Polymeric micelle functionality assessment using mPEG (2K)-DSPE for poorly soluble peptides
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ABSTRACT SUMMARY
Polymeric micelles offer a promising approach for solubilizing poorly water soluble drugs, bypass uptake by reticuloendothelial system, and facilitate tumor targeting. Our research effort involved a mechanistic evaluation of the physical and biological implications of a prototype polymeric micelle carrier (1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (MPEG2K-DSPE). This study demonstrated the concentration dependent formation of nano self-assembly of polymeric micelles for solubilization, assessed impact of biopolymers on the competitive binding interaction with the polymeric micelle carrier and the drug load, evaluated effect of ionic strength and the reaction temperature. These physico-chemical properties were evaluated using fluorescence spectroscopy, dynamic laser light scattering, and NMR. Pyrene was used initially as a probe molecule to understand the behavior of MPEG(2K)-DSPE from polymeric micelle point of view. The net outcome on the solubilization of poorly soluble drug Cyclosporine and cell penetrating peptides RO5493562 and RO5512168 were explored to assess solubility enhancement.

INTRODUCTION
Polymeric micelles belong to a group of nano-carriers well suitable for solubilizing hydrophobic drugs and targeted delivery. These polymeric micelles demonstrate better physico-chemical stability. They self-assemble at a very low critical micelle concentration resulting in nanoparticles facilitating delivery to tumor targets. The use of polymer in the micelle forming molecule is important, as this part of the molecule is exposed on the surface after the self-assembly to form a hydrophilic shell. This protects the particle and associated content, due to steric hindrance. The hydrophilic shell is also involved in interaction with plasma proteins and cell membranes. In synergism with the hydrophilic surface, the inner hydrophobic core is responsible for the stability of the micelle and the hydrophobic drug solubilization capacity. Presented here are experiments performed with pyrene as a model probe compound addressing stability of the micelle under different salt, protein and temperature conditions including binding studies confirming the drugs hydrophobic binding site. Subsequently, the solubility of three representative poorly water soluble peptides i.e. cyclosporine, RO5493562 and RO5512168 were investigated using polymeric micelles

EXPERIMENTAL METHODS
Preparation of micelles: Micelles were prepared by dissolving mPEG2K-DSPE (Corden Pharma, Switzerland) in 10 mM histidine buffered saline pH 7.4 or H2O at a concentration of 50 to 100 mg/ml. the solution was filtered through a 0.22 μm Millex GV PVDF filter (Millipore, Billerica, MA)

Micelle characterization studies
1 mg of dry pyrene (Acros Organics, NJ) crystals in screw capped vials was prepared by drying aliquots of pyrene under nitrogen from a stock solution (10 mg/mL) in methylene chloride. mPEG2K-DSPE was diluted between 0.0325 and 15 mg/ml, and 1 ml of each dilution was added to a pyrene containing vial. Each experiment was either performed with naive micelles or with supplemented buffer with or without other biopolymers. The samples were shaken overnight in darkness. The samples were then consequently filtered through a 0.22 μm PVDF filter to remove any undissolved pyrene. These samples were analyzed for Fluorescence using a Spectramax M5 fluorometer (Molecular Devices, Sunnyvale, CA) from two hundred μl of each filtered solution. Excitation was set at 393 nm and emission wavelength at 390 nm. Critical mPEG2K-DSPE concentration inducing micelle formation was determined based on fluorescence intensity.

Micelle loading capacity
Micelle stock solution of 50 mg/ml mPEG2K-DSPE was incubated with 1mg of dry pyrene overnight as described above. Determination of pyrene loading was determined fluorometrically. Samples were diluted 200 and 400 times in 5 % SDS and measured against a standard curve of 0.08 to 2.5 mg/ml pyrene dissolved in 5 % SDS. mPEG2K-DSPE content was determined by ICP-OES phosphorous measurement without sample digestion.

NMR
Micelle stock solution of 50 mg/ml mPEG2K-DSPE was incubated with 1mg of dry pyrene overnight as described above. Controls were prepared the same way, but without the presence of pyrene. The filtered
solution was directly measured without any modifications on a 400 MHz Ultrashield NMR system (Bruker, MA).

RESULTS AND DISCUSSION

**Figure 1**: Effect of temperature on micelle formation

**Figure 2**: Effect of Sodium Chloride concentration on micelle formation

**Figure 3**: Effect of Human Serum Albumin concentration on micelle formation

**Figure 4**: Effect of Transferrin concentration on micelle formation

**Figure 5**: NMR profile for polymeric micelle entrapping probe compound pyrene

CONCLUSION

mPEG(2K)-DSPE offers better solubilization capacity and a viable option for poorly water soluble peptides. Our efforts to solubilize two representative poorly water soluble peptides yielded an enhanced solubility of Cyclosporine at 5-10 mg/mL and for the cell penetrating peptides RO5493562 and RO5512168 the solubility >4 mg/mL was achieved, at neutral pH conditions. The choice for using mPEG (2K)-DSPE was appropriate especially considering its interactions with competing biopolymers in in-vivo circulation such as albumin and transferrin which did not impact the CMC over the ranges typically observed in blood. It was noted that the ionic strength did not have an impact on the CMC as well. The drug/probe compound confirmed localization of the hydrophobic molecule into the inner core as shown by NMR. The micellization behavior as a function of temperature indicates that the rate of demicellization is higher at a higher temperature thus implicating potential for drug product stability risk at higher storage temperature.