Copolymer Hydrogel Microspheres Consisting of Modified Sulfate Chondroitin-*co*-Poly(*N*-isopropylacrylamide)

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ABSTRACT: Modified chondroitin sulfate (π-CdS) microspheres were synthesized by way of crosslinking-copolymerization reaction with *N*-isopropylacrylamide (NIPAAm), yielding CdS-*co*-PNIPAAm copolymer network. The incorporation of vinyl groups onto the CdS was processed with the use of glycidyl methacrylate (GMA) in an aqueous solution of pH 3.5 under stirring speed of 800 rpm at 50°C. ^{13}C NMR and ^{1}H NMR spectra of CdS treated with the GMA indicated the formation of 3-methacryloyl-1-glyceryl ester of π-CdS and 3-methacryloyl-2-glyceryl ester of π-CdS that are the reaction products resultant of an epoxide ring-opening mechanism via. The synthesis of microspheres was performed via radical reaction of the vinyl groups at the π-CdS with vinyl groups at the NIPAAm in a water−benzyl alcohol microemulsion. The formation of spherical structures is the result of the polymerization-crosslinking reaction of the π-CdS with the NIPAAm monomers at the droplets of water, in view that both reactants have hydrophilic characteristics at the temperature at which the reaction was processed. The pure CdS hydrogel microspheres showed a slightly cracked structure with a lower diameter range while the CdS-*co*-PNIPAAm hydrogel microspheres showed a flat and tight structure with a more regular mass distribution. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 2726–2733, 2011

Key words: biopolymers; hydrogels; emulsion polymerization; microstructure; synthesis

INTRODUCTION

Microspheres made from biodegradable polymers have attracted researchers' attention because of their therapeutic potentials and greater stability of storing. The chondroitin sulfate (CdS) is a glycosaminoglycan or mucopolysaccharide (unbranched chains) that has been successfully used in a series of pharmaceutical formulations, such as tablets, ophthalmic solutions, liquid preparations, soft and hard capsules, hydrogels, and so forth.[1–12] It consists of sequences of alternately sulfated residues of D-glucuronic acid and *N*-acetyl-D-galactosamine linked to each other by β1-4 and β1-3 bonds.[13] A chondroitin chain, which is an important structural component of cartilage, can have more than 100 individual sugars. The varied amount of sulfate groups in CdS chains has been associated to origin of the tissue, its age, and the species.[14] The use of CdS in synthesis of chemically crosslinked hydrogel microspheres can represent innovations from biotechnological point of view, because it is recognized to have an impact in the effective therapy of diseases affecting articular functions, minimizing the pain.[15] However, this type of hydrogel structure is often obtained by way of radical polymerization reaction of the vinyl groups throughout the backbone of polymer chains.[16–21] Although polysaccharides are reactive toward many crosslinking agents, such as di-*n*-methyol compounds, divinyl sulfones, diepoxides that could be reactive with hydroxyl groups of these polymers, there is no way to produce such hydrogel microsphere composed only of CdS via radical polymerization reaction without its original properties being affected. Thus, chemical modification that consists of incorporating vinyl groups vinyl onto the CdS with the use of glycidyl methacrylate (GMA) as the modifier could be an efficient strategy to allow further radical polymerization reaction. Furthermore, the ester groups of the GMA molecule

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allow modified polysaccharide to be hydrolyzed and therefore it is appropriate to undergo either bio- or degradation process. On the other hand, the poor consistence of a hydrogel made only of polysaccharide is another limitation that can seriously affect its applications. In this way, the insertion of a vinyl monomer, which has biotechnological applications, onto the vinyled CdS (π-CdS) by way of copolymerization reaction could help to improve structural characteristics of the material produced. Over the last decades, hydrogels of poly(*N*-isopropylacrylamide) (PNIPAAm) have gained considerable attention in the materials, biotechnological and pharmaceutical fields. It has been widely investigated because of its lower critical solution temperature (LCST) in aqueous media as biological fluids.[22–27] Furthermore, the PNIPAAm has been used for bio- and technological ends, such as separation processes,[28] controlled drug release,[29] and cell culture.[30] In this work, our attention was focused on developing microspheres composed of materials with ever-increasing importance on the biotechnological fields and that exhibit new morphological and structural characteristics. As the use of CdS for materials processing has not been extensively investigated, publications on micrometer-sized hydrogel microspheres based on chemically modified CdS and PNIPAAm have been hardly reported.

