Synthesis and Characterization of pH-Sensitive Crosslinked (NIPA-*co*-AAC) Nanohydrogels Copolymer

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ABSTRACT: A thermoresponsive polymeric nanohydrogels has been synthesized by inverse microemulsion polymerization of *N*-isopropylacrylamide (NIPA) and acrylic acid (AAc) using Aerosol (AOT) as a surfactant, ethylene glycol dimethacrylate (EGDMA) as a crosslinker, and 2,2′-azobisisobutyronitrile (AIBN) as initiator. The effect of concentration of AIBN, EGDMA, and NIPA/AAc weight ratio was investigated. The lower critical solution temperature (LCST) of poly (NIPA-*co*-AAC) can be manipulated to be 40°C and 45°C which was correlated to amount of AAc that was copolymerized with NIPA. FTIR, 1H NMR, TEM, and DSC characterized the nanohydrogels. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 1947–1955, 2012

Key words: nanohydrogels; *N*-isopropylacrylamide; inverse microemulsion polymerization

INTRODUCTION

Polymeric hydrogels are unique materials, which absorb and retain large amounts of water. The presence of crosslinks between the polymer chains makes them insoluble, soft, and elastic. The stimuli-responsive hydrogels display phase transitions in response to small environmental changes, such as temperature, pH, electric field, and light. Among them, thermosensitive hydrogels have potential applications as soft contact lenses,[1] drug delivery systems (DDS),[2–5] medical sensors,[6] biocompatible materials for plastic surgery,[7] sanitary products like disposal diapers,[8] or separation matrices like molecular sieves and adsorption resins.[9] However in general, hydrogels have rather poor mechanical strength and durability for some applications, such as drug delivery matrix, and hence investigating and possibly enhancing these properties will make hydrogels more acceptable for many future applications.[10]

Nanohydrogel is a crosslinked polymeric network ranging in size from 10 to 1000 nm that is swollen by a good solvent.[11] It's to their surface area of nanohydrogels that present interesting properties.

Nanohydrogels have been investigated as a subcategory of particulate DDS.[12–14] As drug carriers, nanohydrogels have controllable size and a uniformly crosslinked network as a container for controlled drug release. Importantly, their surface can be conjugated with receptor-specific molecules to achieve targeting ability. Very recently, stimuli-sensitive nanohydrogels have been extensively investigated as drug carriers since they are able to undergo swelling–deswelling transitions in response to changes in temperature, pH, or ionic strength. Poly(*N*-isopropylacrylamide) (PNIPA) is the most popular synthetic polymer among the thermoresponsive polymers since it displays a sharp phase transition close to 32°C.[15,16] This very sharp transition is attributed to the disruption of hydrogen bonding of water molecules around the amide group of the side polymer chains. The crosslinked hydrogels obtained from this polymer swell under the lower critical solution temperature (LCST) and shrink above it. When *N*-isopropylacrylamide (NIPA) copolymerized with other hydrophilic monomers with specific functional group such as acrylamide[17] and *N*-(hydroxymethyl) acrylamide[18] [the obtained copolymeric hydrogel may have better hydrophilicity and site-specific function compared with PNIPA itself and can be used in different applications.[19,20] The applications of such gel usually involve the chemical modification of poly(NIPA). These modifications are usually performed to introduce functional groups that can increase the LCST toward body temperature to improve the mechanical properties.[21,22] Huang et al.[23] synthesized hydrogel nanoparticle networks containing poly-*N*-isopropylacrylamide (PNIPAM). In their study, PNIPAM-*co*-allylamine NP networks and PNIPAM-*co*-acrylic acid NP networks are formed by covalently crosslinking. Also, Gan and Lyon[24] have synthesized thermoresponsive core–shell PNIPAM NPs via seeding and feeding precipitation polymerization method. The influence of chemical differentiation between the core and the

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shell polymers on the phase transition kinetic and thermodynamic behavior has been examined in their study. Mohan, Y. M. et al.[25] synthesized nanogels comprising of PNIPAM (NG-1), PNIPAM with poly(ethylene glycol)-maleic anhydride (PEG-MA) (NG-2), and PNIPAM and vinyl pyrrolidone (VP) with PEG-MA (NG-3) using simultaneous free-radical crosslinking polymerization in the presence of *N,N′*-methylenebisacrylamide (crosslinker) and ammonium persulphate/ferrous ammonium sulfate (initiating pair). The chemical constituents present in nanogels were confirmed by spectral analysis (FT-IR, ^{1}H NMR). The inherent phase transition property of nanogels was determined by measuring LCST using UV–Vis spectrophotometer. Rapamycin was selected for loading into nanogels because of our interest in using the drug-loaded nanogel formulations for intravascular drug delivery to prevent postangioplasty restenosis. Size of nanogels ranged from 25–28 nm in diameter by transmission electron microscopy (TEM), whereas it ranged from 35–45 nm when determined using dynamic light scattering.

