Synthesis and Study of and Controlled Release from Nanoparticles and Their Networks Based on Functionalized Hydroxypropylcellulose

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ABSTRACT: The structure of hydroxypropylcellulose (HPC) was modified by covalently attaching either vinyl groups linked by degradable esters or methacrylate groups. The vinyl groups allowed for chemical linking of the HPC chains into nanoparticles through a free radical polymerization process above its low critical solution temperature. The HPC nanoparticles with degradable moieties in aqueous solution were monitored using dynamic light scattering technique as a function of time. The degradation rate increased at either low or high pH values. Residual, un-cross-linked vinyl groups on the surface of the nanoparticles were further linked together, forming a network of nanoparticles. The controlled release of biomolecules from this network was correlated with the primary structure that comprised cross-linked polymer chains in each individual particle and the secondary structure that was a system of cross-linked nanoparticles.

Introduction

Hydroxypropylcellulose (HPC) has undergone intensive study as a result of its unique phase behavior, biocompatibility, and industrial applications.[1−7] The ability to tailor HPC hydrogel structure on nanometer dimensions could have a major impact in the applications of the bulk polymer. HPC polymer chains in water have a low critical solution temperature (LCST) at ∼41 °C.[8] HPC and its derivatives have been cross-linked into bulk a hydrogel with various forms including homogeneous gel, porous gel, and gel beads.[8,9] It was recently reported that HPC chains in water can self-associate at temperatures greater than the LCST and form metastable nanoparticle aggregates.[10,11] Surfactant-free HPC nanoparticles with narrow size distributions can be obtained by cross-linking these aggregates.[10,11]

To date, routes to the formation of cross-linked HPC nanoparticles have employed divinyl sulfone (DVS) as a cross-linker between chains of HPC. This cross-linking reaction requires a very basic environment (pH = 12). This high pH can be detrimental to the incorporation of some active drugs, and the final DVS cross-linked nanoparticles undergo little degradation at physiological conditions. We demonstrate that, by attaching either degradable α-hydroxy ester linked vinyl side groups or methacrylate side groups on the HPC polymer chains, HPC nanoparticles can be synthesized at a neutral pH using conventional free radical initiated polymerization. These nanoparticles can be static in the case of methacrylate cross-linked particles or can degrade into linear polymer if cross-linked with groups containing α-hydroxy ester linkages.

In the case of degradable nanoparticles, it is found that low or high pH values accelerate the degradation of the nanoparticles. Unmodified HPC chains are degradable under certain enzymatic environments.[12] The HPC nanoparticles made in this study can, however, degrade through the action of chemical hydrolysis of the labile moieties. Hydrolytic degradation is preferred for biomedical applications because it is less dependent on the implantation site and the patient.[13] A major advantage of a biodegradable system is the elimination of surgery to remove an implanted delivery device after the delivery system is exhausted.[14,15] Degradable hydrogels are particularly useful for delivering proteins and peptides drugs.[16−21]

The residual vinyl groups on the terminus of attached functional groups on the exterior of the nanoparticles have been further linked together to form the nanoparticle network. We hypothesize that such a system has two-level structural hierarchy: the primary network consisting of cross-linked polymer chains inside each nanoparticle and the secondary network formed of a cross-linked system of the nanoparticles.[22] However, it is very difficult to measure hydrogel materials with electron microscope techniques, since the inherent nanoparticle network structure cannot be preserved during the sample preparation steps by either critical drying or freezing-dry methods. The rapid freezing of the water-swollen gel and the subsequent fast evaporation in a vacuum often leads to the collapse of the pore structure due to ice crystal formation.[23] Here we test this proposed hierarchy structure by the study of loading and release of small and/or very large biomolecules contained within the primary and secondary structures of the HPC nanoparticle networks.