EXPERIMENTAL

Materials

Chondroitin sulfate (CdS) was kindly supplied by Company-Solabia, Maringá, Brazil. High retention seamless cellulose tubes (D−0530, lot 103H0525) with average flat width of 32 mm (1.3 in.), MWCO 12400, 99.99% retention for dialysis were purchased from Sigma. *N*-isopropylacrylamide (NIPAAm) (Aldrich, 41,532-4), Glycidyl methacrylate (Acros Organics), benzyl alcohol (Nuclear-Brazil), ethanoxxl (Nuclear-Brazil 99.5%,), HCl (Nuclear-Brazil 36.5 -38 %), sodium persulfate (Sigma), *N,N,N′,N′*-tetramethylethylenediamine−TEMED (Sigma). The 3′ (trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt, TSP-d_4 (Sigma).

Spheres preparation

Vinyled chondroitin sulfate processing (π-CdS)

With the purpose of using a modification process free of impurities or organic residues, the polysaccharide was dissolved in pure water for further reaction by GMA. On the other hand, this implied the formation of a heterogeneous phase system, which is formed of water-soluble CdS and water-insoluble GMA. To overcome this limitation, the formed-as system was kept under high stirring for an interfacial reaction at the interface of the GMA-in-water system. The CdS-modifying solution was prepared with the addition of 22.5 g CS and 4.6 g GMA into 150 mL aqueous solution. The pH of the solution was adjusted to pH 3.5 by dropping concentrated HCl. The formation of an opalescent liquid was observed within 24 h under stirring speed of 800 rpm at 50°C. After that, the product was precipitated in ethanol three times to remove any residues of impurities, or unreacted GMA. The modified material was separated by filtration under reduced pressure and dialyzed in Milli-Q® water at 5°C. The water was renewed every 12 h for a total period of 72 h.

Reaction of π-CdS with NIPAAm for hydrogel spheres

Spheres-forming emulsion was prepared with the addition of 50 mL aqueous solution consisting of 15% π-CdS into a benzyl alcohol phase. The volume of benzyl alcohol corresponded to three times the volume of water. The NIPAAm was introduced under a bubbling stream of nitrogen for 15 min. The concentrations of the NIPAAm were always 2.5 and 5%, with respect to weight of π-CdS. The final mixture was then stirred vigorously at 6000 rpm by a high shear lab mixing (Quimis®, Dispersor Extratur®) and then 0.1 m*M* sodium persulfate (SP) was introduced as an initiating agent. The stirring speed was increased up to 12,000 rpm and the spheres-forming process was started off with the introduction of 0.127 m*M* TEMED as the catalytic agent for 15 min. The temperature of the reacting system was kept at 25°C with the use of a thermobath. Afterward, 500 mL of acetone was introduced to aggregate the particles for further vacuum filtration. Finally, resultant product was dried by room temperature within 4 h for further visualization by scanning electronic microscopy.

FTIR spectroscopy for π-CdS characterization

FTIR spectra of GMA, CdS, and π-CdS were recorded on a Bomen FT-IR model MB100 spectrometer. All the samples were analyzed as dry powders which have been kept at 60°C for ~ 2 days. Measures were performed in triplicate and a total of 128 scans were run for a resolution of 4 cm^{-1}.

^1H NMR and ^{13}C NMR spectra for π-CdS characterization

NMR spectra were performed on a Varian Mercury plus BB 300 MHz spectrometer at a frequency of 300,059 MHz for ^1H nucleus. D_2O solutions

containing 50 mg L^{-1} of GMA, CdS, and π-CdS with 0.05% 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4), as the internal standard (0 ppm), were prepared in NMR tubes of 5 mm diameter. An angle pulse of 90° with a relaxation delay of 30 s was used to acquire the spectra. The signal associated with water was suppressed by irradiation during the relaxation.

Solid-state ^{13}C-CP/MAS NMR spectra for radical reaction of the vinyl groups at the π-CdS

Solid-state ^{13}C-CP/MAS NMR spectra of Cds, π-CdS, and CdS hydrogel were obtained on Varian spectrometer model Oxford 300 by applying a frequency of 74.475 MHz. The samples were placed into a 4-mm rotor; other important parameters were adjusted as follows: pulse angle θ = 37°, spinning rate of 12 kHz, contact time of 3 ms, and relaxation time of 3 s.

