Hydrophobic drug delivery: micelles of poly(ethylene glycol-*b*-propylene sulfide) as vehicles of Cyclosporin A

Diana Velluto and Jeffrey A. Hubbell
Laboratory for Regenerative Medicine & Pharmacobiology(LMRP), École Polytechnique Fédérale de Lausanne(EPFL), Switzerland.

Statement of Purpose: Many existing and emerging potent drugs have a poor water solubility. In this regard the development of new vehicles that can solubilise therapeutic agents and be safe for the human administration is of great importance. Micelles self-assembled from amphiphilic block copolymers is the focus of much interest as vehicles for the solubilization and controlled delivery of water insoluble drugs. The potential of poly(ethylene glycol-*b*-propylene sulfide) (PEG-PPS) block copolymers micelles for the solubilisation and controlled delivery of Cyclosporin A (CsA) has been investigated in this study.

Methods: Three different PEG-PPS diblock copolymers, with degree of polymerization of 44 on the PEG and 10, 20 and 42 on the PPS respectively (abbreviated as PEG_{44}-PPS_{10}, PEG_{44}-PPS_{20}, $PEG_{44}PPS_{42}$) were synthesized in our own laboratory by the anionic polymerization. The molecular weight was determined by size-exclusion chromatography (SEC) on a Polymer Laboratories column in THF and confirmed from ^1H NMR measurements. The polymeric micelles have been formed by solvent displacement method using CH_2Cl_2 as organic solvent or by dissolving the copolymers in hot water, obtaining in both cases a 10 mg/mL polymeric micelles suspension. Size and shape of the aggregates have been determinated by Dynamc Light Scattering measurements and Cryo-TEM images. The critical micellar concentration (CMC) of copolymers was measured by fluorescency using pyrene method.
The CsA, provided by Fluka, has been loaded in different amounts during the micelles formation. The micellar solution was then centrifugated to remove the CsA precipitates. The CsA levels were measured using SEC obtaining a nice separation between the polymer and the drug. CsA concentrations were estimated by UV detection at 210nm.
The releasing of the drug was also investigated: the CsA polymeric micellar formulation was placed in a Spectra/Por dialysis bag (MW cut-off = 6,000-8,000 g mol^{-1}) that was located in water while stirring at 37°.

Results/Discussion: The synthesis of PEG-PPS block copolymers has been reported previously (Napoli A.;Tirelli N.;Kilcher G.;Hubbell J.A. *Macromolecules* 2001, *34*, 8913-8917). In the present study copolymers with a hydrophilic fraction (f_{PEG}) higher than 0.35 have been synthetised. A previous investigation (data not reported) showed the influence of the f_{PEG} on the PEG-PPS aggregates morphology: higher values of f_{PEG} easily lead to micelles formation.
Micelles formed by PEG_{44}-PPS_{10}, PEG_{44}-PPS_{20} and PEG_{44}-PPS_{42} diblock copolymers, using the cosolvent displacement method, have been demonstrated to solubilize the CsA which reached a level of 1,915 mg/mL in aqueous media by PEG-PPS micelles (Table n.1) and precipitated in water in the absence of the polymers. Among PEG-PPS block copolymers of different PPS block lengths, maximum CsA : polymer weight ratio was achieved by PEG_{44}-PPS_{42} block copolymer showing direct relationship between the hydrophobicity of the micelles core and the CsA loading.

Table n.1: Characteristics of CsA-loaded PEG-PPS micelles prepared by methylene chloride displacement method.

PEG-PPS polymer	Size (nm)	Initial level of CsA (mg)	CsA loading mg/mg	Encaps.Eff. (%)
PEG_{45}-PPS_{10}	12	1	0.0305 ± 0.002	60.9 ± 4.1
PEG_{45}-PPS_{20}	23	4	0.130 ± 0.030	66.0 ± 7.3
PEG_{45}-PPS_{42}	26	6	0.1915 ± 0.030	63.8 ± 9.2

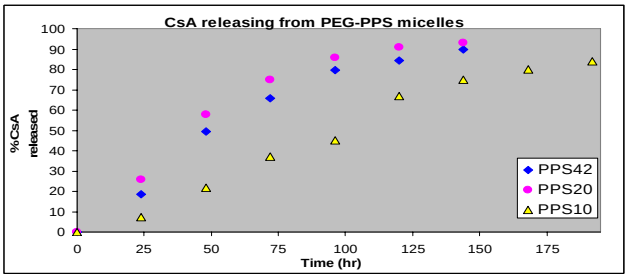
Experiments have also shown that CsA is quite soluble in the melted copolymers (at 60°) and the addition of hot water to the mixture leaded to micelles loading CsA formation. This easy method is favorable for the PEG_{44}-PPS_{10} polymer more than for the other investigated polymers (Table n.2).

Table n.2: Comparison of encapsulation efficiency obtained by both methods for the investigated block-copolymers

POLYMER	PEG_{45}-PPS_{42}	PEG_{45}-PPS_{42}	PEG_{45}-PPS_{42}
METHOD	Enc.Eff.(%)	Enc.Eff.(%)	Enc.Eff.(%)
CH_2Cl_2 evaporation	60.9 ± 4.1	66.0 ± 7.3	63.8 ± 9.2
Hot water suspension	72.7 ± 8.8	56.3 ± 8.1	7.5 ± 2.5

The rate of the release of CsA from the PEG-PPS micelles has been determined in vitro: they can release their entire drug content during 6 days or even slower, depending on the polymers composition (Figure 1).

Figure 1: CsA releasing profile



Conclusions: PEG-PPS micelles provided adequate aqueous CsA levels and showed controlled drug delivery properties in invitro models. Thus, PEG-PPS micelles may have a good chance to stay stable, and hold into their drug content for prolonged periods in blood circulation.