Experimental Section

Materials. Dry hydroxypropylcellulose (HPC) powder (average M_w = 30 kDa) was received from Nippon Soda Co., Ltd., and used as received. Sodium metabisulfite (BIS) and potassium persulfate (KPS) were purchased from Aldrich Chemical Co. and used as received. The substitution level of the HPC polymer for this study was MS = 3.9, where MS was the average number of molecules of propylene oxide combined per anhydroglucose unit. Water for all reactions, solution preparation, and polymer purification was distilled and purified to a

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resistance of 18.2 MΩ using a Millipore system and filtered through a 0.22 μm filter to remove particulate matter.

a. Synthesis of Methacrylated Hydroxypropylcellulose. In a typical synthesis, 5.0 g of hydroxypropylcellulose (MW = 30 kDa) was dissolved into 200 mL of dry *N*,*N*-dimethylacetamide under an argon atmosphere. Next, 1.1 g of methacryloyl chloride was added to the solution. (Note: the water content of the commercially obtained HPC was about 3.5 wt %. Therefore, excess methacryloyl chloride was added to consume the water.) The reaction was allowed to stir for 48 h. After this time, the solution was poured into 1 L of cold diethyl ether to precipitate the polymer. The collected polymer was then reprecipitated three times from 50 mL of hot acetonitrile. The polymer was placed under vacuum to remove excess solvent. ^{1}H NMR was used to measure the amount of methacrylation (usually 2.5−10% of the HPC repeat units) of the polymer by integrating the geminal protons of the methacrylate group (δ (D_2O) 6.1 and 5.6), relative to the methyl protons (δ (D_2O) 1.1) of the isopropyl units on the polymer chain.

b. Synthesis of a Degradable Cross-Linker. In a typical reaction, a 1 mol % excess of hydroxyethyl methacrylate (**1**) was reacted with 2-bromoacetyl bromide (**2**) in chloroform. Excess potassium carbonate was used for base. The reaction proceeded in near quantitative yields in 16 h. δ ($CDCl_3$): 6.15 (1H, s, =C−H), 5.60 (1H, s, =C−H), 4.4 (4H, m, HEMA OCH_2-CH_2O), 4.32 (2H, s, Gly CH_2), 1.97 (3H, s, methacrylate CH_3).

The reaction supernatant was filtered from remaining solids, and solvent was removed under reduced pressure. Without further purification the formed ester product (**3**) was reacted with excess succinic acid and sodium succinate in *N*,*N*-dimethylacetamide at 95 °C for 3 h. The reaction was then cooled to room temperature and poured into cold deionized water. The product (**4**) separated from water and was collected. The product was dissolved in chloroform and dried over $MgSO_4$. Chloroform was removed under reduced pressure to yield a viscous oil with a slight yellow color. δ ($CDCl_3$): 6.15 (1H, s, =C−H), 5.60 (1H, s, =C−H), 4.68 (2H, s, Gly CH_2), 4.43 (4H, m, HEMA OCH_2CH_2O), 2.75 (4H, m, succinate CH_2-CH_2) 1.97 (3H, s, methacrylate CH_3). The general reaction was illustrated in Scheme 2.

c. Synthesis of Degradable Cross-Linker-Functionalized Hydroxypropylcellulose. The general reaction is shown in Scheme 3. Typically, the needed portion of cross-linker needed for a particular functionalization level of the hydroxypropylcellulose was reacted with excess oxalyl chloride in chloroform for 3 h. The ratio of cross-linker to chloroform was about 2 g to 25 mL. After this time the chloroform was removed under reduced pressure to yield the acid chloride derivative of the degradable cross-linker. δ ($CDCl_3$): 6.15 (1H, s, =C−H), 5.60 (1H, s, =C−H), 4.68 (2H, s, Gly CH_2), 4.43 (4H, m, HEMA OCH_2CH_2O), 2.63 (2H, t, succinate CH_2CH_2COCl), 2.41 (2H, t, succinate CH_2CH_2COCl), 1.97 (3H, s, methacrylate CH_3) The acid chloride was then added to a stirring solution of hydroxypropylcellulose in *N*,*N*-dimethylacetamide. The remaining procedure followed that of (a). The level of polymer functionalization was 2.5−10% of the HPC repeat unit.

d. Synthesis of Surfactant-Free Hydroxypropylcellulose Nanoparticles from Methacrylated or Degradable Cross-Linker Attached Hydroxypropylcellulose. 1.33 g of 5% methacrylated or biodegradable cross-linker attached hydroxypropylcellulose (polymer concentration of 0.33 wt %) was dissolved in 400 mL of deionized water. After the polymer had completely dissolved, the solution was purged with argon gas for 20 min. The solution was then warmed to a temperature above the lower critical solution temperature of the solution (45 to 65 °C). Next, 4 mg of sodium metabisulfite and 4 mg of potassium persulfate were added to initiate the reaction. The reaction was stirred at the selected temperature for 20 min. After this time, the polymer was concentrated to about 2.5 wt % by ultracentrifugation and redispersion. The particle size was measured by dynamic light scattering method using a laser light scattering spectrometer (ALV, Germany) equipped with an ALV-5000 digital time correlator.