RESULTS AND DISCUSSION

Reaction of polysaccharide by GMA

The modification of polysaccharides with the use of the GMA occurs by way of transesterification and/or epoxide ring-opening reaction mechanisms.[31–35] The occurrence of either reaction mechanisms (or both mechanisms) depends on pH and chemical nature of the solvent and polymer.[36] In the present investigation, the CdS was treated with the GMA in a solution of pH 3.5. In such conditions, the GMA reacts with both carboxylic and hydroxyl groups (both groups are found in CdS) through epoxide ring-opening mechanism in a forward and irreversible reaction route. The reaction products that result of an epoxide ring-opening via are two isomers: 3-methacryloyl-1-glyceryl and methacryloyl-2-glyceryl esters. At basic conditions, the GMA reacts with hydroxyl groups by way of both the transterification and the epoxide ring-opening mechanisms, whereas the transterification via occurs in both the forward and reverse directions.

π-CdS characterization

Figure 1 shows the FTIR spectra of GMA, CdS, and π-CdS in the spectral range of 4000–500 cm^{-1}. The bands at 1715 and 1638 cm^{-1} in the spectrum of Ch-π, corresponded to the stretching vibrations of the carbonyl groups (νC=O) and carbon−carbon double bonds (νC=C) of conjugated ester groups, are evidence of the attachment of chemical groups issued from GMA onto the glycosaminoglycan structure of the π-CdS.

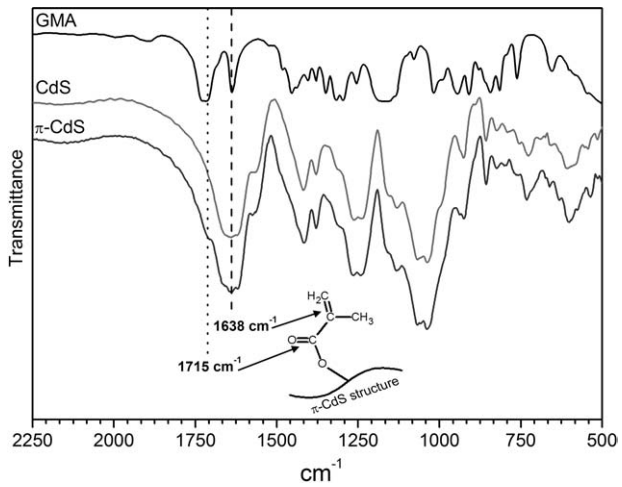

Figure 1 FTIR spectra of GMA, CdS and π-CdS in the spectral range of 4000–500 cm^{-1}.

Figure 2 shows the ^{1}H NMR spectra of CdS and π-CdS. The signals appearing at δ 6.18 (1A-numbered atom) and at δ 5.58 (1a-numbered atom), in the spectrum of π-CdS, was associated to vinyl carbon-linked hydrogen. The high-intensity signal at δ 1.95, in the same spectrum, was ascribed to hydrogen of methyl groups at the vinyl carbons, and the signals at δ 4.30 and δ 3.65 were attributed to glyceryl spacer issued from GMA. The appearance of these signals indicates the formation of 3-methacryloyl-1-glyceryl ester of π-CdS and 3-methacryloyl-2-glyceryl ester of π-CdS that are the reaction products resultant of the epoxide ring-opening mechanism via.

Figure 3 shows the ^{13}C NMR spectra of CdS and π-CdS. The corresponding signals of the vinyl carbon were clearly observed at δ 138.7 and δ 130.1 in the spectrum of π-CdS, methyl carbon at δ 20.3, and carbonyl carbon at δ 171.5. The signal at the spectral region of δ 78–62 indicates the glyceryl spacer. The corresponding signals of the four- and six-numbered carbons were detected at δ 72.2 and at δ 65.2 for 3-methacryloyl-2-glyceryl ester of π-CdS. The corresponding signal of the five−numbered carbon that indicates the formation of 3-methacryloyl-1-glyceryl ester of π-CdS was observed at δ 68.1. Both the ^{1}H NMR and the ^{13}C NMR data demonstrated that the reaction in which the GMA molecule is coupled to backbone of the π-CdS occurs via an epoxide ring-opening mechanism.

