Enzymatically-degradable, sulfated poly(N-isopropylacrylamide) nanoparticles for drug delivery applications

Rush L. Bartlett II, Mathew R. Medow, Brandon L. Seal.
Purdue University, West Lafayette Indiana 47907

Statement of Purpose: Thermoresponsive N-isopropylacrylamide nanoparticles have been well characterized for biotechnology applications[1]. However, the limited ability of these particles to efficiently and selectively release drugs at specific locations has hampered their usefulness. This study characterizes a range of reaction conditions for optimal drug delivery in physiological electrolyte environments at physiologically relevant temperatures. These nanoparticles also exhibit no effect on hemocompatibility or coagulation at tested concentrations. Also, to our knowledge, we have demonstrated the first documented case to crosslink small peptide chains into N-isopropylacrylamide nanoparticles during precipitation polymerization. Enzymatic degradability of the particles will facilitate local release of cationic therapeutics.

Methods: A standard polyNIPAm protocol was used to produce control poly(NIPAM-BIS) and poly(NIPAM-AMPS-BIS) nanoparticles[2]. N-isopropylacrylamide (NIPAm), N,N'-methylenebis(acrylamide) (BIS), 10% sodium dodecyl sulfate water solution (SDS), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), potassium persulfate, and several peptide sequences were purchased or synthesized to make the nanoparticles in aqueous free radical precipitation polymerization. Peptides were functionalized with acryloyl chloride chemistry prior to incorporation into the nanoparticles to facilitate crosslinking. Samples were purified against pure water in a Spectra/Por 7 dialysis membrane, MWCO 15,000. ζ potentials and sizes (dynamic light scattering) were measured by a Nano-ZS90 nanoseries Zetasizer in folded capillary cells. Peptide purity and functionalization was validated by maldi mass spectrometry and HPLC. Colloidal stability was determined with a SpecraMax M5 plate reader. Particles morphologies were verified on a FEI/Philips CM-100 Transmission Electron Microscope at 100Kv using uranyl acetate stain at pH 4.5. Hemocompatability was determined measuring clotting time and hemolysis of citrated whole bovine blood.

Results: Characterization of poly(NIPAm-BIS-AMPS) revealed hydrodynamic radii ranging from 100 to 500 nm depending on temperature and incorporation of AMPS as indicated in Figure 1.

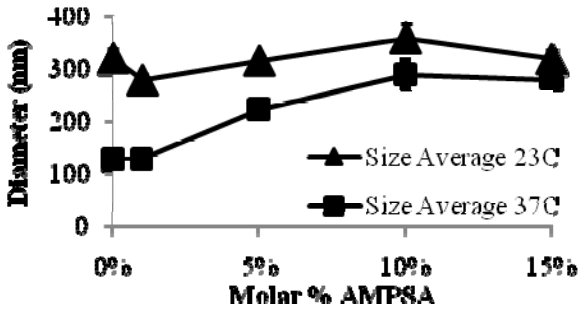

Figure 1. Hydrodynamic diameter of poly(NIPAm-BIS-AMPS) measured by Dynamic Light Scattering

Although particles increased in size with higher AMPS incorporated, they still exhibited standard spherical morphologies as indicated by TEM in Figure 2.

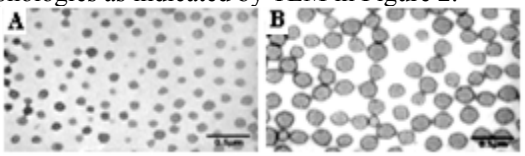

Figure 2. TEM images at 28.5K magnification of p(NIPAm-BIS) without AMPS(A) and with 10% AMPS(B)

Also noted was that the aspect swelling ratio of these particles did not change by varying reaction condition surfactant amounts but was reduced with increasing AMPS incorporation. Of special *in vivo* significance, particles containing AMPS had no statistically significant interaction with blood as measured by hemolysis and coagulation assay when dosed at 1 mg particles per 1 ml blood. It was observed that poly(NIPAm-BIS) particles aggregated when maintained at physiological temperature and ionic strength In contrast, the addition of 2.5% to 10% AMPS drastically increased the colloidal stability of these particles under physiological conditions as verified by spectrophotometry and increased ζ potentials.

After characterization of poly(NIPAm-BIS-AMPS), our research has moved to develop a selectively degradable poly(NIPAm-Peptide-AMPS) nanoparticle for enhanced local drug delivery via enzymatic degradation of the particles. Incorporation has been indicated by the amino acid UV sensitive fluorescence marker dansyl glycine and is shown in Figure 3.

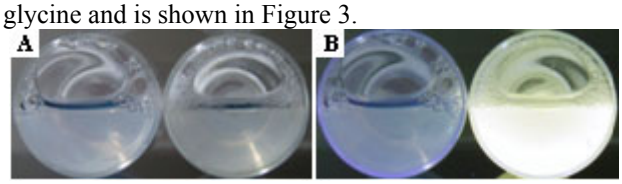

Figure 3. Visible (A) and UV (B) excitation showing presence of peptide in nanoparticle crosslinks (vial on the right).

We have shown that acryloyl chloride modified peptide chains can be incorporated into the reaction complex to form nanoparticles with diameter of 300-400 nm in at 23ºC. These particles exhibit thermal properties similar to particles crosslinked with BIS. Additional research will investigate how the charge, length, and composition of peptide crosslinkers affect particle properties.

Conclusions: We have characterized several properties of poly(NIPAM-BIS-AMPS) nanoparticles and have shown hemocompability when using various amounts of AMPS in the polymerization feed. We have also demonstrated the ability to modify and incorporate peptide chains as crosslinks within the poly(NIPAM-AMPS) system. Future work will focus on testing the selectivity of enzymaticaly degradable crosslinks for site specific drug delivery *in vitro* and *in vivo*.

References: 1.(Lyon L.A. Chem Soc Rev. 2009;38:865-874.) **2.**(Hu ZB. Ange Chem Int Ed. 2003;42:4799-4802.)