Surface hydrolysis mediated PEGylation for the passivation of pNIPAM Nanogels

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Statement of Purpose: Since their inception, nanogels of thermosresponsive polymers, such as poly(N Isopropylacrylamide) (pNIPAM), have been investigated for temperature sensitive drug delivery. However, their implementation into intravenous systems is complicated by the body's natural response against foreign bodies. In order to combat this poly(ethylene glycol) (PEG) has proven a potent passivator to extend the circulation half-life in the blood stream by limiting protein adhesion[1]. The issue is that the methods used to this point for the incorporation of PEG into these pNIPAM nanogels have proven to adversely affect the swelling response. This is because the inclusion of PEG comonomers allows it to pervade the system, interfering with the temperature response. In order to limit these effects, PEG needs to be localized to the surface. To do this we turn to Surface Hydrolysis. This technique concentrates reactive carboxyl groups to the surface of acrylamide (AAm) containing nanogels. These carboxyl groups can then be used to attach amine functionalized PEGs with EDC - NHS chemistry.

Methods: Nanogels are synthesized via an emulsion polymerization. In short, NIPAM, AAm and a surfactant (sodium dodecyl sulfate (SDS)) are combined in water with a crosslinker (N'N' methylene bisacrylamide (MBAM)) at 70° C and purged with nitrogen. The reaction is initiated by injecting ammonium persulfate (APS). The resulting suspensions were either purified via dialysis against ultrapure 18.2 megohm water or further manipulated with Surface Hydrolysis. Surface hydrolysis entails raising the temperature of unpurified suspension to 60° C and mixed with an equal volume of 1 N NaOH. The solution is kept at 60° C for 3 days then brought back down to pH of 7 with hydrochloric acid. These solutions are then purified via dialysis. PEGylation was performed with EDC NHS chemistry. In short, dried particles were suspended in borate buffer, pH 8.4, and mixed with 1-Ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) and sulfo-N hydroxysuccinimide(NHS). Amine functionalized PEG is then mixed in and allowed to react overnight. Excess PEG and extraneous salts were then removed via dialysis. Temperature dependence of diameter and zeta potential of all samples were measured with a Malvern Zetasizer nano ZS.

Results: The process of surface hydrolysis takes a previously neutral material and subjects it to harsh conditions, the effects the various steps can be visualized by the measurement of zeta potential. The results from these studies are shown in figure 1. These results show a drastic increase in zeta potential after Surface Hydrolysis and a return to neutral after the subsequent PEGylation for two different feed ratios of AAm to total monomer feeds.

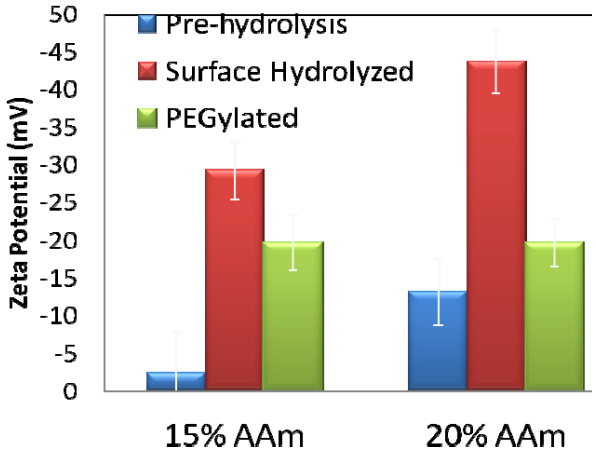

Figure 1. Zeta potentials of surface hydrolysis mediated PEGylation at various steps in the process.

The swelling results of PEGylated compared to untreated 20% AAm p(NIPAM-co-AAm) nanogels are represented in figure 2. There is unnoticeable change to the lower critical solution temperature (LCST) and a positive effect on the overall swelling response.

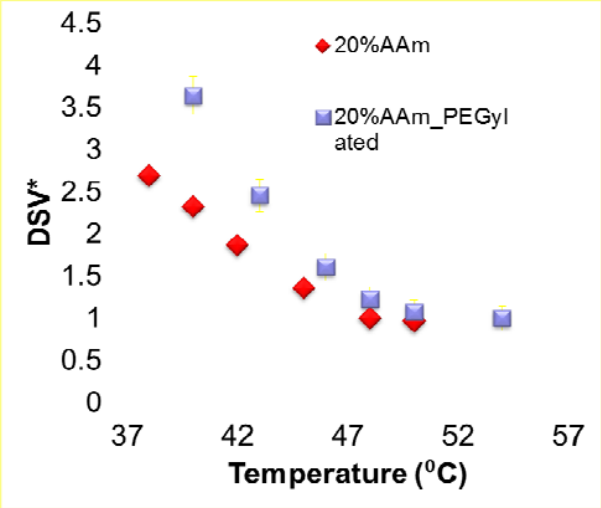

Figure 2. Swelling results of 20% AAm p(NIPAM-co-AAm) nanogels.

Conclusions: The zeta potential results demonstrate the successful surface hydrolysis and PEGylation of p(NIPAM-co-AAm). The swelling results show an increase in overall swelling, however the LCST remains unaffected. The increased swelling being only an impact of the increased hydrophobicity of the carboxyl groups formed during Surface hydrolysis. This has proven to be an effective means to introduce PEG to these neutral systems without greatly affecting their response.

References

1.Gref, R. et al. Colloids and Surfaces B: Biointerfaces 18, 301–313 (2000).

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