomer emission (355 nm), with a simultaneous decrease in the excimer emission band (440 nm). All these changes indicate that both drugs complex with β-CD at both concentrations. The emission intensity variation shows the formation of a 1:1 complex for both NP and SAL (Figure 7), as for the other drugs.

For these two drugs however, there is a major difference between the binding constant in dilute and concentrated systems (Table 2). This could arise from drug aggregation (naproxen excimer formation is indeed observed) but also from inner filter effects, which cannot be avoided in these conditions, hence, these results should be treated with care.

The binding constant of dilute salicylate systems (440.9 ± 30.1 g/g) (or 587.1 ± 43.3 M^{-1}) (Table 2) is much higher than those reported previously for hep2,6 β-CD (140 ± 15 M^{-1}).[56] Comparing with the other drugs used in this study, the binding constant of naproxen is the highest. The high binding constant of NP to hep2,6 β-CD, even in the presence of F127, gives further support to the hypothesis that the micellar growth observed at 7 and 9% CD with 5% F127 (Figure 4) is due to the decrease of NP concentration inside the micelle, as a direct result of its complexation with hep2,6 β-CD.

In dilute systems, in the presence of F127, the addition of hep2,6 β-CD changes the fluorescence of these drugs, giving evidence of drug complexation. The binding constant of SAL and NP decreases in the presence of polymeric micelles. Therefore, given that in dilute solutions of NP and SAL no partition into F127 is observed (cf. section 2.1), only two binary systems are present: drug/CD and probably F127/CD. Hence, the decrease must be due exclusively to the binding of F127 to hep2,6 β-CD. In these conditions (625 ± 25 (g/g) and no partition), the concentrations of free β-CD determined at 2% NP are 5.1% and 7.0% for total β-CD concentrations of 7 and 9%, respectively, and 9.0%−11.0% free β-CD for total β-CD of 11−13%. These values could justify the effect observed from SANS (Figure 4), since at 2% NP the micelles are not broken up with 7−9% β-CD (i.e., below 7% effective) and micellar break up is only observed from 11 to 13% (above 7% effective). At 1% NP instead, for 9% total β-CD, there is 8.0% free β-CD, thus above 7%, which could therefore explain why the micelles are broken up (Figure 4). In this perspective, and with this yet limited set of data, this suggests that hypothesis (2) alone could explain the weakened disruption of the micelles by β-CD in the presence of drug.

Therefore, although the high affinity of both drugs, NP and SAL, to hep2,6 β-CD may decrease the disruptive action of β-CD on the micelles at low β-CD, with further addition of β-CD instead, the micelles may be more readily broken up by β-CD, since the β-CD/F127 binding increases. This again demonstrates that a fine balance of forces is involved, which controls micelle aggregation and break up, but this also opens up very interesting perspectives in terms of finely tuning the release of the drug at specific sites in these ternary mixtures.

Conclusion

In this contribution, we have used a combination of SANS and fluorescence spectroscopy to gain more insight into the complexation behavior of the Pluronic F127, heptakis(2,6-di-*O*-methyl) β-cyclodextrin, and four selected drugs (namely, lidocaine (LD), pentobarbital (PB), sodium naproxen (NP), and sodium salicylate (SAL)) in binary and ternary mixtures. In particular, we have attempted to explain the structural micellar changes observed in the SANS patterns by an analysis of the interactions involved (reflected by binding constants and partitioning coefficients), the

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consideration of the chemical structure of the drugs, and the modifications occurring in their microenvironment upon addition of polymer, β-CD, or both. The main findings are summarized below.

The binding constants of the drugs to the Pluronic micelles, determined by fluorescence spectroscopy, were found to be directly correlated to the amount of charge, following NP $\approx$ SAL < PB < LD. All drugs were found to lie at the micellar interface, with a slightly deeper localization of lidocaine (LD) inside the micelles. The hydrophobic character (and absence of very hydrophilic groups) of LD gave rise to a substantial growth of the micelles, while charge repulsions in NP and SAL led to the shrinking of the micelles. PB was found to adopt a more superficial position, with the hydrophobic substituents inside the apolar region of the micelle, the polar ring protruding into the palisade zone and probably into the solution; taking into account the size of both parts of the drug, its interaction with the micelle can be defined as "anchored" to the micelle through its lateral chains, therefore affecting only marginally the overall structure of the aggregates.

All drugs were found to form complexes with β-CD; the strength of the binding constant (at low drug concentration) followed the order: LD < PB < SAL < NP. While hep2,6 β-CD has been shown to gradually break up F127 micelles through β-CD/polymer complexation, this effect is weakened in the presence of drug, provided the drug is above a critical concentration ($\sim$2% PB with 5% F127). In the case of NP, a low drug concentration (1%) did not alter micellar breakup by β-CD (from 9% β-CD and above); however, at a higher drug concentration (2% NP), gradual addition of β-CD led to micellar growth, up to 11% β-CD where micellar breakup occurred. These effects are attributed to a preferential β-CD/drug complexation over β-CD/polymer interaction, as deduced from the binding constants. However, it is clear that a fine-tuning of the component ratio controls the balance of forces and the mechanism of interaction. In the case of PB, the results suggest that the effect is not exclusively related to the partitioning of the drug inside the micelles or its binding to β-CD, but the presence of the drug in the aggregate also modifies the polymer/β-CD binding, contributing to the overall effect. This study is a first attempt to shed light on the nature of these complex interactions, but further work should enable us to understand better the balance of forces, in order to be able to control and adjust the behavior for targetted drug delivery.

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Supporting Information Available: Graphs showing the fluorescence spectra of lidocaine and pentobarbital at varying pH and in a range of solvents (hexane, dichloromethane, chloroform, methanol, acetone, acetonitrile, ethanol, propanol, butanol, octanol, and water) and a table showing the position of emission maxima of both drugs in these solvents. This material is available free of charge via the Internet at http://pubs.acs.org.