Experimental report

18/01/2017

Proposal: 9-12-401 **Council:** 10/2014

Title: Unravelling the mechanisms of complexation between Tetronics and cyclodextrins

Research area: Chemistry

This proposal is a new proposal

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Samples: Tetronics, cyclodextrins

Instrument	Requested days	Allocated days	From	To
D22	3	2	04/12/2015	06/12/2015

Abstract:

Cyclodextrins (CDs), cyclic oligosaccharides consisting of 6, 7 or 8 glucopyranose units, can thread onto polymer chains and form inclusion complexes referred to as pseudopolyrotaxanes (PPR) - the starting point of versatile supramolecular constructs. These complexes have applications in drug delivery, as injectable gels and responsive molecular machines. Following-up a substantial body of work in the area of PPRs, we are focusing in this study on Tetronics, X-shaped PEO-PPO block copolymers (related to the more well-known family of Pluronics) and their complexes with a range of cyclodextrins. We propose to use time-resolved SANS experiments; combined to a stopped-flow set-up - to elucidate the mechanisms of CD threading, in particular: the early-stages of complexation, the competitive processes involved when two types of CDs are present and the impact of temperature on the complexation process.

Unravelling the mechanisms of complexation between Tetronics and cyclodextrins

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EXPERIMENTAL CONDITIONS. Kinetic SANS measurements were carried out on D22, combined with a stopped-flow unit. The wavelength λ was set at 6 Å, sample-to-detector distance 4 m, with a collimation at 5.6 m and a detector offset of 400 mm to maximize the available *q* range ($1.2 \cdot 10^{-2} < q < 0.26$ Å^{-1}). A 7×10 mm^2 sample aperture was used, and the sample path length in the Biologic SFM-300 stopped-flow apparatus was 0.1 mm. Raw data were corrected for electronic background and empty cell and normalized by water using *Lamp* ILL software. The acquisition times were calculated according to the geometric series defined in Valero et al. *J Phys Chem B*. **2012,** *116*, 1273. Generally, sixty eight frames were measured for a total time of 653 s, after which 20 additional frames were measured with 6 s of exposure each. The stock solutions of surfactant and CD were prepared by weighing the required amounts of surfactant, CD and deuterated water. Appropriate volumes of stock solutions (total of 250 µL) were then mixed in the stopped-flow cell with a flow rate of 3 mL/s to obtain the target concentrations.

SYSTEMS STUDIED. The following kinetic experiments were carried out:

- **Poloxamine T904:** T904 + γCD, T904 + βCD, T904 + αCD, T904 + DIMEB, T904 + RAMEB, T904 + αCD + DIMEB (different proportions of the CDs)
- **Poloxamine T1107:** T1107 + αCD, T1107 + DIMEB, T1107 + RAMEB
- **Poloxamine T1307:** T1307 + αCD, T1307 + DIMEB, T1307 + RAMEB
- **Poloxamine T90R4:** T90R4 + βCD, T90R4 + γCD
- **TPGS:** TPGS + DIMEB

(In each case the corresponding static experiment was carried out)

RESULTS. As an example we show here the kinetics of de-micellization of TPGS with DIMEB and the main conclusions. The experiments were conducted at 40ºC, to compare with poloxamines, mixing solutions of TPGS and DIMEB (final concentrations: 1% in surfactant and 3% in CD). The results are shown in **Figure 1.** For comparison purposes, the static measurement of TPGS 1% is included. The shortest time reached is 100 ms and the micelles have largely disappeared by this time, while large aggregates can be detected as an uprising curve at low *q*, which lies over the curve of 1% TPGS measured under static conditions. The kinetics of micelle formation as studied by scattering techniques has been reviewed in-depth by Lund et al. (*Adv. Polym. Sci.* **2013**, *259*, 51), mainly focusing on block copolymer micelles. In general, the exchange process monomer/micelle takes place on the microsecond time scale, and likewise the kinetics of

inclusion complexes of CDs, the fitness of the guest molecule to the cavity being a key factor to control the reaction rate.

To get some insight into the demicellization process from the TR-SANS data, we propose here a simple model in which the overall scattering is split up into two contributions, one due to the aggregates, considered as spherical, with an average sld equivalent to that of a micelle (calculated as $4.7 \cdot 10^{-6}$ Å^{-2}) and another one to the complex, which is modelled as a small sphere of sld equivalent to that of one TPGS molecule and three of DIMEB, $8.4 \cdot 10^{-7}$ Å^{-2}, in other words a binary mixtures of hard spheres. **Figure 2** shows some of the most relevant findings obtained with this model.

Some conclusions can be derived from the inspection of these plots. On the one hand, the constant value of the size and of the complex ($R \approx 25$ Å) and its volume fraction and size match reasonably well the size obtained by DLS or SANS, proving that the complex is totally formed after 100 ms. The second inference is the changing size of the aggregates. At 100 ms the size is close to that of the micelles (60 Å), and their volume fraction, about 0.006, is considerably smaller than the 0.025 calculated for 1% TPGS solution alone (not shown), indicating that there are still some micelles. After a few seconds, at the same time that the volume fraction of micelles diminishes, the aggregates become larger, reaching a maximum size. Little can be said about the composition of these aggregates, but the presence of a certain amount of DIMEB cannot be ruled out. Anyhow, at sufficient long times these aggregates either disappear or their volume contribution becomes negligible. It seems clear that the kinetics of complexation of TPGS with the CD is a fast process that implies the prompt reduction in the concentration of free unimers when mixing the micelles and DIMEB solutions in the stopped flow chamber. This produces the immediate drop in the number of micelles and justifies that no micelles can be detected even at 100 ms.