Gelling capacity of the π-CdS in water

The chemical modification of the CdS by reaction with glycidyl methacrylate (GMA) can reduce its solubility in water due to originally hydrophobic characteristics of both isomers. Thus, the capability of the π-CdS to undergo a gelling process in water was verified by radical polymerization of the vinyl

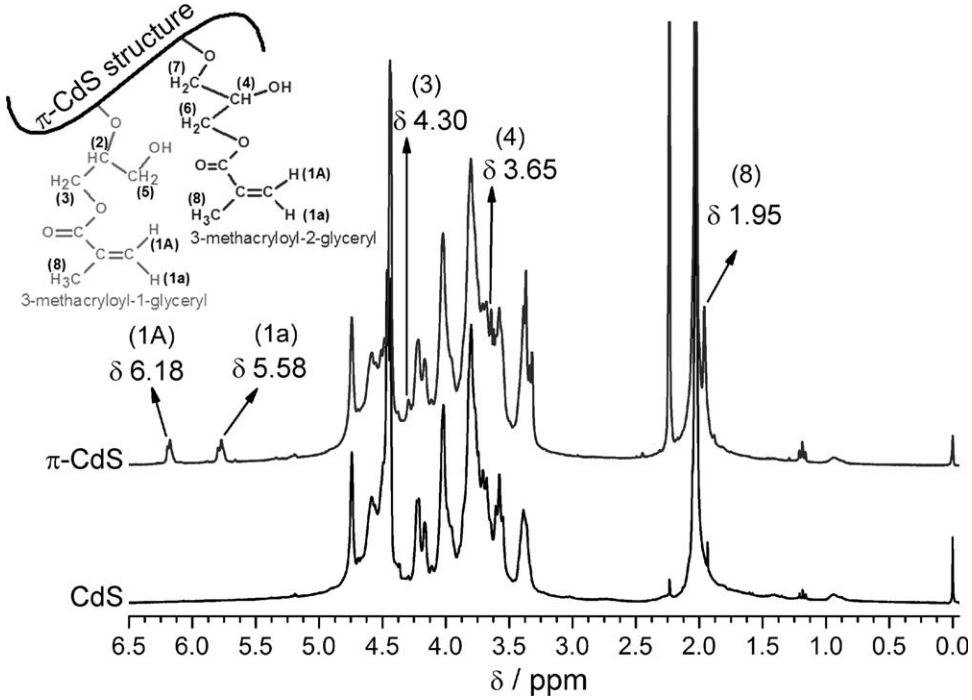

Figure 2 ^{1}H NMR spectra of CdS and π-CdS in the spectral range of 6.5–0 ppm.

groups at the glycosaminoglycan structure of the polysaccharide with the use of sodium persulfate as the initiator. The reaction was characterized by means of ^{13}C-CP/MAS NMR analysis (shown in Fig. 4) that can give an overview of the gelling process of the π-CdS by way of ^{13}C-resonances of the

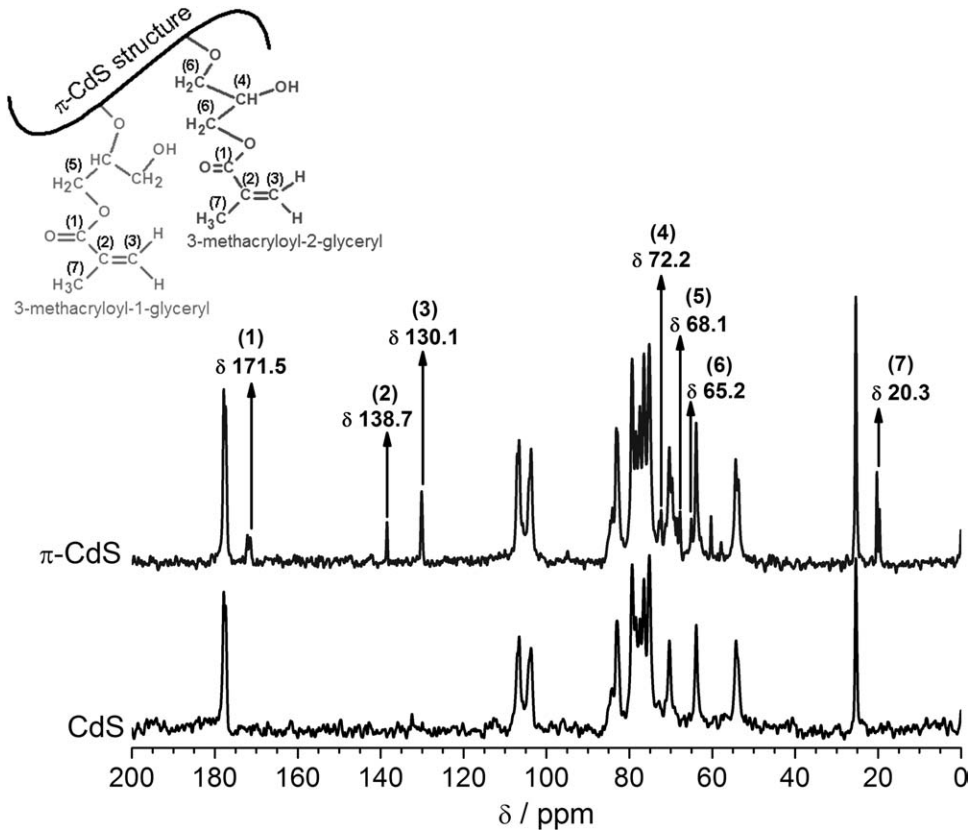

Figure 3 ^{13}C NMR spectra of CdS and π-CdS in the spectral range of 200–0 ppm.

