# Tuning Polymeric Micelle Stability with Selective Polyethylene Glycol Density

Jennifer Logie[1,2], Christopher K. McLaughlin[1], Shawn C. Owen[1,2], Molly S. Shoichet[1,2,3].

1. Chemical Engineering and Applied Chemistry, 2. Institute of Biomaterials and Biomedical Engineering, 3. Department of Chemistry, University of Toronto 160 College St, Rm 514, Toronto, Ontario, M5S 3E1

**Statement of Purpose:** Polymeric micelles continue to be a viable strategy for the encapsulation and delivery of hydrophobic chemotherapeutics. Polymers used are typically comprised of a hydrophobic, biocompatable block which makes up the core of the micelle and a poly(ethylene glycol) (PEG) hydrophillic corona[1]. PEG has been shown to be biodegradable, prevent premature clearance by the mononuclear phagocyte system, and limit protein adsorption and opsonization of nanoparticles when used as a surface coating[2]. While these systems are beginning to show clinical promise, many formulations require complex preparations involving excipients due to a lack of particle stability[3].

Here, we show that by varying the PEG graft density on our novel graft copolymer, poly(D,L-lactide-co-2-methyl-2-carboxytrimethylenecarbonate)-g-poly(ethylene glycol) (P (TMCC-co-LA)-g-PEG), we can tune stability and improve handling of our particle system. Increasing coronal PEG density helps to prevent particle aggregation and dissociation under both serum-containing conditions and through the freeze-drying process.

**Methods:** P(TMCC-co-LA) was synthesized as previously described[4]. PEG was grafted onto the polymeric backbone using peptide coupling chemistry with diisopropylcarbodiimide (DIC) and hydroxybenzotriazole (HOBt). Varying the equivalents of PEG achieved densities averaging between 0.5 and 6 PEG chains/backbone. Polymers were characterized by $^1$H NMR and GPC. The thermodynamic stability of particles was assessed using the standard pyrene procedure to determine their critical micelle concentration. Polymeric micelles were synthesized using a dialysis self-assembly procedure. Micelle size, polydispersity and zeta potential was determined using a Malvern Zetasizer Nano ZS. The cytotoxicity of polymers was evaluated using an LDH assay on MDA-MB-231 cells after incubation with micelles and a hemolysis assay on red blood cells. Stability of particles through freeze-drying was assessed by micelle characterization after resuspension with and without the use of stabilizing excipients. Kinetic stability in 20% Fetal Bovine Serum (FBS) was determined using size exclusion chromatography (SEC) of particles incubated at various time points- 0, 6, 24, 48 and 72 h. Micelle peak areas were monitored over time and then integrated to quantify dissociation.

**Results:** High PEG density formulations did not require the use of excipients to prevent aggregation and stabilize them through the freeze-drying process. The more extended, brush-like conformation of high PEG density particles increases the polymer-induced forces at the particle surface, sterically stabilizing particles. Particle characterization revealed formulations with low polydispersity and an increase in size with increasing PEG density, confirmed by both TEM and DLS. Zeta potentials became increasingly neutral with higher PEG densities, potentially providing benefits in *in vivo* by preventing immune response associated with negatively charged species. All formulations showed no cytotoxicity and did not cause hemolysis.

Interestingly, the thermodynamic stability remained constant among formulations, with critical micelle concentrations around 0.5 μM. The low CMCs of all formulations provides evidence that the change in ratio between hydrophobic and hydrophilic components does not influence the thermodynamic stability of the micelle, and confirms that micelles are not as susceptible to dilution upon intravenous injection.

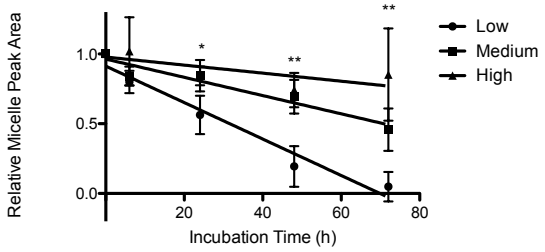


**Figure 1.** Change in peak area of different PEG-density micelles as a function of incubation time. Decrease in peak area indicates micelle dissociation (n=4, mean ± standard deviation, * $p<0.05$, ** $p<0.01$ by one-way ANOVA followed by Bonferroni post-hoc test)

Serum stability revealed exceptional kinetic stability, with high PEG density particles showing almost no destabilization over a 72 h incubation in serum as shown in Figure 1. Medium and low PEG density particles had half lives of 71±12h and 32±5 h respectively. Drug loading studies of docetaxel showed that only the hydrophobic block determined loadings (~10%), and increasing the PEG density did not significantly change the drug loading with respect to the hydrophobic block.

**Conclusions:** Stability studies showed that increasing PEG density improved kinetic stability, prevented aggregation upon dehydration and maintained good thermodynamic stability. Future work will include further modifications to the hydrophobic core to increase drug loading and coupling of targeting ligands to the corona.

**Acknowledgements:** The authors are grateful to the Natural Science and Engineering Research Council of Canada (NSERC) and the Canadian Institute for Health Research (CIHR) for funding.

**References: 1.** Wang et al. Annu Rev Med. 2012:63:185-198. **2.** Walkey et al. J Am Chem Soc. 2012:134:2139-2147. **3.** D'Addio et al. Adv Drug Deliver Rev. 2011:63:417-426. **4.** Lu et al. Macromolecules. 2010:43: 4943-4953