

## Preparation of Microgels Using Thiol-Ene “Click” Chemistry

Andrew K. Fraser, Han Shih, Chien-Chi Lin.

Department of Biomedical Engineering, Purdue School of Engineering and Technology,  
Indiana University-Purdue University at Indianapolis, Indianapolis, IN, USA

**Statement of Purpose:** Local delivery of bioactive molecules with tunable release rates is an attractive approach in disease treatment and tissue engineering applications. One way of achieving this task is through encapsulating drugs in biodegradable polymeric matrices that produce sustained, uniform, and targeted release.<sup>[1]</sup> The objective of this study is to develop a new photochemistry for synthesizing microgels. Our laboratory has recently developed a visible light-mediated thiol-ene photo-click chemistry for forming step-growth hydrogels. Gelation is achieved in seconds to minutes using macromer poly(ethylene glycol) norbornene (PEGNB), crosslinker dithiothreitol (DTT), and photoinitiator eosin-Y.<sup>(1)</sup> In this work, we aim to capitalize on this innovation by synthesizing step-growth microgels without using cytotoxic solvent or surfactant.

**Methods:** Step-growth thiol-ene microgels were synthesized using water-in-oil emulsion. The aqueous macromer solution was prepared from 10 kDa PEG-tetra-norbornene (PEG4NB), cross-linker DTT (40mM), and a non-cleavage type photoinitiator eosin-Y (0.1mM). Hexane or mineral oil was used as the organic phase. Two surfactant systems including Tween80/Span80 and Pluronic-F68 were used to evaluate their effect on microgel size. To synthesize the microgels, 100  $\mu$ l of macromer solution was added to a test tube containing 1.0 ml of organic phase. After 20 seconds vortexing to suspend the macromer solution into micro-droplets, the mixture was immediately exposed to visible light ( $\lambda=400\sim700$  nm) for 2.0 minutes to initiate polymerization. Following polymerization, the microgels were washed twice in isopropanol and three times in water. Gel diameters were characterized by image analysis. Additional reaction conditions (i.e., surfactant concentrations, mixing times, polymer concentrations, and polymer molecular weights) were tested to optimize the mineral oil method. Macromer solution viscosity was measured by a viscometer.

**Results:** With hexane as an organic phase and Tween80/Span80 as surfactants, microgels were formed with a mean diameter of 69.9  $\mu$ m (Figure 1). Without Tween/Span in the hexane, a stable suspension of macromer droplets did not form and only bulk gels were formed following photopolymerization. In the mineral oil system without using surfactant, larger microgels were formed (average diameter  $\sim 122.0$   $\mu$ m). However, the average diameter of the microgels decreased to 55.5  $\mu$ m and 27.0  $\mu$ m with the addition of Tween/Span or Pluronic, respectively (Figure 1). In addition to the significant size variations, we also found that microgels formed in hexane contained droplets of the organic solvent suspended in the gel network (Figure 2A). These trapped solvent droplets diffused out of the microgels (97%) after 24 hours. Interestingly, we did not find droplets of organic phase trapped in the microgels when mineral oil was used as an

organic phase (Figure 2B). We also characterized the effect of emulsion conditions on microgel size in the mineral oil/pluronic system. Although increasing mixing time had little effect on the average gel diameter, it did decrease the polydispersity of microgel diameter. On the other hand, increasing surfactant concentration caused an exponential decay in microgel size by reducing surface energy to stabilize droplets (Figure 2C). The composition of the macromer solution also effects particle diameter. Increasing either the concentration or molecular weight of the PEG4NB polymer caused an increase in average gel size. Figure 2D illustrates that this increase in size directly correlates to solution viscosity.

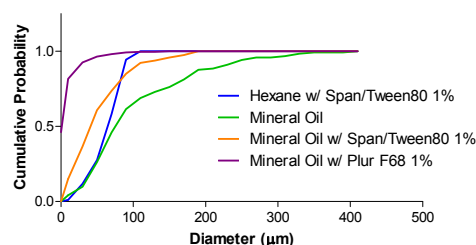


Figure 1: Microgel diameter distribution using different emulsion methods.

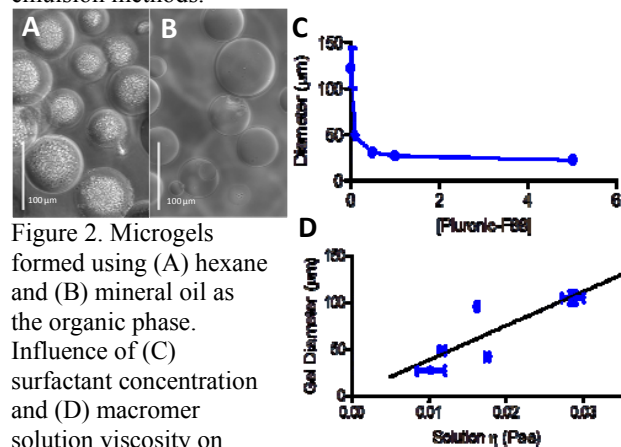


Figure 2. Microgels formed using (A) hexane and (B) mineral oil as the organic phase. Influence of (C) surfactant concentration and (D) macromer solution viscosity on particle size.

**Conclusions:** We have successfully synthesized step-growth microgels using a visible-light mediated thiol-ene photo-click reaction. Comparison of reaction conditions revealed that mineral oil with Pluronic-F68 surfactant is a better synthesis method because it produced smaller, more uniform microgels without using toxic components. Adjusting mixing time, surfactant concentration, and macromer solution viscosity significantly affected the particle size. Ongoing work is focused on synthesizing multilayer microgels using interfacial thiol-ene photo-click reactions.

**References:** [1] Tibbitt MW, et al. *J Biomed Mater Res A*. 2012;100(7):1647-54. [2] Shih H, Lin C.C. *Biomacromolecules*. 2012;13(7):2003-2012.