Experimental report

23/09/2025

Proposal: 9-10-1883 **Council:** 10/2024

Title: Addressing vector-borne parasitic diseases with new drug delivery systems based on mixed polymeric micelles and gels

Research area: Chemistry

This proposal is a new proposal

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Samples: Tetronic
Miltefosine
Pluronic
Pentamidine

Instrument	Requested days	Allocated days	From	To
SAM	2	1	22/05/2025	23/05/2025
D33	2	0		
D11	2	0		

Abstract:

Leishmaniasis is a neglected tropical disease with high morbidity and mortality, considered the second leading cause of death worldwide by a parasitic agent. The available drugs exhibit high toxicity and long duration schedules that can be overcome with more efficient formulations. Our objective is to elucidate by SANS the structures formed at physiological conditions by PEO/PPO-based block copolymers (Pluronic and Tetronic) in combination with pentamidine and miltefosine (MF), an alkylphospholipid with activity against various parasite species. Due to its amphiphilic nature, MF forms mixed micelles and gels with the polymers, depending on the concentration, temperature and pH (Tetronic). The combination Tetronic-Pluronic produces also mixed micelles in which the loading capacity can be tuned by the proportion of the co-surfactants. The experiments will be conducted at the diluted and concentrated regimes, using the copolymers and their mixtures combined with both drugs. The ultimate aim is the design of drug delivery systems based on dual combination therapy in which the drugs act at lower concentration, reducing the side effects while keeping their therapeutic action.

EXPERIMENTAL CONDITIONS

The proposal was allocated 24 h of beam time in the SAM diffractometer. The experiments were designed to study:

1) The structure of mixed micelles formed by Tetronic and Pluronic block copolymers at the diluted regime, specifically, Tetronic 1107 and Pluronic F127 and L68.

2) The effects of the incorporation of pentamidine isethionate (PTM) and miltefosine (MF) to the systems in (1) (diluted regime)

3) The systems in (1) and (2) at the concentrated regime (gels)

The experiments were conducted at 20ºC and 37ºC in deuterated PBS buffer. Samples were transferred onto 1 mm path-length "banjo" cells. The neutron wavelength, λ, was 6 Å, and detector distances of 0.84 and 6.8 m where used to sample the q-range from 0.005 to 0.5 $Å^{-1}$. The raw data were corrected for electronic background, empty cell and normalized by the solvent (GRASP software), and the SANS patterns at the different distances merged in a single file on site for further data processing.

SYSTEMS STUDIED

The following sets of experiments were carried out:

Diluted regime:

- Surfactants: F127 1%, T1107 1%, L68 400uM and combinations Tetronic + Pluronic
- Combinations surfactant + drug: 1% F127 or 1% T1107 + PTM and/or MF at different drug concentrations, 1% F127 and 100um L62 + PTM and/or MF at different concentrations

Concentrated regime:

- F127 and/or T1107 (total concentration 25%) alone and combined with MF and MF + PTM at different concentrations

PRELIMINARY RESULTS

The analysis of the SANS data and model development is ongoing. As an example, we include here the case of Tetronic T1107 with MF and PTM in PBS at the diluted regime (1% of surfactant). The experiment was conducted at 37ºC (above the critical micelle temperature of the polymer) to mimic the physiological conditions (Figure 1). The scattering patterns correspond to core-shell micelles, typical of PEO-PPO-based block copolymers. In the presence of MF, mixed micelles form, as reported by Dirany et al. (https://doi.org/10.1016/j.molliq.2023.121654), with a smaller size. The further addition of PTM reduces slightly the dimensions of the aggregates. The fact that the MF is located preferentially at the core, according to our NMR evidence (gradients and 2D-NOESY), permits combining the fitted parameters (core radius, shell thickness, volume fraction and scattering length densities of micelle core and shell) in a system of equations, from which the number of drug molecules drugs and polymer per micelle can be deduced (Figure 1). With F127, the addition of MF and PTM produces similar changes to those of T1107.