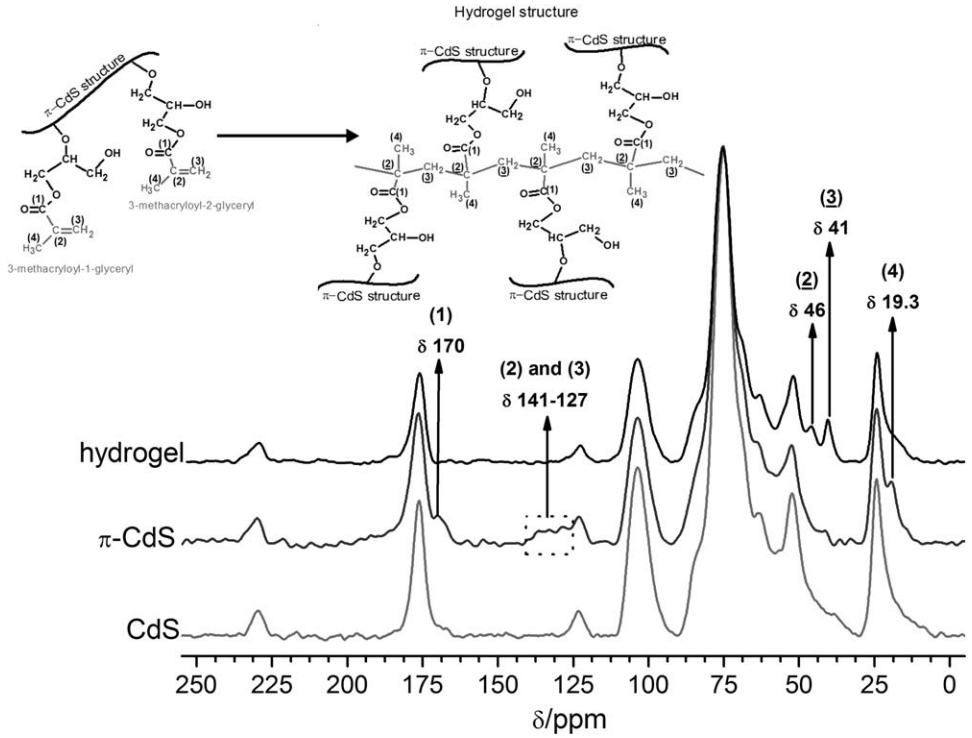

Figure 4 ^{13}C-CP/MAS NMR of CdS, π-CdS and π-CdS hydrogel in the spectral range of 250–0 ppm.

methacrilate-conjugation. The signal at δ 41 in the spectrum of π-CdS hydrogel was ascribed to saturated carbon groups (—CH_2—CH_2—), indicative of the consumption of π-bonds at the π-CdS structure. Over the hydrogel processing, the vinyl carbon groups (>C=C<) of the π-CdS are converted to saturated carbon groups (—CH_2—CH_2—). The signal at δ 170, assigned to carbonyl groups (>C=O) of nonconjugated system, and the absence of the signal at the spectral range of δ 130−120 in the spectrum of hydrogel are the resulted of the radical crosslinking reaction.

Hydrogel microspheres from π-CdS and NIPAAm

The crosslinking-copolymerization reaction of the π-CdS with NIPAAm monomers was characterized by FTIR analysis, demonstrated in Figure 5. The shift in the >C=O stretching band from 1710 cm^{-1} in the spectrum of π-CdS to 1740 cm^{-1} in both the spectra of CdS-*co*-PNIPAAm hydrogel was due to the loss of conjugation of the ester groups, an strong evidence of the occurrence of the crosslinking-copolymerization reaction. The wide-ranging band at 1665 cm^{-1} in both spectra of CdS-*co*-PNIPAAm hydrogel was attributed to the overlap of the >C=O stretching bands of esters (issue from π-CdS), and N—H vibrations of secondary amides groups (issued from PNIPAAm). This finding is an additional evidence of crosslinking-copolymerization reaction of π-CdS with NIPAAm monomers. The whole gelling process starts off with the homolytic cleavage of the sodium persulfate molecule which is accelerated with the introduction of TEMED. Under a bubbling stream of nitrogen at 25°C, the sodium persulfate is decomposed by homolytic cleavage to form sulfate radical ions. As the NIPAAm monomer is the vinyl substance more reactive in the emulsion, the sulfate radical ion attacks the monomer before the π−CdS. The new NIPAAm radical reacts then with another NIPAAm monomer in the same manner as the sulfate radical ion did. The radical persulfate moieties react then with the vinyl groups of the π-CdS to yield a three-dimensional copolymer network.

