Experimental report

28/02/2019

Proposal: 9-10-1527 **Council:** 4/2017

Title: Mixed systems of poloxamines and TPGS for transdermal gel delivery

Research area: Chemistry

This proposal is a new proposal

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Samples: poloxamines, poloxamers, TPGS

Instrument	Requested days	Allocated days	From	To
D22	2	1	03/04/2018	04/04/2018

Abstract:

The development of efficient nanocarriers for drug delivery demands a careful structural characterisation in order to identify the relevant architectural parameters which lead to a measured biological response. Based on our track record in the area of polymeric surfactants, the objective of this proposal is to explore the synergy of gels of TPGS and poloxamines (Tetronics) as novel drug carriers, with modulable cargo release. The experiments pursue two well-defined objectives:
i) To investigate the effect of the presence of gentramicin, naproxen and acyclovir on the structure and phase behaviour of mixed systems TPGS-poloxamine. The combination of both surfactants produces gels at physiological temperature, and the purpose is their use for the transdermal delivery of these drugs, which act respectively as antibiotic, antiviral and anti-inflammatory, having different HLB and water solubility.
ii) To investigate the effect that pH has on the delivery and stability of the gels and cargo release by pH-jump TR-SANS. This involves as a first step the investigation under static conditions of the different systems.

EXPERIMENTAL CONDITIONS. Kinetic and static SANS measurements were carried out on the D22 instrument at the ILL. For the kinetic experiments a stopped-flow unit (Biologic SFM-300 was used). The wavelength λ was set at 6 Å, sample-to-detector distance 5 m, with a collimation at 5.6 m and a detector offset of 400 mm to maximize the available *q* range. The sample path length in the 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. For the kinetic experiments 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 surfactants and drugs were prepared by weighing the required amounts of substance and deuterated water. Appropriate volumes of stock solutions (total of 502 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 sets of experiments were carried out:

Kinetic runs

Acidification (HCl) and dilution of T904 5%
Acidification (HCl) and dilution of T908 5%
Acidification (HCl) and dilution of mixtures T908 + TPGS 5%
Acidification (HCl) of T908 5% + gentamicin (GM)
Acidification (HCl) of T908 + TPGS 5% + gentamicin (GM)

Static measurements (at different temperatures, ranging from 25 to 55 ºC)

TPGS, T904, T908 alone at different concentrations
Mixtures T904+TPGS and T908+TPGS in the absence and presence of GM, at acidic or natural conditions
Mixtures of methylparaben (MP) + T904 or T1107 at different concentrations
Mixtures of valproic acid (VAL) + Pluronic F127 or T1107 at different concentrations

RESULTS. The studies of kinetics of demicellization using a pH-jump revealed a very fast process with any of the surfactants considered (T904 or T908), faster than expected, which implies an instantaneous delivery of the cargo contained in the micelles. As an example we show here the kinetics of de-micellization at 45º of T908, a large PEO-PPO-based block copolymer that forms micelles of 10 nm in radius (**Figure 1**). 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. As the protonation of the amino groups of the Tetronics take place in a much shorter time, the equilibrium of micelle formation would be shifted towards the free, positively charged unimers at pH=2. We believe that this is the main mechanism of the disruption of the micelles, as the micellar core is virtually dehydrated and consequently the protonation of the connecting diamino spacer hindered.

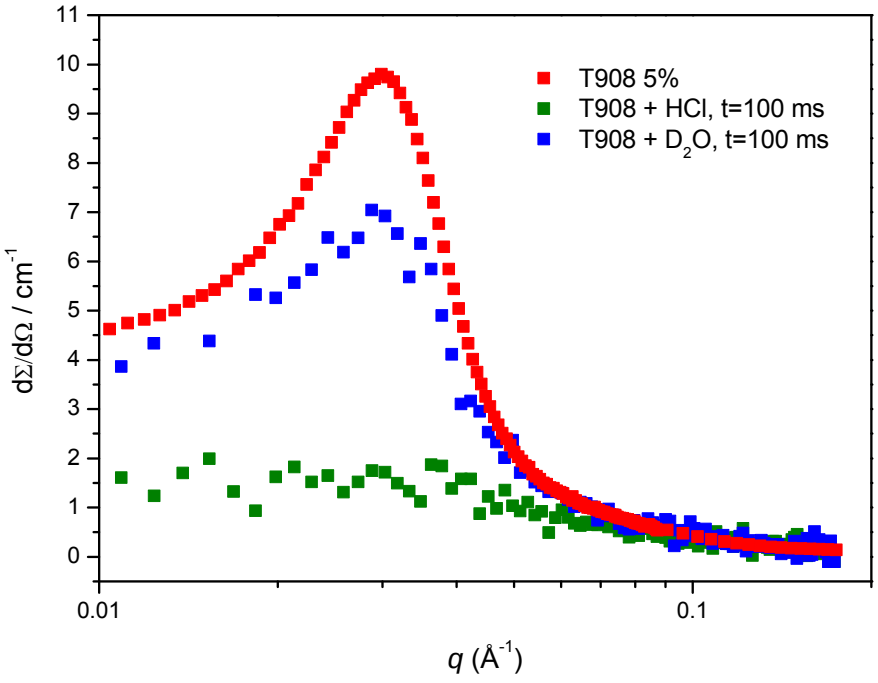

Figure 1. Kinetic SANS curves for 1:1 dilution of T908 to 5% (blue trace) and acidification to pH=2 (green trace). The red trace correspond to 10% T908 in D_2O.