e. Loading of Fluorescein-Labeled Bovine Serum Albumin into Hydroxypropylcellulose Nanoparticles and Subsequent Nanoparticle Network. Into 30 mL of deionized water, 100 mg of hydroxypropylcellulose functionalized with either degradable cross-linker or methacrylate side groups was dissolved. The reaction was purged for 20 min with inert gas. Next, fluorescein-labeled bovine serum albumin was added to the solution so that the total protein added was 5% w/w relative to hydroxypropylcellulose. The solution was warmed to above the lower critical solution temperature of the solution (45 to 65 °C). Sodium metabisulfite and potassium persulfate were added to the solution (0.2−0.4 wt % relative to polymer). The reaction was allowed to proceed for 20 min and then cooled to room temperature. About 1.5 mg of sodium metabisulfite and 1.5 mg of potassium persulfate were added to the suspension. The suspension was agitated to dissolve the initiator and accelerator. The sample was then centrifuged at 25 000 rpm for 30 min using a Beckman LE-80 ultracentrifuge. The formed plug at the bottom of the tube was allowed to sit overnight before removal. UV−vis analysis of the supernatant allowed for the determination of the amount of BSA loaded into the nanoparticles. The supernatant was free of color by visual inspection, indicating that virtually all of the BSA was entrapped inside the formed nanoparticles. The formed networks were carefully removed as one piece and studied according to procedure g.

f. Preparation of Hydroxypropylcellulose Nanoparticle Networks Containing FITC BSA in the Secondary Network Space. Nanoparticles formed using the nonsurfactant method containing either degradable cross-linker side groups or the methacrylate side groups had residual polymerizable groups. These groups were used to form the networks. Typically, 15 g of a suspension of nanoparticles in water (∼2.5 wt %) was weighed into a 25 mL ultracentrifuge tube. The suspension was purged with an inert gas for 5 min. Next, about 18 mg of the BSA was added to the suspension, and the suspension was agitated to dissolve the BSA. About 1.5 mg of sodium metabisulfite and 1.5 mg of potassium persulfate were added to the suspension. The suspension was agitated to dissolve the initiator and accelerator. The sample was then centrifuged at 25 000 rpm for 30 min using a Beckman LE-80 ultracentrifuge. The formed plug at the bottom of the tube was allowed to sit overnight before removal. UV−vis analysis of the supernatant allowed for the determination of the amount of BSA loaded into the network. For all cases, approximately 1.15 mg of BSA was loaded into networks containing 375 mg of dry mass of nanoparticles.

g. Release Study of BSA from Hydroxypropylcellulose Nanoparticles Networks. Typically, a mass of fully hydrated network (100−375 mg dry mass) of known BSA content was rinsed 5 times with 100 mL portions of phosphate buffered saline (pH 7.4) warmed to 37 °C. This removed any surface BSA present on the network sample. The sample was then placed into a 20 mL scintillation vial containing 10 mL of phosphate buffered saline. This sample was incubated at 37 °C for the duration of the study. Aliquots were removed periodically for UV−vis analysis. All aliquots were returned to the sample container after analysis.

h. Controlled Release of Small and Large Biomolecules from HPC Nanoparticles Network at the Same Time. 5% methacrylated HPC polymer and 8 mg of sodium metabisulfate as initiator were added into 135 mL of water under nitrogen gas. At 43.5 °C, just above the HPC phase transition, 8 mg of potassium persulfate (KPS) was added. After 1 min, 15 mL of bromocresol green (BCG) solution (10 ppm) was added. The reaction was carried on for 30 min. The HPC nanoparticles were formed with BCG entrapped. The colloidal dispersion was ultracentrifuged at 30 000 rpm for 40 min to collect nanoparticles.