Microemulsions are isotropic, optically transparent, and thermodynamically stable dispersion systems consisting of water, oil, and suitable surfactants. Microemulsion polymerization has been widely studied since 1980s, which can be divided into oil-in-water (direct), water-in-oil (reverse), and water-in-oil-in-water (double) according to the precursor microemulsions.[26–29] Microemulsion polymerization has been widely used to synthesize particles of controlled size and shape, and provide a simple tool to fabricate hydrogel micro/nanoparticles.

Nurettin and Mohit[30] synthesize completely charged hydrogel micro/nanoparticles in w/o microemulsion of a biocompatible phospholipid. This synthetic method yields hydrogel particles with a biocompatible phospholipid coating and makes surfactant removal unnecessary. In some cases it can enhance penetration of carrier hydrogel particles when used as delivery devices.[31–34]

Recently, polymerization of acrylates in water-in-oil and bicontinuous microemulsions has attracted considerable interest to prepare microporous and nanopolymer materials.[35–38] Most of literature on microemulsion containing ionic surfactants has dealt with system which requires alcohol or some other cosurfactant and\or salt for their conformation and stability.[39–41]

The goals of this study are to investigate the synthesis of copolymeric hydrogels Poly(NIPA-*co*-AAC) by inverse microemulsion polymerization with the particle size in nanoscale and their characterizations. The pH-sensitivity and swelling behavior of the nanohydrogels were studied (Figure 1).

EXPERIMENTAL

Material

Acrylic acid (AA) and *N*-isopropyl acrylamide (NIPA) were used as monomers, 2,2′-azobisisobutyronitrile (AIBN) as a free-radical initiator, ethylene glycol dimethacrylate (EGDMA) as a crosslinker agent, and aerosol (AOT) sodium bis (2-ethylhexyl sulfosuccinate) as a surfactant. High purity grade toluene, ethanol, acetone, and diethyl acetate (purchased from Aldrich) were used as received. The Fluka chemical company (USA) supplied all chemical.

TECHNIQUES

Synthesis of copolymer nanohydrogels

The microemulsion was prepared using water in oil system. Before the polymerization the reaction medium was purged with nitrogen for 20 min to remove oxygen. 0.5 g of (AOT) was added to 5 mL of hexane (oil phase). The solution was stirred at a speed of 300 rpm and purged with nitrogen. *N*-isopropyl acrylamide with different amounts of acrylic acid (20 and 40*M* ratio) were added by dropping funnel at 60°C during 1 h. The solution was stirred at a rate of 300 rpm under a nitrogen atmosphere. A crosslinking agent, (EGDMA), was added to the solution followed by the addition of AIBN initiator. The reaction proceeded for 7 h. Different nanohydrogel samples were obtained with varying the (AAc) monomer concentrations, initiator, crosslinking agent.[42,43] The copolymer was purified using selective precipitation with excess acetone and diethyl ether to destabilize the micelles.

After the purification process, the nanohydrogels were dispersed in ethyl acetate during 2 h with magnetic stirring.[44]

Measurements

The prepared coplymeric nanohydrogels were dissolved in $CDCl_3$ and analyzed using Varian ^{1}H NMR spectrometer model JNM-EX (300 MHz) for determining their chemical structures. In addition, the prepared nanohydrogels were characterized by FTIR-spectroscopy using FTIR-spectrophotometer type (Mattson-infinity series bench tab 961, PerkinElmer Spectrum GX-USA) as a spectroscopic technique for elucidating the structure. An FT-IR is a method of obtaining infrared spectra by first collecting an interferogram of a sample signal using an interferometer, then performing a Fourier Transform on the interferogram to obtain the spectrum. It is a spectral instrument that collects and digitizes the interferogram, performs the FT function and displays the spectrum.