As an example of the static measurements we include here the study by SANS of the incorporation of methylparaben (MP), a bactericide and preservative, to the polymeric micelles of T904 and T1107. This study is extensively described in a just-accepted paper (Zornoza et al. J. Molecular Liquids, 2019). Briefly, we have observed that the presence of MP reduces the critical micelle temperature (CMT) of both poloxamines and induces the formation of larger micelles at room temperature compared to the plain surfactants. A remarkable temperature dependent effect on the structure of the micelles has been detected, which progressively evolve from core-shell spheres to rods as the temperature increases (Figure 2). The incorporation of the preservative into the micelles modifies its reactivity against alkaline hydrolysis, resulting in a decrease of its reaction rate constant in which the dominant factor for the reduction in the hydrolysis rate is the incorporation

into the micelle core, rather than the length of the hydrophilic polyethylene oxide (PEO) arms.

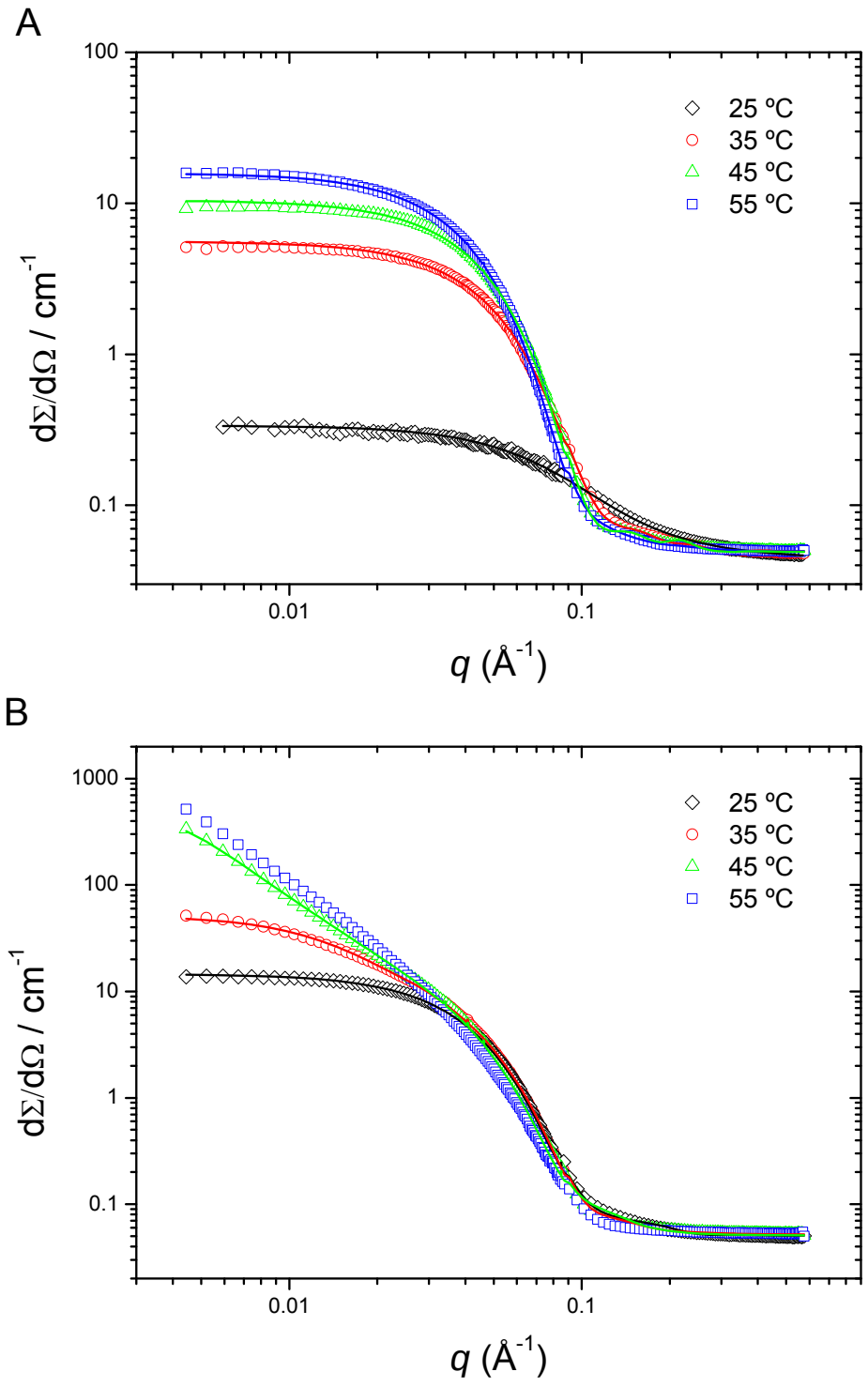

Figure 2. SANS curves for T904 1% in D_2O in the absence (A) and presence of 0.3% MP (B). Solid lines represent the fits to core-shell spheres (25ºC) and core-shell rods (35, 45ºC).

The rest of the kinetic and static experiments are currently under analysis by using combined NMR and DLS data and will be the subject of a publication in the near future.