In the second step, the nanoparticles were redispersed and mixed with 25 mL of water, 10 g of 50 ppm BSA, 5 mg of sodium metabisulfate, and 5 mg of KPS. The dispersion was ultracentrifuged at 30 000 rpm for 40 min and formed a plug of nanoparticles at the bottom of the centrifuge tube. After 18

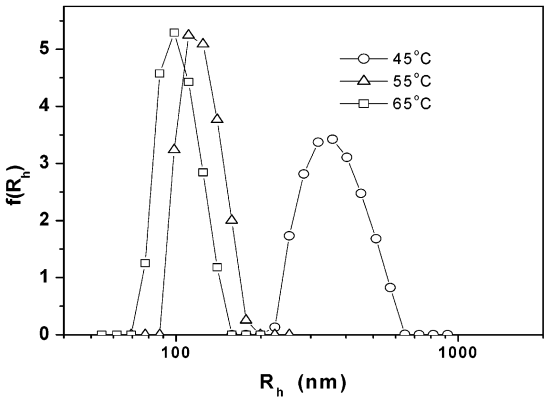
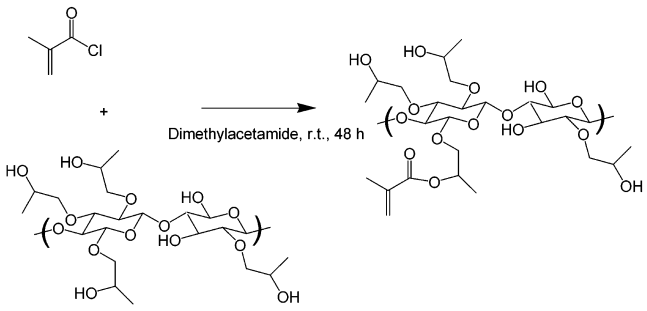


Figure 1. Distributions of hydrodynamic radius of HPC nanoparticles in water prepared using methacrylated HPC at reaction temperature of 45, 55, and 65 °C. The light scattering angle is 60°.

Scheme 1



h at room temperature, the nanoparticle network was formed with the BSA entrapped between the particles.

The HPC nanoparticles networks containing BCG within the nanoparticles and BSA between particles were immersed in a PBS buffer solution with pH = 7.4 at 37 °C. The time-dependent drug release was monitored by a UV/vis spectroscopy. The characterization absorptions for the BSA and the BCG are at 496 and 620 nm, respectively.

Results and Discussion

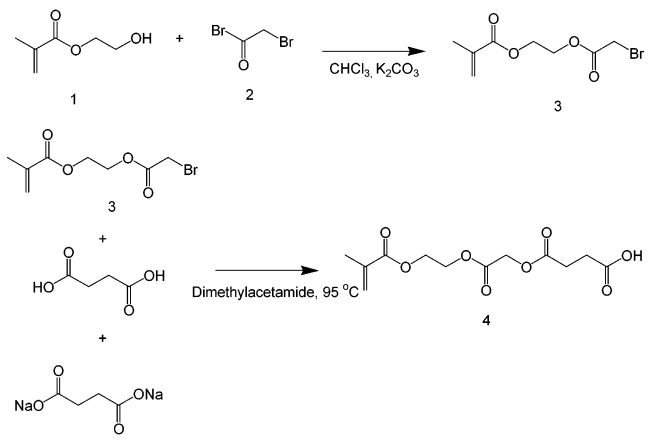
1. Synthesis of Nanoparticles Based on HPC Modified with Methyl Acrylate Moieties. The HPC chain structure is modified by attaching methacrylate moieties as side groups. Scheme 1 shows the general synthetic outline for this modification. This allows for chemical cross-linking of the nanoparticles through a free radical polymerization process.

An aqueous solution of methacrylated hydroxypropylcellulose (polymer concentration of 0.33 wt %) is prepared without surfactant. As the solution temperature is raised above the LCST, individual HPC chains aggregate into nanoparticles. Addition of potassium persulfate initiates radical polymerization of methacrylate side groups of the modified HPC, resulting in nonreversible nanoparticle formation. The formed nanoparticles are easily collected by ultracentrifugation.