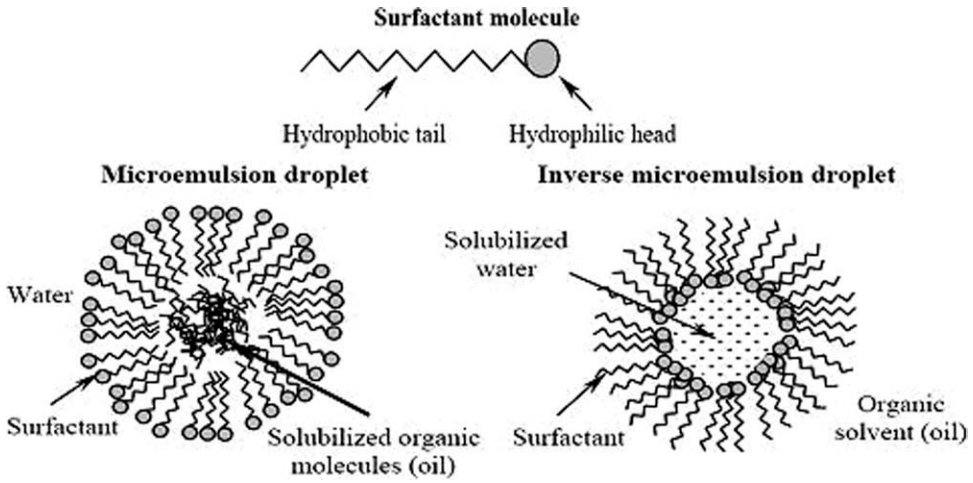

Figure 1 Schematic diagram of micelles and microemulsions.

The cloud point or LCST of poly (NIPA-*co*-AAC) nanohydrogel samples were determined by differential scanning calorimetry (DSC), (TA 2920 Modulated DSC, TA instruments). The pH of solutions was adjusted with very small amounts of 0.1*M* hydrochloric acid and sodium hydroxide and determined using a digital pH meter (pHS-4CT, Shanghai Dazhong Analytical Instrument).

TEM of the colloidal nanohydrogel particles were taken using a JEM-100S Transmission Electron Microscope (TEM, Japan). The TEM sample was prepared by mixing one dilute drop of prepared aqueous particles or latex dispersed in 5 mL acetone to become slightly turbid solution onto the copper grids and allowing it to dry. The grid was dried under IR lamp. The images of representative areas were captured at suitable magnifications which clarify the morphology and the size of the nanoparticles.

Swelling behaviors of (NIPA-*co*-AAC) nanohydrogels

The equilibrium swelling was performed to characterize the pH-responsive behavior of poly (NIPA-*co*-AAC). To determine the equilibrium swelling behavior, 100 mg freeze-dried poly (NIPA-*co*-AAC) was dispersed in 10 mL buffer solution at pH values of 4.00–8.00 and ionic strength of 0.4. The pH values of solutions were determined by a pH meter and size of (NIPA-*co*-AAC) was measured by laser particle size analyzer before and after swelling. The swelling degree was defined as the volume ratio of poly (NIPA-*co*-AAC) after and before swelling.

RESULTS AND DISCUSSION

Hydrogels, biomaterials formed by three dimensional networks of hydrophilic polymers, absorb large amounts of water. Hydrogel nanoparticles are of interest due to their size and biocompatibility. Potential applications of nanogels, mainly those with the ability of react to stimulus.[45] include their use as DDS.[46] It was reported that, nanogels can be prepared using a variety of methods, but those prepared with synthetic polymers have been obtained almost exclusively by polymerization of vinyl monomers with crosslinkers, using emulsions, microemulsions, and micelles as templates. The use of vinyl monomers in the presence of surfactants is interesting despite it is difficult in purification.

Inverse microemulsion techniques have been employed in the synthesis of polymeric nanoparticles for the ability to create submicron hydrophilic polymer particles with improved polydispersities for use in drug delivery applications. The DeSimone group[47] utilized inverse microemulsion polymerization techniques to synthesize stable, biocompatible polymeric nanohydrogels <200 nm in size, for antisense and gene delivery to HeLa cells via the exploitation of charge. These particles showed a narrow size distribution with polydispersity of <10% for the nonionic hydrogels.[47] Furthermore, Horgan and Vincent describe a method of producing 5–15 nm sterically stabilized organic nanoparticles via an inverse microemulsion technique. This method allows for facile surface modification as well, leading to the possibility of these particles to be used for drug delivery and other biomedical applications.[48]

In this study, inverse microemulsion polymerization was used to synthesize two (PNIPA-*co*-AAc) copolymers nanohydrogels with ratios of 80 : 20 mol/mol (CO1) and 60 : 40 mol/mol (CO2), respectively.

