THERMALLY RESPONSIVE INTERPENETRATING POLYMER NETWORK NANOPARTICLES

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Introduction

Stimuli-responsive polymer hydrogels which are capable of responding to changes in their environment such as pH (1), osmotic strength (2), or temperature (3) have received growing attention in recent years for applications in a wide variety of areas including controlled drug delivery (4). Also, due to the size limitations imposed by the body's natural defense mechanisms, such as the reticuloendothelial system, polymer systems 0.3 μm in diameter and smaller will be required in order to create effective *in vivo* drug delivery systems (5-7). Under normal conditions these stimuli-responsive particles exist in a collapsed state where diffusion or release of an encapsulated agent is limited or prevented by the small size of openings in the polymer mesh that comprises the nanoparticle. However, upon activation or change in a specific environmental factor, these particles swell, thereby increasing their mesh size and allowing for the release of any encapsulated agents. Due to the highly buffered nature of blood serum, thermally responsive nanoparticles appear to be the most likely candidates for use in injectable systems because this environmental factor is much easier to change and control.

Within the field of thermally responsive hydrogel polymers two general types exist. Those which exhibit a negative or inverse swelling response, which collapse on heating such as poly(N-isopropylacrylamide) particles (8), and those which exhibit a positive swelling response and expand in response to increase in temperature, such as poly(acrylic acid) copolymer particles (9). A further subcategory of positively responsive polymer nanoparticles are those which exhibit a rapid volume transition over a very narrow temperature range usually related to the polymer's upper critical solution temperature. Interpenetrating Polymer Network (IPN) nanoparticles comprised of poly(acrylic acid) (PAA) and polyacrylamide (PAAm) are one of the few systems that exhibit this unique UCST-like behavior. For applications involving traditional controlled drug delivery the UCST-like behavior of PAA/PAAm IPN nanoparticles would be preferred because they can be formulated to remain in a collapsed or "off" state at temperatures at or below the normal body temperature and then rapidly and fully expand into the "on" state with a small increase in temperature above the normal body temperature.

IPN's are comprised of two or more polymer networks that are not chemically crosslinked with one another, but are interpenetrating or physically entangled within one another such that they can not be separated. PAA/PAAm IPN nanoparticles are able to achieve UCST-like swelling behavior due to their unique structure and the presence of secondary hydrogen bonding complexes that develop between the PAA and PAAm networks. At lower temperatures hydrogen bonding forces dominate and maintain the particles in a collapsed state, however as temperature is increased these bonds are weakened and a hydrophilic front is established within the polymer (10). These two effects work synergistically together to rapidly swell the particles leading to the UCST-like behavior that is seen in these materials.

Experimental

Materials

Acrylamide (AAm), acrylic acid (AA), N,N'-methylenebisacrylamide (MBAAm), polyethylene glycol laurylether (Brij 30), and cyclohexane were obtained from Aldrich, ammonium persulfate (APS) was obtained from FisherBiotech, and sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was obtained from Fluka. All were used as received for the preparation of the polyacrylamide and poly(acrylic acid) polymers.

Methods

PAAm/PAA IPN polymer nanoparticles were synthesized by a two stage sequential inverse emulsion polymerization method. This inverse emulsion solution consisted of an 81% cyclohexane continuous phase, with a 13% surfactant phase (AOT and Brij 30 in a 2:1 ratio), and a 6% aqueous phase. In a typical experiment, a 3-neck round bottom flask equipped with a condenser, nitrogen purge line, and overhead mechanical stirrer was first charged with the entire volume of cyclohexane to be used in the polymerization. To this the entire emulsifier phase was added and dissolved under vigorous stirring. For the first stage of the sequential IPN polymerization only half of the total aqueous phase, 11.7 wt% AAm, 4 wt% MBAAm, 5.3 wt% APS, and 79 wt% deionized water, was added. This mixture was then purged with nitrogen gas for 30 minutes to remove oxygen, after which the polymerization was initiated thermally by immersion of the reaction vessel in a 60˚C bath and allowed to react to completion (typically 2 hours). Upon completion of the first stage of the IPN synthesis method, the second stage was then started by adding the other half of the aqueous phase, which consisted of 11.7 wt% AA, 4 wt% MBAAm, 5.3 wt% APS, and 79 wt% deionized water, to the same 3-neck round bottom flask as before. The vessel was again purged with nitrogen gas and allowed to react at 60˚C for two hours, thus resulting in the formation of the final PAAm/PAA IPN nanoparticles.

PAAm and PAA homopolymer nanoparticles and PAAm-co-PAA copolymer nanoparticles were all also made using the same inverse emulsion polymerization system as the IPN particles except that the aqueous phases were combined and added in just one step. In the case of the co-polymer nanoparticles, both aqueous phases (containing both AAm and AA monomers) were polymerized together in one step. In the case of the homopolymer nanoparticles both phases were again combined but only contained AAm monomer in the case of PAAm homopolymer nanoparticles and AA monomer in the case of the PAA nanoparticles.

All four types of nanoparticles where then collected and purified by removal of the cyclohexane phase with elevated temperature and reduced pressure in a rotary evaporator. This was followed by precipitation of the particles out of the emulsifier phase with the addition of excess ethanol and subsequent pelting and washing (3 times) by centrifugation. Finally, the remaining polymer pellet was then resuspended in deionized water for further analysis.