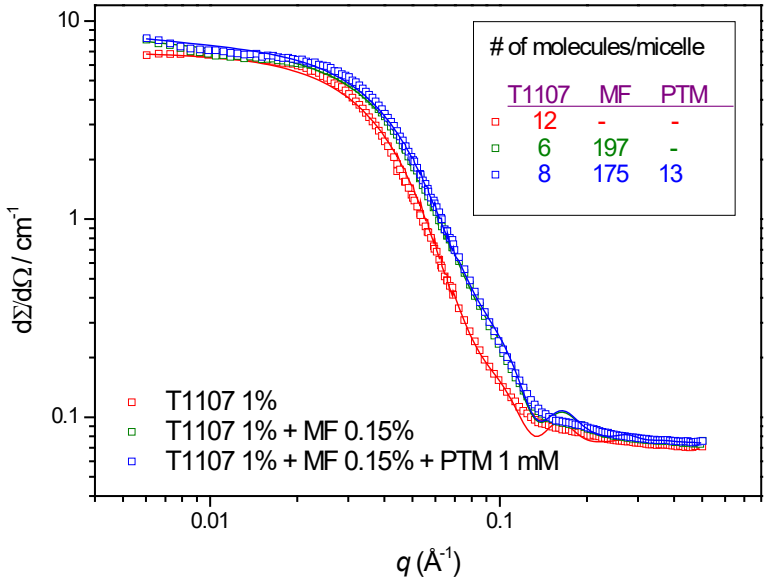

Figure 1. SANS patterns for 1% T1107 at different drugs loads (37ºC, d-PBS) and fits to core-shell spheres with a hard-sphere structure factor (data fitted with Sasview 6.0)

As an example of the results at the concentrated regime, the patterns for F127 and T1107 and their combination are shown in Figure 2. The micelles of these surfactants are known to self-organise in different types of paracrystal networks (fcc and bcc, respectively). A preliminary calculation using the models implemented in Sasview for these cells provides a *dnn* (nearest neighbour distance) of 20.4 nm (F127), and 14.5 nm (T1107). Interestingly, the mixture of both surfactants does not render a pattern as the sum of the individual ones, the best fit corresponding to an fcc-type, in which the *dnn* reduces to 18.2 nm. This value is in-between those of the copolymers considered individually. The actual type of packing is apparently governed by the co-surfactant that forms more compact micelles (the linear F127, in this case), although more data are needed to confirm this hypothesis.

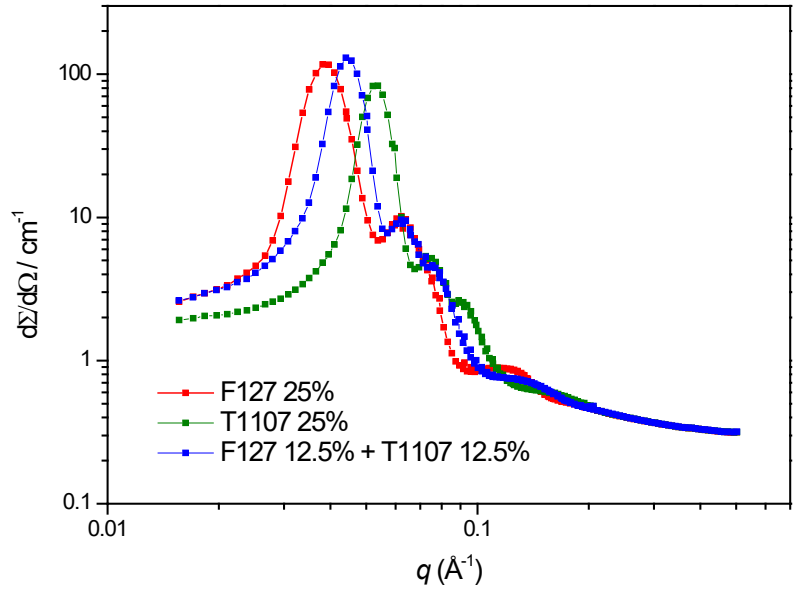

Figure 2. SANS patterns for T1107, F127 and their mixture (25% total) at 37ºC, in d-PBS.