Chemical structure and morphology of poly (NIPA-*co*-AAC) nanohydrogels

The FTIR spectrum of poly (NIPA), poly (AAC), and poly (NIPA-*co*-AAC) is illustrated in Figure 2 The

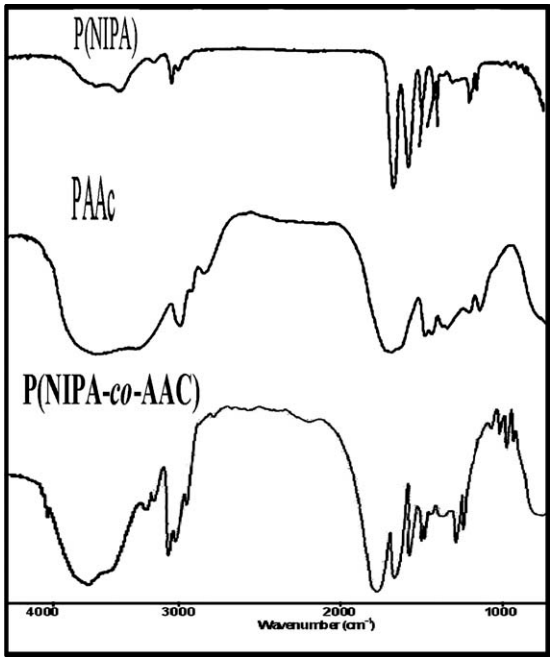

Figure 2 IR-Spectra of poly (NIPA), poly (AAC), and poly (NIPA-*co*-AAC) nanohydrogel.

spectrum of poly (NIPA-*co*-AAC) confirms the presence of the most important functional groups of the monomers (NIPA and AAc) in the copolymer structure. Thus, we observe bands at 2940 and 3350 cm^{-1} that are due to the vibration of the amide proton NH— and carboxylic —OH group of acrylic. In addition, we have band at 1650 cm^{-1} for C=O from NIPA and AAc. The signal at 1150 cm^{-1} was assigned for C—N of amid group. Similar spectra were obtained for other samples of nanohydrogels.

Figure 3 represents the ^1H NMR spectra of poly (NIPA), poly (AAC), and poly (NIPA-*co*-AAC). The spectrum of poly (NIPA-*co*-AAC) shows signal at 3.9 and 10.2 ppm refereed to the copolymer contains both NIPA and AAc monomers, respectively[49]; also, the disappearance of vinyl proton at 5–6 ppm confirmed the copolymerization. The copolymer compositions were calculating using ^{1}HNMR spectrum, By comparing the integration peak of —OH at 10.2 ppm of acrylic acid moiety with that of —CH of NIPA moiety at 3.6 in the copolymer backbone. The compositions of NIPA and AAc in the copolymer are 79.5–20.5 mol %, respectively. Table I demonstrates the compositions of copolymer nanohydrogels prepared. Figure 3 shows the chemical shifts attributable to the other function group.

Figure 4 shows TEM images of the poly (NIPA-*co*-AAC), CO1, particles obtained by polymerization with different concentration of initiator and crosslinker. The copolymer designated as CO1-1 to CO1-5 at crosslinker/initiator, (1–3%/1) and vice verse, respectively. In Figure 4(a), all particles copolymerized from the solution of (1% EGDMA and 1% AIBN), shows sphere-shaped particles have an average diameter of 60–80 nm. Figure 4(b) shows that high concentrations of (AIBN) resulted in nanohydrogels with diameters of 60–50 nm as reported in Table I. These results show that with an increasing concentration of AIBN, the resulting poly (NIPA-*co*-AAC) particle sizes decreased up to concentration of AIBN 3% respect to the total monomer concentration.[49] Figure 4(c) shows that as EGDMA concentration increases the particle size is slightly increases as listed in Table I. It is well-known that the swelling of polymer network depend not only on the pore of the network but also depend on surface area of polymer network.[50,51] In this study, the swelling of polymer network depend on surface area of absorbed polymer. This results may be explained by the fact that the more crosslinking concentration the stiffer the crosslinking network is and the smaller the cavities produced this enhance the collapse of polymer chains and made it stable in nanosize, i.e., the increase of crosslinking agent will lead to more polymer chains connected to each other, which will increase the particle sizes. At the same time, the smaller cavities will provide larger absorption surfaces, which give higher swelling rate of the polymeric nano network, i.e., the increase of crosslinking agent will lead to more polymer chains connected to each other, which will increase the particle sizes. Figure 4(d,e) represents particle size distribution of P(NIPA) crosslinked with 1% EGDMA and 1% AIBN and PAAc which is crosslinked with 1% EGDMA and 1% AIBN.

Effect of AAc concentration on LCST

In general, the solubility of most polymers increases with increase in temperature. However, in the case of polymers exhibiting LCST, increase in temperature decreases the polymer's water solubility due to predominating hydrophobic interactions.