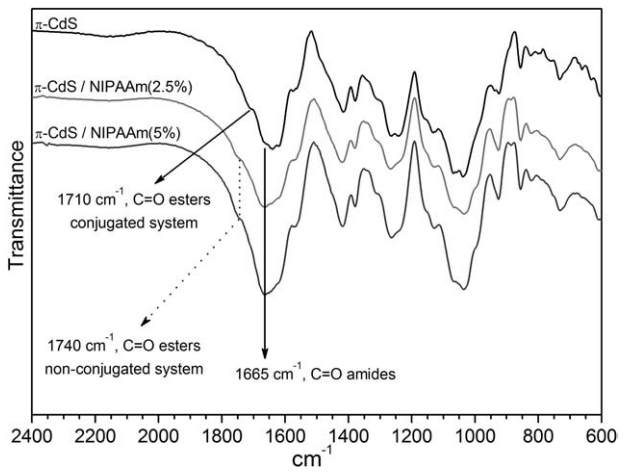

Figure 5 FTIR spectra of π-CdS and CdS-*co*-PNIPAAm hydrogel consisting of either 2.5 or 5% NIPAAm.

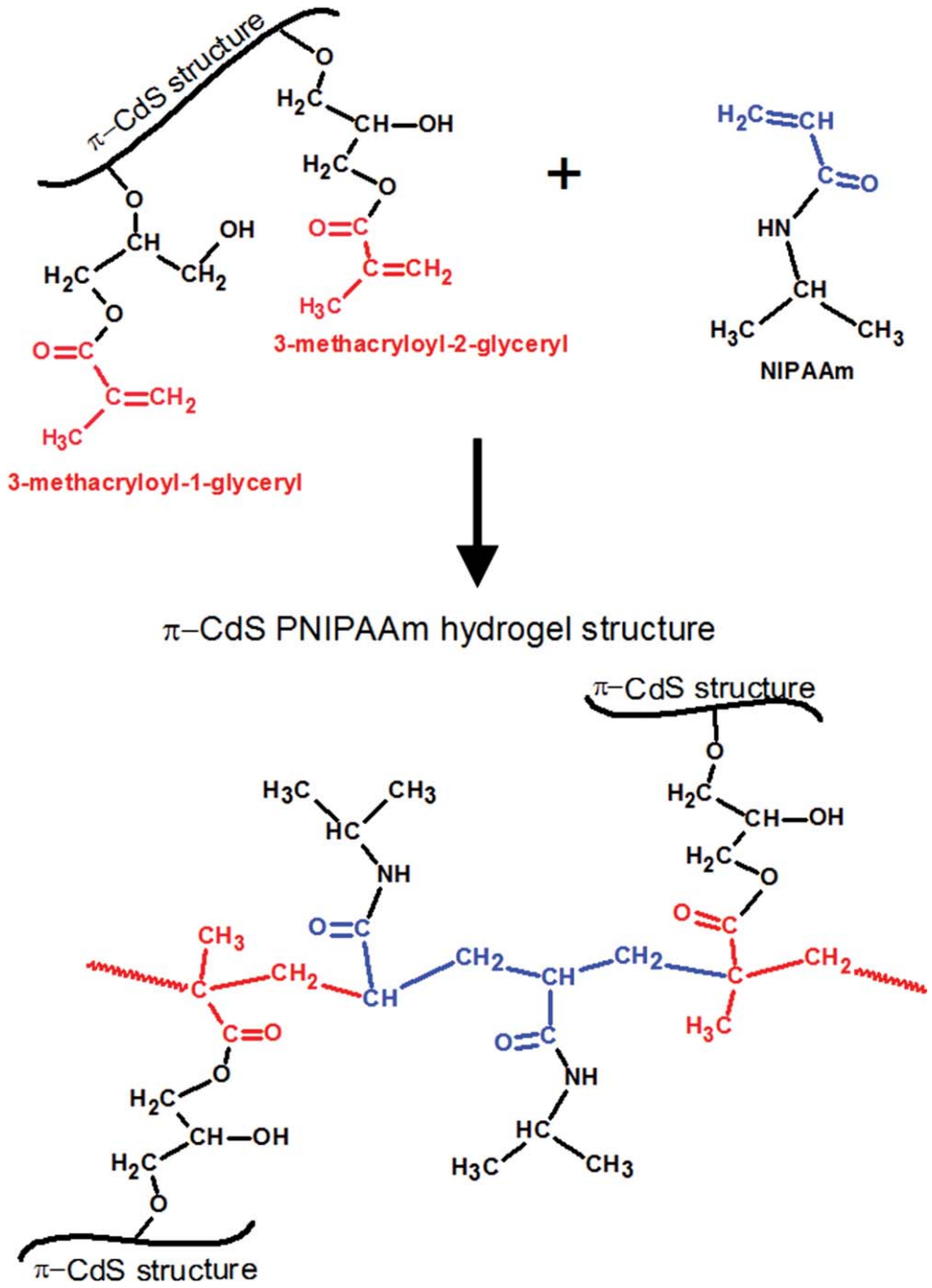

Figure 6 A schema of the copolymer network that forms the CdS-*co*-PNIPAAm hydrogel microsphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

A simple schema of the copolymer network that forms the CdS-*co*-PNIPAAm hydrogel was represented in Figure 6.

Morphology and characteristics of pure CdS and CdS-*co*-PNIPAAm hydrogel microspheres

Figure 7 shows the SEM photomicrographs of (a–b) pure CdS hydrogel microspheres, (c) 2.5% NIPAAm-containing CdS-*co*-PNIPAAm hydrogel microspheres, and (d) 5% NIPAAm-containing CdS-*co*-PNIPAAm hydrogel microspheres at different magnifications. The formation of such a structure with a spherical shape was associated to a random movement of water droplets inwards the benzyl alcohol phase, or external phase, that was caused by high stirring speed. There should be a formation of a multiple emulsion with a benzyl alcohol–water–benzyl alcohol configuration, within of each which the substance is stable as a one-phase system. The confinement of a phase in a two-phase microemulsion system has been already reported in the literature.[37,38]

