Modulating Pluronics micellar rupture with cyclodextrins and drugs: effect of pH and temperature

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Abstract. Micelles of the triblock copolymer Pluronic F127 can encapsulate drugs with various chemical structures and their architecture has been studied by small-angle neutron scattering (SANS). Interaction with a derivative of β-cyclodextrin, namely, heptakis(2,6-di-O-methyl)-β-cyclodextrin (DIMEB), induces a complete break-up of the micelles, providing a mechanism for drug release. In the presence of drugs partitioned within the micelles, competitive interactions between polymer, drug and cyclodextrin lead to a modulation of the micellar rupture, depending on the nature of the drug and the exact composition of the ternary system. These interactions can be further adjusted by temperature and pH. While the most widely accepted mechanism for the interaction between Pluronics and cyclodextrins is through polypseudorotaxane (PR) formation, involving the threading of β-CD on the polymer backbone, time-resolved SANS experiments show that de-micellisation takes place in less than 100 ms, thus unambiguously ruling out an inclusion complex between the cyclodextrin and the polymer chains.

1. Introduction

Triblock copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO), known as Pluronics or Poloxamers, are polymeric surfactants able to form micellar aggregates in solution [1]. They can solubilise drugs in an aqueous environment, and present similar features to natural carriers in terms of size, structure and transport properties, making them excellent candidates for drug delivery applications [2].

Natural cyclodextrins are cyclic oligosaccharides, formed by 6, 7 and 8 glucose units: α-, β- and γ-cyclodextrins, respectively [3]. Their derivatives, obtained by modifying the external hydroxyl groups with various types of substituents (such as hydroxyethyl, methyl and other functional groups), have been synthesized and are available commercially. Cyclodextrins present a conical shape with a hydrophobic inner cavity and the hydroxyl groups of the glucoses placed on both external sides, which confer them a polar character, and therefore aqueous solubility, while their interior is mostly hydrophobic. The twice-methylated derivative of β-cyclodextrin, namely, heptakis(2,6-di-O-methyl)-β-cyclodextrin (DIMEB), has been shown to induce a full rupture of Pluronic micelles [4-6], and the break-up mechanism is very selective on the type of cyclodextrins [5]. In addition, cyclodextrins are well known to form complexes with a wide range of drugs [7], therefore the ternary systems of...
DIMEB, Pluronic and drug leads to interesting 3-way competitive interactions [6]. Finally, structures and interactions can be modulated by temperature and pH, as we show in this paper. Responsiveness to these various triggers and parameters, and thus the ability to tune the self-assembly structures, make these systems good candidates for the development of smart drug carriers for controlled release.

In this work, we study the drug-loading effect of two selected drugs of different structures on Pluronic F127 micelles: pentobarbital (PB) and naproxen (NP), both as sodium salts. We then examine the effect of the loaded drugs on the ability of DIMEB to break-up F127 micelles at various pH (pH 1, pH 13 and natural pH) and temperatures (25°C and 37°C) and provide some clues on the mechanism of break-up.

2. Experimental

2.1. 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. Heptakis(2,6-di-O-methyl)-β-cyclodextrin, naproxen sodium salt and pentobarbital sodium salt were obtained from Sigma-Aldrich UK.

2.2. Methods. Small-angle neutron scattering (SANS) measurements were carried out on LOQ at the ISIS facility (Rutherford Appleton Laboratory, Didcot, UK). The instrument uses incident wavelengths from 2.2 to 10.0 Å, sorted by time-of-flight, and a sample-detector distance of 4.1 m, providing access to scattering vectors $q$ from 0.009 to 0.287 Å$^{-1}$. Kinetic SANS measurements were carried out on the D22 instrument at the ILL using a stopped-flow equipment [8]. The wavelength was set at 6 Å, the sample-to-detector distance was 4 m, providing a $q$-range from $1.2 \times 10^2$ Å$^{-1}$ to 0.26 Å$^{-1}$. The scattering measurements were made with an acquisition time for each frame $n$ of $t_n$ following a geometric series during 10 minutes:

$$t_n = a^{n-1}t_1, \quad T_n = \frac{1-a^n}{1-a}t_1$$

where $T_n$ is the accumulated time after mixing, with $t_1 = 100$ ms and $a = 1.1$. Sixty eight frames were measured for a total time of 651.7 s, after which 20 additional frames were measured for 1 s exposure each. Additional details can be found here [9].