Thus, LCST is a characteristic facet of "inversely" thermoresponsive polymers. It is defined as the temperature at which the polymer solution undergoes a phase transition from a soluble state (i.e., random coil form) to an insoluble state (i.e., collapsed or globule form) on elevating the temperature. In fact this coil-to-globule transition of poly (NIPA) has been clearly seen on a nanometer scale using atomic force microscopy (AFM).[52]

Poly (NIPA), like other LCST polymers, is fully hydrated with an extended chain conformation below 32°C and gets extremely dehydrated and compact above 32°C. The huge promise in the thermoresponsive property of poly (NIPA), however, lies in the fact that its LCST phase transition occurs close to the body temperature.[53] In general, the LCST of thermoresponsive polymers can also be

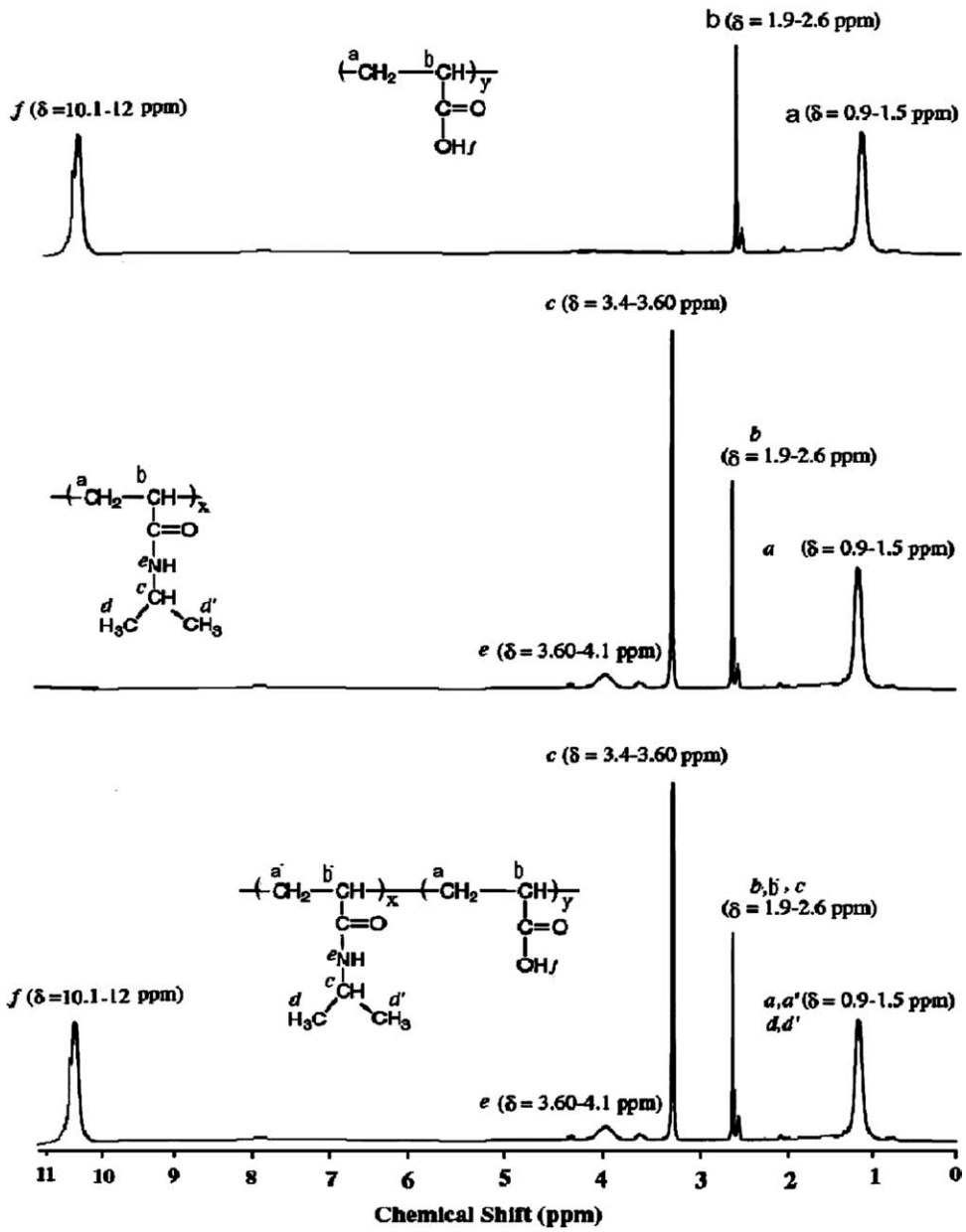

Figure 3 ^{1}H NMR-spectra of poly (AAC), poly (NIPA), and poly (NIPA-*co*-AAC) nanohydrogel.