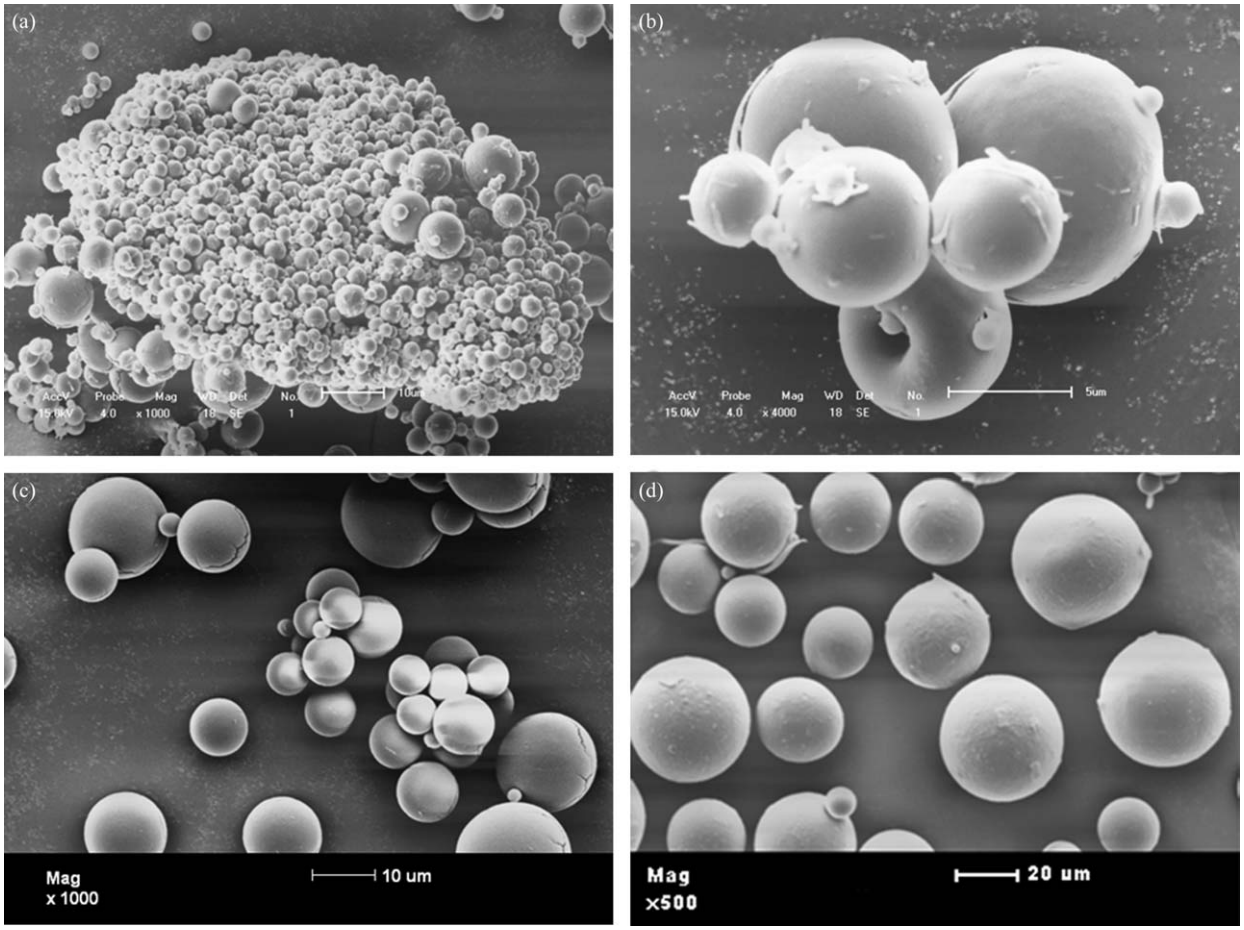

Figure 7 Scanning electronic microscopy (SEM) photomicrographs of (a–b) pure CdS hydrogel microspheres, (c) 2.5% NIPAAm CdS-*co*-PNIPAAm hydrogel microspheres, and (d) 5% NIPAAm CdS-*co*-PNIPAAm hydrogel microspheres at different magnifications.

The crosslinking-polymerization reaction is assumed to occur at the droplets of water, in view that both the π-CdS and the NIPAAm monomers possess hydrophilic characteristics at 25°C, temperature in which the reaction was processed. The morphological aspects of CdS-*co*-PNIPAAm hydrogel microspheres clearly differs those of the pure CdS hydrogel microspheres. The pure CdS hydrogel microspheres showed a slightly cracked structure while the CdS-*co*-PNIPAAm hydrogel microspheres had a flat and tight structure with a more regular mass distribution. Note that the CdS-*co*-PNIPAAm hydrogel microspheres acquired a higher diameter range: from 2 to 20 μm for 2.5% NIPAAm-containing microspheres, from 8 to 44 μm for 5% NIPAAm-containing microspheres, and from 1 to 10 μm for pure CdS microspheres. At the constant stirring speed of 12,000 rpm, the volume of water droplets formed at the phase interior of benzyl alcohol is understood to be always regular, and with invariable dimensions. In this logic, the increased diameter of CdS-*co*-PNIPAAm hydrogel microspheres could be then associated to the isopropyl moieties of the PNIPAAm that require an additional volume at interior of the network to be accommodated in a stable and organized configuration.