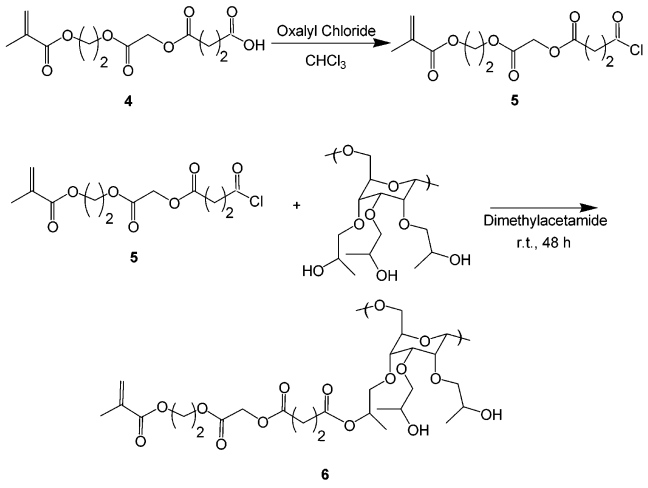
Figure 1 shows plots of three distributions of nanoparticle sizes from three different nanoparticle populations. These three samples were prepared at three different temperatures. The data clearly indicate that lower temperatures lead to larger average particle sizes. Therefore, simply raising or lowering the temperature allows for tailoring of HPC nanoparticle sizes when using this strategy.

2. Degradable HPC Nanoparticles. The degradable HPC nanoparticles are prepared using degradable

Scheme 2



Scheme 3



polymerizable side groups. Scheme 2 shows the general synthetic outline of the degradable side group possessing the proper functionality for HPC modification. The hydrolytic susceptibility of the α-hydroxy ester is far greater than those of normal esters at physiological pH. Hence, their utility in controlled release applications of various pharmaceuticals is expected.[24]

Scheme 3 illustrates the synthesis of modified HPC polymer with polymerizable groups that contain degradable, α-hydroxy ester linkages. HPC modified in this way can also be used to prepare nanoparticles without the need for surfactant. The methods are identical to those used to prepare nondegradable HPC nanoparticles, and the nanoparticles also show similar trends between nanoparticle size and temperature of synthesis.

The typical degradable characteristics of these nanoparticles in pH = 1 aqueous solution at 37 °C are illustrated in Figure 2a using a dynamic light scattering technique. There is a general increase of the particle size and the broadening of the particle size distributions. Since the swelling capacity of a gel nanoparticle is dependent on cross-linking density (i.e., as cross-linking density decreases, swelling capacity increases), this average size increase is expected. The increase in volume of the nanoparticles corresponds with the hydrolysis of cross-linkers within the gel, thus reducing the cross-links over time. This should have valuable impact on the application of controlled delivery for the device.

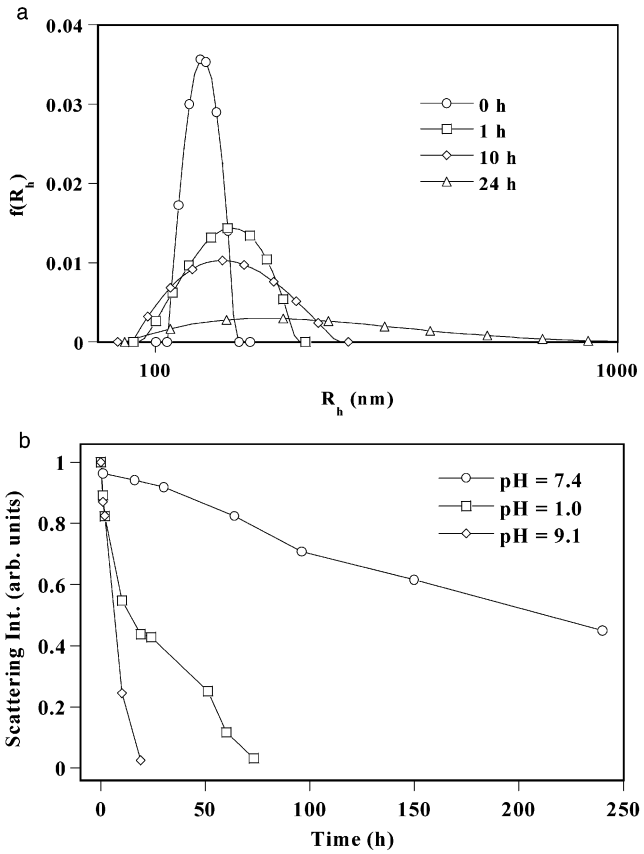


Figure 2. (a) Degradation of HPC nanoparticles in pH = 1 aqueous solution at 37 °C. The particles were prepared using HPC with degradable cross-linker side groups. The scattering angle is 60°. (b) The normalized light scattering intensity $[R_{vv}(q)]_t/[R_{vv}(q)]_0$ decreases as a function of time for degradable HPC nanoparticles at 37 °C in aqueous solutions of pH = 1, 7.4, and 9.