tuned to such useful values, by simply adjusting the ratio of hydrophilic and hydrophobic segment of the polymer.[54–57] Modification of LCST of poly (NIPA) with more hydrophilic monomer (AAc) will favor hydrogen bonding in preference to hydrophobic interactions and will increase the LCST of the copolymer.[58–60] Interestingly, copolymerizing poly (NIPA) with more hydrophobic monomers (e.g., *N*-butyl methacrylate) resulted in the formation of a dense skin during the de-swelling process (i.e., when the temperature was raised above LCST).[61–64] This dense skin formed at high temperatures blocked release of drugs loaded in the matrix, resulting in

TABLE I
Effect of Initiator and Cross-Linking Agent Concentration in Particle Size and Composition of Nanohydrogels CO1 Determined by ^{1}H NMR

Sample	NIPA (mol %)	AAc (mol %)	EGDMA (%)	AIBN (%)	Size (nm)
P(NIPA)	100	0	1	1	200
P(AAc)	0	100	1	1	300
CO1-1	79.5	20.5	1	1	66
CO1-2	79.5	20.5	2	1	70
CO1-3	79.5	20.5	3	1	75
CO1-4	79.5	20.5	1	2	56
CO1-5	79.5	20.5	1	3	52

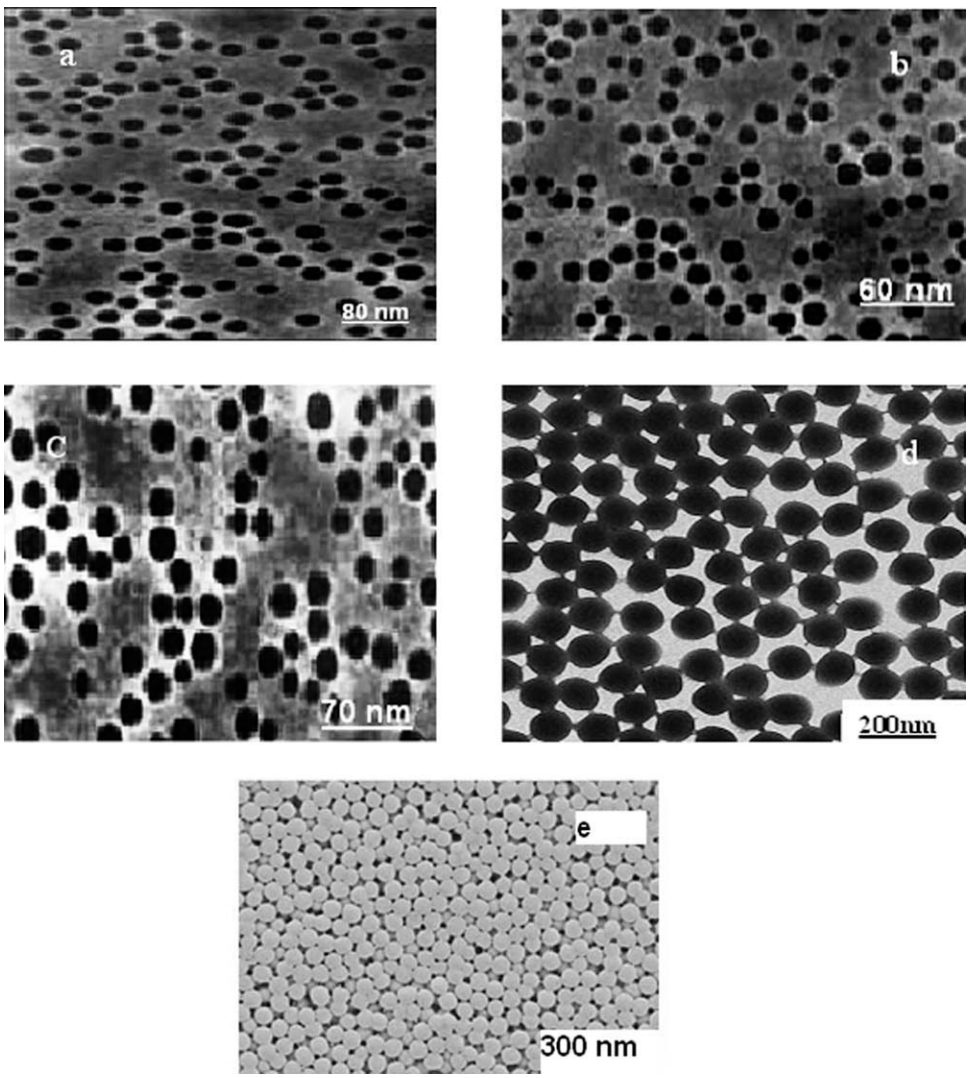

Figure 4 TEM of synthesized nanohydrogel (a) (1% EGDMA and 1% AIBN); (b) (1% EGDMA and 2% AIBN); (c) (2% EGDMA and 1% AIBN); (d) P(NIPA) (1% EGDMA and 1% AIBN), and (e) PAAc (1% EGDMA and 1% AIBN).

the abrupt tapering off of drug release, modulated by temperature.