3. Results and discussion

We first studied the effect of the pH (1, 13 and natural pH) and temperature (25°C and 37°C) on the properties of the micelles loaded with 1% PB or NP.

Figure 1A shows the scattering from solutions of 5 wt% F127 micelles at 25°C in the presence of 1 wt% PB at pH 1 and pH 13 (data at natural pH have been published elsewhere [6]), and also at 37°C and natural pH. The scattering from F127 shows typical features of spherical micelles [10]. At pH 13, the addition of 1% PB hardly affects the scattering, as it had also been observed at natural pH (ca. 9) [6]; only a slight shift of the curve to higher $q$-values and decrease of the low-$q$ intensity suggest a small decrease in size and increase in inter-micellar repulsions, possibly due to charge, since at both pH values the drug is dissociated. Overall however, although PB partitions within the micelles [6], it hardly affects their shape and size, thus suggesting a rather superficial localisation at the micelle/water interface. At pH 1 instead (the drug is in its molecular form), the scattering increases dramatically and stronger features are seen in the scattering pattern, showing that the partitioning of PB induces micellar growth. At a higher temperature of 37°C (and natural pH), the micelles are larger (Figure 1A), with a higher aggregation number [4], and, so are the micelles in the presence of PB; however, as seen at 25°C and both at natural pH and pH 13, the addition of PB has a very limited impact on micellar size.
In Figure 1B, the effect of adding DIMEB to the same solutions is shown. At pH 1 and 13 (at 25°C), the addition of 9% DIMEB leads to a complete rupture of the micelles, as had been observed at natural pH, both in the presence and absence of 1% PB [6]. At 37°C instead, the disruption of the micelles is quite limited; they decrease in size but are not ruptured (as inferred from the persisting strong features in the scattering pattern). Interestingly, while the micelles with 1% PB at pH 1 and 25°C are larger than at 37°C, they are ruptured much more substantially by the same amount of DIMEB. In other words, the temperature more efficiently opposes demicellisation than low pH. However, we had also shown that with a higher amount of PB (2%), the effect of DIMEB was substantially weakened at 25°C and pH 9 [6]. There is a clearly a balance of repulsive-attractive forces involved, which either hold the micelles together or oppose micellisation, and are modulated by the partitioning of the drug, the interaction of both the polymer and the drug with DIMEB, pH and temperature. Both pH and temperature affect the affinity between the various players (polymer chains, drug and cyclodextrins).

Figure 1. SANS curves from 5% F127 micelles alone and in the presence of either PB or NP, at various pH (pH 1, pH 13 and natural pH) and temperatures (25 and 37°C): A. Micelles with 1% PB at pH 1 and pH 13 (25°C), and at pH 9 (37°C). B. Micelles with 1% PB as in A and in the presence of 9% DIMEB. C. Micelles with 1% NP at pH 1 and pH 13, 2% NP at pH 13 (25°C), and with 1% NP at pH 7 (37°C). D. Micelles with 1% NP at pH 13 (25°C) and with 7% DIMEB; and at pH 7 (37°C) with 9% DIMEB.

We next studied the effect of NP on the micelles (Figure 1C and 1D). At pH 13 (25°C), the effect is similar than at neutral pH [6] (Figure 1C): adding NP (1 and 2%) decreases the size of the micelles, which is a clear sign of the partitioning of the drug inside the micelles, leading to charge repulsion and thus to a reduction of the aggregation number. Instead, at pH 1, adding 0.5% NP induces a growth of the micelles. At this pH the drug is in molecular form, therefore likely to partition deeper inside the micelles, and thus leading to a very different outcome than when in its charged form. At 37°C and
neutral pH, the effect is similar to 25°C (and pH 7 or 13): micelles decrease in size when adding NP (but overall they are larger to start with at higher temperature).

As shown in Figure 1D, adding 7% DIMEB to the solutions containing 1% NP at pH 13 leads to full de-micellisation (as had also been observed at pH 7 [6]). Instead, it had been observed that with a higher amount of drug loaded (2% NP) and pH 7, the addition of the same amount of DIMEB induced an increase in micellar size [6], thus DIMEB offsets the disruptive effect of the partitioning of NP inside the micelles, possibly because of the preferential interaction of DIMEB with the drug, ‘snatching’ drug molecules from the micelles to form a β-CD drug complex [6]. However, at 37°C and pH 7 (Figure 1D), as also observed above with PB, the effect of DIMEB is substantially weakened by the presence of 1% NP, since the addition of 9% DIMEB leads only to a partial reduction in micellar size but no break-up.