Figure 2b shows that the normalized light scattering intensity $[R_{vv}(q)]_t/[R_{vv}(q)]_0$ decreases as a function of time for all samples at various pH values. It is known that $R_{vv}(q) \rightarrow CM_w$ as $C \rightarrow 0$ and $q \rightarrow 0$, where C is the particle concentration and M_w is molecular weight of the particle.[25] As shown in Figure 2a, the average particle size becomes larger due to the decrease of degradable cross-linkers. The decrease of the scattering intensity is due to the decrease of the number of particles in water. The data in Figure 2b demonstrate that either low or high pH can accelerate the degradation of the nanoparticles. The degradation rate of the nanoparticles at pH 9 or pH 1 is much faster than at pH 7.4, similar to previous results of degradable bulk hydrogels.[20] This can be understood by considering that the α-hydroxy ester is much more stable at a neutral pH than at acidic or basic environment.

3. Synthesis and Study of HPC Nanoparticle Networks. Nanoparticle networks have been synthesized from nanoparticles prepared from the surfactant-free method. Residual methacrylate or degradable groups are present both within and on the exterior of HPC nanoparticles. As the nanoparticles are collected by ultracentrifugation, potassium persulfate in addition to sodium metabisulfite is added. Hence, the residual methacrylate or degradable groups on the exterior of the nanoparticles are linked together to form the secondary structure of the network.

Building Nanoparticle Networks with Different Sized Particles. An important characteristic of these nanoparticle networks is that the interstitial space between particles (mesh size) within the network can be adjusted by changing the particle size.[21] The mesh size of the network decreases as the particle size decreases for closely packed polymer nanoparticle networks. This property can be used to tune the release rate of chemical entities from the network. Figure 3a shows plots for the release of fluorescein-labeled (FITC) bovine serum albumin (BSA) from HPC nanoparticle networks of different particle size compositions. All three samples show a "burst" release in the first 5 h of release. However, for the network with the smallest size particles (48 nm) the "burst" was significantly lessened. Although the complete release of BSA was not seen in