LCST is a key parameter of the thermally responsive nanohydrogel. It determines the potential application of nanohydrogel in targeted drug delivery.[65]

LCST of nanohydrogels were measured in our research by DSC thermograms are shown in Figure 5. The LCST for P(NIPA), CO1, and CO2 were 32°C, 40°C, and 45°C, respectively. The result indicated that LCST increased with the increase of AAc wt %, as shown in Figure 5. The hydrophilicity of AAc is known to be better than that of NIPA. The more hydrophilic the moiety of AAc in nanohydrogels, the stronger it produces a hydrogen bond interaction in aqueous solutions. This stronger bond requires more energy to destroy it and results in the increase of LCST. Thus, LCST of nanohydrogels could be adjusted by modulating wt %.

Swelling behaviors of poly (NIPA-*co*-AAC)

Polyelectrolyte hydrogels are based on charged networks that contain ionized groups. Negatively or positively charged hydrogels usually exhibit different degrees of equilibrium swelling at different pH values depending on the ionic composition of the polymers. Since PAAc is organic acid and is in the molecular state at pH $<$ (pK_a = 4),[66,67] there is hydrogen bonding between the COOH group of the PAAc and —CONH group of the PNIPA. Figure 6 demonstrates that the transmittance of the nanohydrogel dispersions increased with the increase of the PH value. It is found that the transmittances of poly (NIPA-*co*-AAC) nanohydrogels rise sharply at pH 6.5–7.0 for CO1 and pH 8–8.5 for CO2. At low pH, there is a hydrogen bonding between the polymer chains of poly (NIPA-*co*-AAC) nanohydrogels, thus leading to a lower transmittance in acetone aqueous

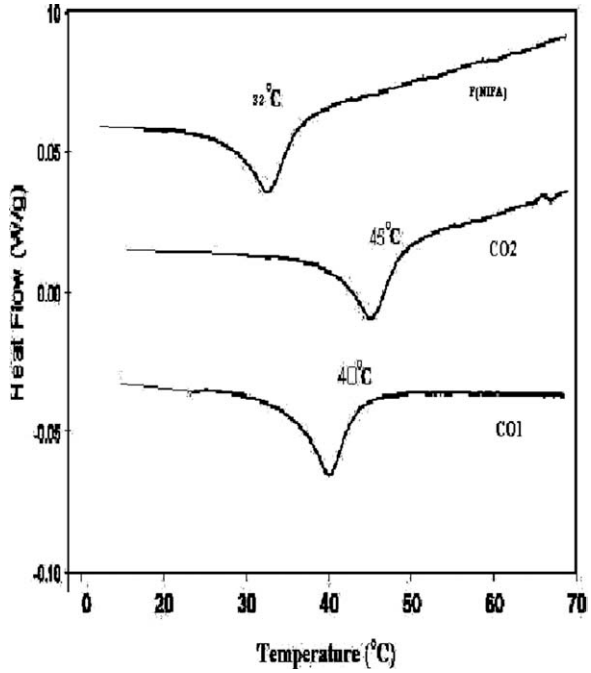

Figure 5 DSC thermogram of P(NIPA) and CO1 and CO2 nanohydrogel.

solution. As pH increases, PAAc becomes ionized, and the ionized PAAc weakened the H-bonding between PAAc and NIPA and strengthened the water-solubility of PAAc, so that the poly (NIPA-*co*-AAC) particles swell and the nanohydrogel dispersion becomes transparent.

Effect of PH on the swelling capacities

The swelling capacities of the prepared nanohydrogels were measured at 25°C in different pH solutions as reported in the experimental section. It was noted that, the swelling capacity was increased with an increase in pH up to 12. Increasing the pH of the external solution increases the ion dissociations of COOH and, consequently, the charges on the polymeric chains increases. To investigate the swelling behaviors of poly (NIPA-*co*-AAC), 100 mg freeze-dried polymer was dispersed in 10 mL buffer solution with pH value of 5 at 25°C and the size of gel were measured after 24 h. (The pK_a of AAc is approximately 4).[66,67] When the environmental pH is lower than pK_a, COOH group assumes in a state of protonation and appears hydrophobic character. Whereas, when pH is greater than pK_a, COOH presents a hydrophilic character. As a result, the pH variation of environment can lead to the volume change of poly (NIPA-*co*-AAC). The pH sensitivity of poly (NIPA-*co*-AAc) is shown in Figure 7. It can be seen that the size of poly (NIPA-*co*-AAC) increases sharply when pH value changes from 5 to 12. Apart from this pH range, the change of particle size is slight with pH variation. Similar to poly [methacrylic acidgrafted-poly (ethylene glycol)] P(MAA-*g*-EG) anionic hydrogel nanoparticles, the