The rest of the kinetic experiments is currently under analysis, following the approach described above. The general trend is quite similar in all cases (fast kinetics and vanishing of the micelles in less than 100 ms), irrespectively of the type of poloxamine.

The data shown in this report are included in a draft manuscript on TPGS and cyclodextrins.

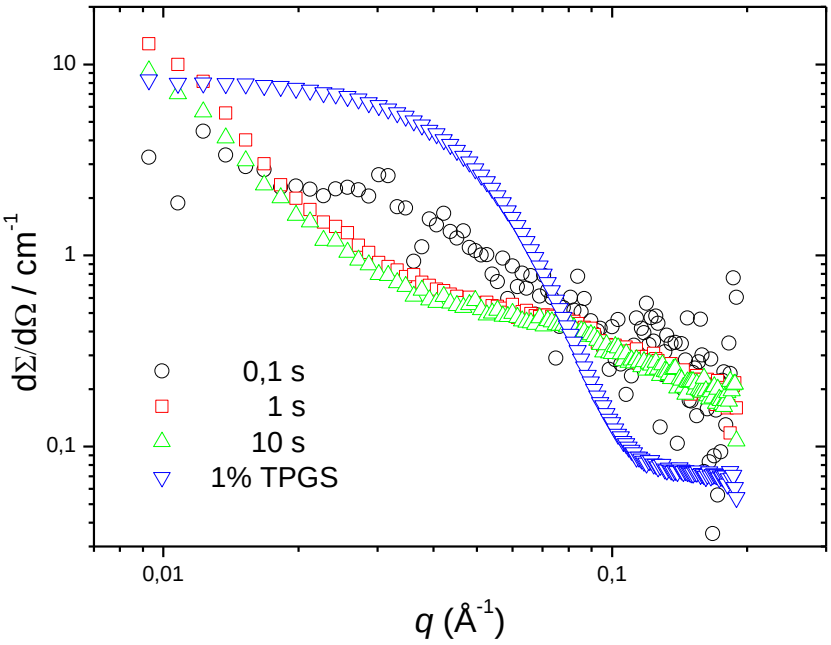

Figure 1. TR-SANS patterns for TPGS 1% + DIMEB 3% in D_2O at different times.

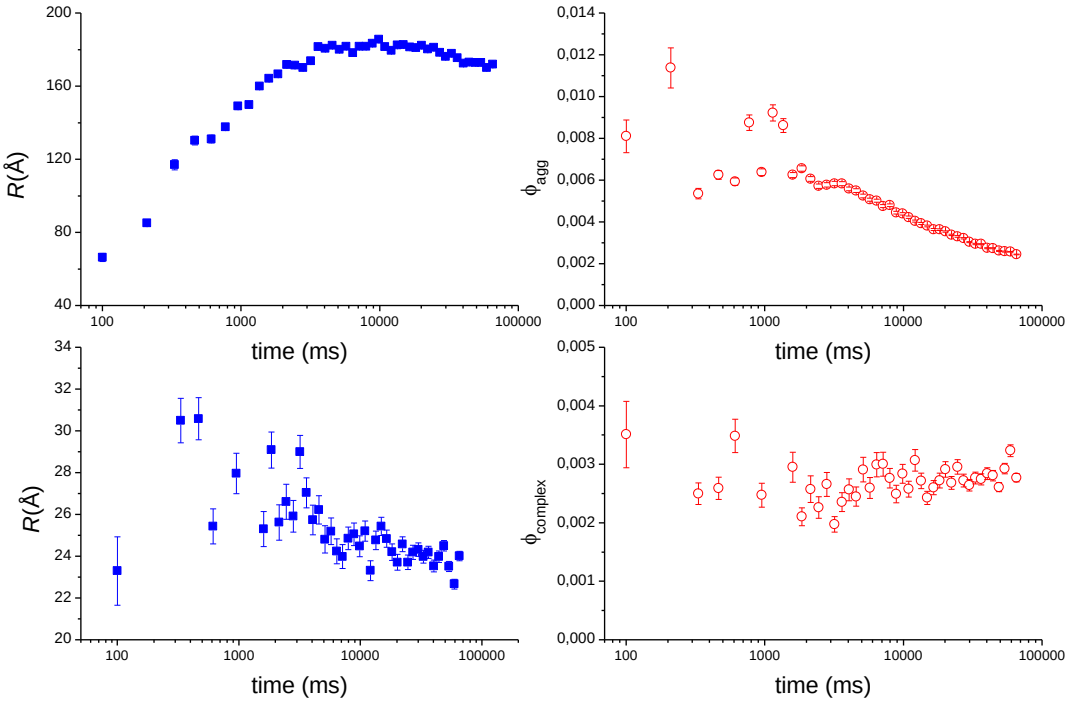

Figure 2. TR-SANS analysis of the kinetics of 1%TPGS + 3%DIMEB in D_2O, modeled as a binary mixture of hard spheres (see text). Up: radius and volume fraction of the aggregates; b) Down Radius and volume fraction of the complex.