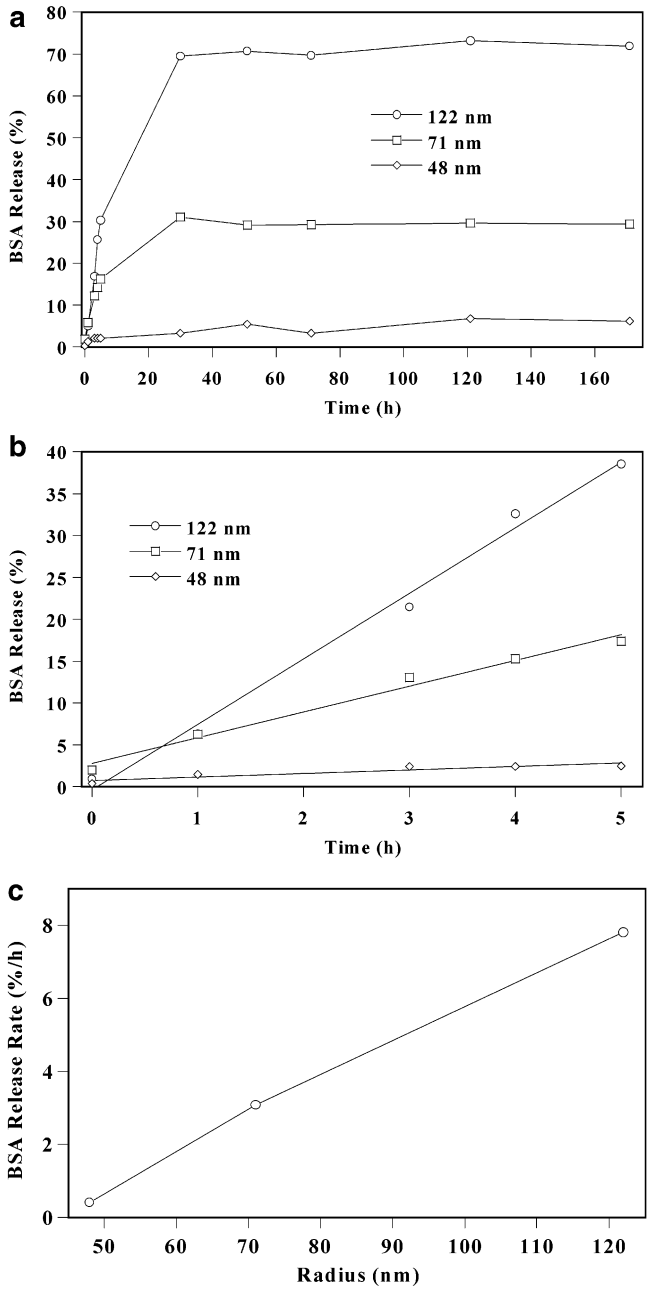


Figure 3. (a) Release of FITC-labeled BSA from HCP nanoparticle networks using with varying particle size at pH = 7.4 and 37 ° C. All networks are of the same size containing 375 mg dry mass of HPC. (b) Initial rates or "burst" release of FITC-labeled BSA from HPC nanoparticle networks with varying particle size at pH = 7.4 at 37 °C. (c) Initial rates of release of FITC-labeled BSA from HPC nanoparticle networks vs particle size.

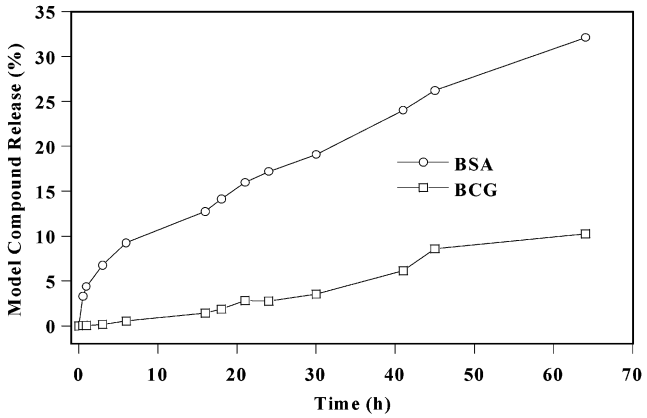


Figure 4. Time-dependent drug release from HPC nanoparticle network made with methacrylated HPC at reaction temperature of 43.5 °C was monitored by UV/vis spectroscopy. The HPC nanoparticles network that contains bromocresol green (BCG) in its naoparticles and bovine serum albumin (BSA) between particles was immersed in a PBS buffer solution with pH = 7.4 at 37 °C. The characterization absorptions for the BSA and the BCG were at 496 and 620 nm, respectively.

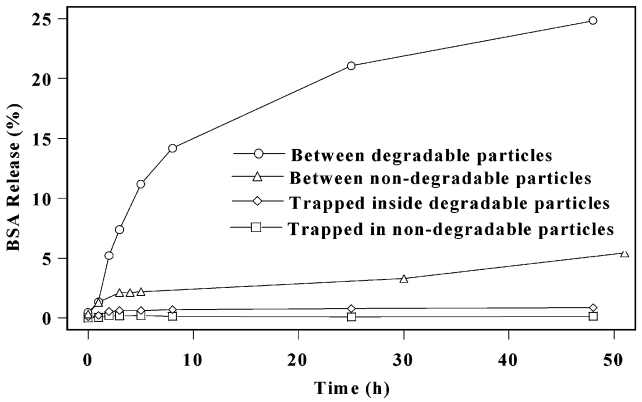


Figure 5. Plot showing release profiles of BSA from (a) between particles of a degradable HPC nanoparticle network (particle size = 54 nm, total expected release = 172 ppm, network dry mass = 375 mg) and (b) between particles of a nondegradable HPC nanoparticle network (particle size = 45 nm, total expected release = 153 ppm, network dry mass = 375 mg). (c) Release of BSA from within particles of a nondegradable HPC nanoparticle network (particle size = 57 nm, total expected release = 1875 ppm, network dry mass = 100 mg). (d) Release of BSA from within particles of a degradable HPC nanoparticle network (particle size = 62 nm, total expected release = 1875 ppm, network dry mass = 100 mg).

any of the samples, the amount of BSA released clearly depends on the particle size contained in the network.