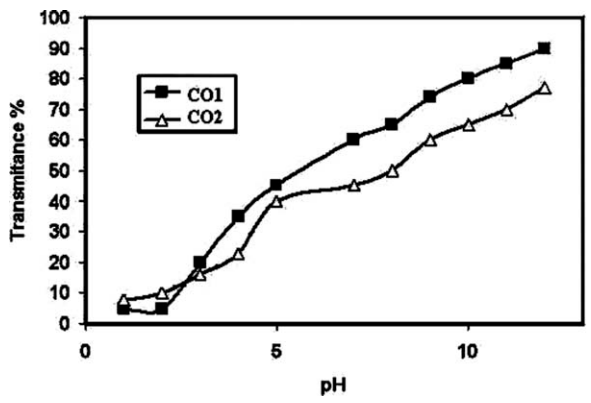

Figure 6 Effect of pH value on the transmittance of the CO1 and CO2 nanohydrogels.

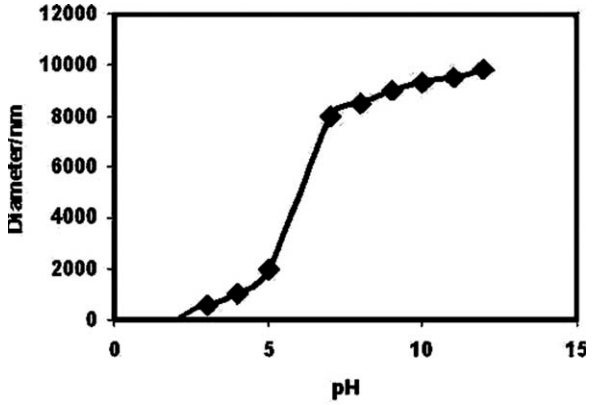

Figure 7 Effect of pH value on swelling behavior of CO1–3, 100 mg freeze-dried CO1–3 was dispersed in 10 mL buffer solutions with ionic strength of 0.4 and then swelled for 24 h at 25°C.

TABLE II
Effect of Cross-Linker Dosage on the Swelling Degree of CO1 Nanohydrogel at pH = 7

Designation	EGDMA (mol %)	Diameter before swelling (nm)[a]	Diameter after swelling (nm)	Swelling degree[b]
P(NIPA)	1	200	2000	10
P(AAc)	1	300	1500	5
CO1-1	1	66	2200	33
CO1-2	2	70	3000	43
CO1-3	3	75	4000	54
CO1-4	1	56	3500	62
CO1-5	1	52	3300	63

[a] 100 mg freeze-dried poly (NIPA-*co*-AAc) was dispersed in 10 ml buffer solution with ionic strength of 0.4 and then poly (NIPA-*co*-AAc) were swelled for 24 h at 25°C.

[b] The swelling degree was defined as the ratio of poly (NIPA-*co*-AAc) after and before swelling.

crosslinker dosage has a great effect on the swelling degree of P(DMAEMA-g-EG).[68] In this article, Table II shows the effect of crosslinker concentration on the equilibrium swelling degree of poly (NIPA-*co*-AAC). When the crosslinker dosage is 3 mol % of EGDMA, the equilibrium swelling degree of the prepared nanohydrogel reaches the maximum. When the crosslinker dosage is 1 mol %, the degree of crosslinking is very low, this makes the particles themselves loose. It is obviously that the concentration of the initiator has not affect on the equilibrium swelling degree of synthesized nanohydrogel. The data in Table II reveals also that, the swelling of P(NIPA) hydrogel is greater than the swelling of P(AAc) hydrogel. This may be attributed to the presence of pendent chains or dangling chains in the polymeric network of P(NIPA) which increase the swelling capacity of the network.

CONCLUSIONS

In the study of the formation of copolymer nanohydrogels, the size of prepared nanohydrogels depends strongly on the ratio of crosslinker/initiator. The diameter of nanohydrogels decreased with the increase of initiator concentration, while increased with in the increase of crosslinker concentration. The LCST for P(NIPA), CO1, and CO2 were 32°C, 40°C, and 45°C, respectively. The result indicated that LCST increased with the increase of wt % of AAc, i.e., increase hydrophilicity. The synthesized nanohydrogels have pH sensitive character. Copolymer nanohydrogel performs pH-responsive swelling behavior, which is strongly influenced by the crosslinker dosage. These environment-sensitive characters of crosslinked (NIPA-*co*-AAC) copolymer will endue this kind of nanohydrogels great potential application in the environment-sensitive DDS.

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