Two possible mechanisms have been proposed to explain the modulation of the disruptive effect of DIMEB in the presence of drugs [6]:

- Hypothesis (1): competition between drug and F127 for complexation with CD reduces the availability of CD molecules to rupture the micelles
- Hypothesis (2): the partitioning of the drug affects the ability of the micelles to interact with F127.

More insight into the mechanism of rupture and the rules controlling it has been obtained for a range of compositions by measuring the binding constant of the drugs to the micelles and to DIMEB [6], and provides scope for a more in-depth discussion of the mechanisms.

The most widely invoked mechanism reported in cyclodextrins-polymer interaction is pseudopolyrotaxane (PR) formation, a type of inclusion complex involving the cyclodextrins threading onto the polymer backbone [11], acting as a rotor. Our first hypothesis was that this type of structure could be at the origin of micellar break-up [4]: by threading onto the Pluronic chains, and preferentially locating on the hydrophobic PPO block (the micellar core), the cyclodextrins would solubilise the micelles [4, 12]. However, our recent NMR studies [13] have revealed that the CH₃ group of PPO interacts weakly with the inner cavity protons H₃ and H₅ of the cyclodextrins, as well as with the CH₂ group in position 6. We proposed instead the formation of a “loose” inclusion complex, between DIMEB and the lateral CH₃ group of the PPO group from the polymer [13]. This would also justify the highly discriminative nature of the rupture mechanism on CD substituents [5, 13].

More compelling evidence was recently provided by time-resolved SANS (TR-SANS) measurements on the naked micelles [9]. We report here new TR-SANS results on the drug-loaded micelles.

![Figure 2](image-url)

**Figure 2.** Small-angle neutron scattering data from 4 wt% F127 micelles with drug (○), either 1% PB (A) and 1% NP (B) in the presence of 5% DIMEB at different time points after mixing: after 0.1 s (frame 1, ■), frame 1.5 s (frame 10, ▲), 111.1 s (●), 622.1 s (frame 68, ×), 642.1 s (frame 88, ⬤).
Figure 2A and B show the effect of adding 5% DIMEB to 4% F127 solutions containing either 1% PB or NP. The first frame, taken less than 100 ms after mixing, reveals a drastic, instantaneous change in the scattering pattern, which reflects a shrinking of the micelles, as described above. This clearly demonstrates that the disruption of the micelles by DIMEB occurs in less than 100 ms. The threading of cyclodextrins onto linear hydrophobic polymers to form PRs is a lengthy process (over 10 s [14]); therefore, these measurements unequivocally rule out the formation of a PR as the mechanism of micellar break-up, as previously demonstrated for the free micelles [9,13]. Interestingly however, in the presence of NP and PB, scattering patterns measured at increasing times show a slight evolution of the disruption of the micelles. This had not been observed in the absence of drugs [9] and thus hints to a slightly different (marginally slower) mechanism of rupture in the presence of the drug, possibly involving loaded drug-cyclodextrin interaction as an intermediate step in the process, occurring over a few minutes. In the absence of drug, no changes were detected after 100 ms [9]. After ca. 620 s (ca. 10 min), the pattern has stabilised and overlaps perfectly with the equilibrium mixture of Pluronic, drug and DIMEB (Figure 2B).

4. Conclusion
We have shown that DIMEB can be used as a trigger to rupture Pluronic micelles. The partitioning of drugs modulates this behaviour, as well as the temperature and the pH. While the detailed mechanism is still under study, we have unambiguously ruled out the widely-invoked polypseudorotaxane formation as the mechanism of rupture and propose instead the formation of a loose complex between the polymer chains and the cyclodextrins. The same instantaneous micellar disruption reported before [9] is demonstrated here for the drug-loaded micelles, thus ruling out PR formation, however a further (slight) shrinking of the aggregates is observed here for the first time, suggesting a slightly different mechanism in the presence of drugs.

More insight into the balance of interactions controlling the break-up needs to be obtained in order to further develop this system as a smart drug carrier.

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