Figure 3b shows the "burst" release profiles of each of the samples in Figure 3a. This suggests a relationship between particle size and the initial rate of release of BSA. Figure 3c is a plot of the slopes of each of the plots in Figure 3b as a function of particle size. It is noted that more data in the initial release stage could be measured to improve the statistical analysis of the elution rates. These data suggest that diffusion of BSA within the network can be controlled through tailoring of the network mesh size by manipulating the nanoparticle size.

Primary and Secondary Networks. It is hypothesized that the polymer gel nanoparticle network has two levels of structural difference: the primary network consisting of cross-linked polymer chains inside each nanoparticle and the secondary network formed of a cross-linked system of the nanoparticles. The mesh size of the primary network can be much smaller than that of the secondary network. This hypothesis can be tested by the study of the controlled release of small and large molecules from the primary and the secondary network simultaneously. As a demonstration, a HPC nanoparticle network was formed with a small molecule (bromocresol green) (BCG) entrapped within the particles and a large chemical entity (BSA) entrapped between the particles.

This nanoparticle network was then immersed in a PBS buffer solution at pH = 7.4 and at 37 °C. The time-dependent drug release was monitored by a UV−vis spectroscopy. The characteristic absorptions for the BSA and the BCG were at 496 and 620 nm, respectively. As shown in Figure 4, the HPC nanoparticle network can simultaneously release a small molecule (BCG) and a large molecule (BSA). These data show a sharp departure from normal diffusion kinetics seen normally with bulk gels. BSA's release from the network is almost an order of magnitude greater than that of BCG, though the size of BCG is over 2 orders of magnitude smaller. The results show that contained within the same network are two distinct release sites operating under two distinct mechanisms. This demonstrates not only the two-level structure in the nanoparticle network but also the potential versatility of the nanoparticle networks in the arena of controlled delivery.

Nanoparticle Networks with Degradable Moieties. Chemical entities can also be entrapped within the primary structure (i.e., each particle) and incorporated into a nanoparticle network. Since these entities are initially located within the particle, diffusion from the particle is primarily effected by the cross-linking density or rate of degradation of the network due to degradable cross-links. Once the chemical entity has exited the nanoparticle, its release from the network will be governed primarily by the secondary structure (i.e., mesh and particle size). Figure 5 contains plots of BSA release from three different situations: BSA within the particles without degradable links, BSA within the particles with degradable cross-links, and BSA in between particles with degradable cross-links. Clearly apparent is the effect of BSA residing within the particle as opposed to between the particles. The release of BSA from within particles of degradable HPC nanoparticle networks had an almost completely negligible "burst" release when compared to the total amount of BSA loaded into the network. Furthermore, the release appears to be continuing, probably as a result of the slow degradation of the nanoparticles and subsequent release of BSA. This is compared to the nondegradable HPC nanoparticle network where the release has completely stopped.

Contrasting this is the significant "burst" release of BSA from degradable HPC nanoparticle networks where the BSA originates from the secondary structure (i.e., between particles). The fact that the network is degradable has little to no effect on the release rate of BSA. This shows that the diffusion of chemical entities within the secondary structure is not affected by the degradation of the network, since the diffusion is much faster than degradation. This however would not be the case where smaller particle sizes, hence smaller mesh size, would occlude smaller chemical entities. It should be noted that only about 50% of the BSA has been released

from the secondary structure of this network by diffusion. The remaining release will most likely be affected by the degradation of the network.

Conclusions

The hydroxypropylcellulose (HPC) chain structure has been modified by attaching methacrylate or degradable moieties as side groups. This allows for chemical cross-linking of the nanoparticles through a free radical polymerization process in aqueous solutions at temperatures above their LCST. It is found that low or high pH values accelerate the degradation of the nanoparticles. The residual methacrylate or degradable groups on the exterior of the nanoparticles have been further linked together to form a nanoparticle network. It is hypothesized that such a system has two-level structural hierarchy: the primary network consisting of cross-linked polymer chains inside each nanoparticle and the secondary network formed of a cross-linked system of the nanoparticles. This hypothesis is tested to be correct by the study of loading and release of small and/or very large biomolecules contained within the primary and secondary structures of the HPC nanoparticle networks. From this study, it is demonstrated that nanoparticle networks of various compositions and/or different biodegradable cross-linker types and amounts containing a specific active compound can be combined together. The resulting combination has provided an overall synergistic effect of drug delivery due to the differences in degradability and diffusion rates for the primary and secondary networks.

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