Results

The relative size and morphology of the polymer nanoparticles was examined using a LEO 1530 field emission scanning electron microscope (FE-SEM) operating at 10kV. Particles were prepared for examination in the SEM by lyophilization in a Labconco Freezone 4.5 manifold lyophilizer. After lyophilization, a white powdered material was collected and a small sample of this material was mounted on a strip of double-sided conductive carbon tape that was affixed to an aluminum SEM mount. This mount, along with the polymer nanoparticle powder was then sputter coated for 30 seconds using a Pelco Model 3 sputter-coater with a gold-palladium target at a deposition rate of 10 nm/min. Figure 1 is an image of a batch of PAA/PAAm IPN nanoparticles that was synthesized in this work and

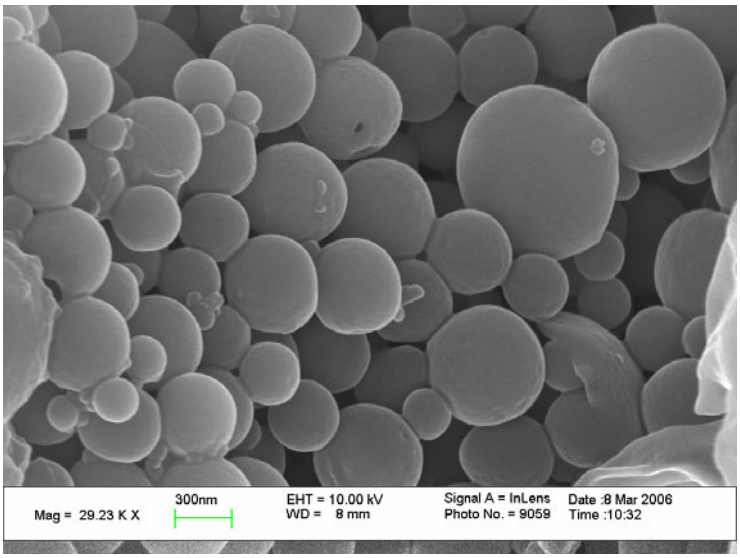

Figure 1. Scanning electron microscopy image of dried and gold-palladium coated PAA/PAAm IPN nanoparticles. Scale bar (green) represents 300nm.

is representative of the typical size and morphology of the various nanoparticles that were prepared using the inverse emulsion system described in this paper.

Dynamic light scattering (DLS) studies of the polymers were conducted across a range of temperatures from 25-55˚C using a Brookhaven ZetaPlus DLS operating at a 90˚ scattering angle with a 635 nm 35 mW diode laser source. Figure 2 illustrates swelling behavior of the four types of polymeric nanoparticles that were examined in this study. The volume swelling ratio was calculated as the volume of the particle in solution at a give temperature (as determined by DLS) divided by the volume of the particle in solution at the initial temperature of 25 ˚C (as determined by DLS). The 50/50 (AA mol% / AAm mol%) IPN exhibited a positive UCST-like swelling response at approximately 40 ± 5 ˚C. Both the poly(acrylic acid) and 50/50 (AA mol% / AAm mol%) PAA-co-PAAm nanoparticle systems exhibit a slight and gradual swelling transition as the temperature was increased from 25 ˚C to 55 ˚C. The polyacrylamide nanoparticle system exhibited little to no change in the volume swelling ratio as a function of temperature.

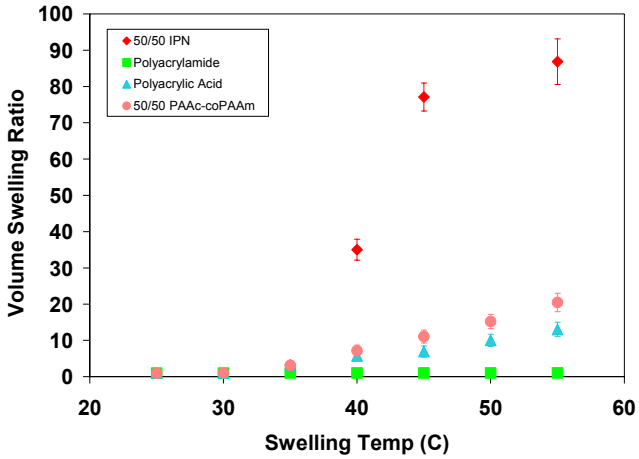

Figure 2. The change in the volume swelling ratio of the various polymeric nanoparticle formulations as a function of temperature. Error bars represent one standard deviation, n=3.

Discussion

This report illustrates the successful synthesis of PAA and PAAm homopolymer, random copolymer, and IPN nanoparticles. SEM was used to confirm that the particles had a spherical morphology with average size in the biologically relevant region of 300 nm diameter and under. Furthermore, dynamic light scattering studies clearly demonstrated the unique UCST-like swelling behavior of IPN nanoparticles. The rapid and large volume swelling ratios that were achieved by the PAA/PAAm IPN nanoparticles was much greater than what was achieved by the component polymers alone (i.e. PAA nanoparticles or PAAm nanoparticles) or in a different polymer structure such as PAA-co-PAAm random copolymer nanoparticles. These results further support the application of IPN nanoparticles in the field of thermally responsive drug delivery systems.

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