[23] In addition, there should be a movement of PNIPAAm chains in the direction of the external phase (closer to alcohol−water interface) because of the hydrophobic characteristics of the isopropyl groups of the PNIPAAm. It is then reasonable to consider that the PNIPAAm chains tend to be preferentially accommodated at the regions closer to sphere surface rather than at the sphere center.

CONCLUSIONS

Hydrogel microspheres based on the π-CdS were synthesized by way of a crosslinking-copolymerization reaction with the NIPAAm monomers. The incorporation of vinyl groups issue from GMA onto the CdS was processed with the use of GMA in an aqueous solution. ^{1}H NMR and ^{13}C NMR spectra revealed the formation of 3-methacryloyl-1-glyceryl ester of π-CdS and 3-methacryloyl-2-glyceryl ester of π-CdS that are reaction products resultant of the epoxide ring-opening mechanism via. The synthesis of microspheres occurred via radical reaction of the

vinyl groups at the π-CdS with NIPAAm in the presence of sodium persulfate in a water–benzyl alcohol microemulsion at 12,000 rpm. The formation of the spherical structure results of the polymerization-crosslinking reaction of the π-CdS with NIPAAm monomers at the droplets of water in view of both reactants have hydrophilic characteristics at the temperature at which the reaction was processed. The pure CdS hydrogel microspheres showed a slightly cracked structure while the CdS-*co*-PNIPAAm hydrogel microspheres had a flat and tight structure with a more regular mass distribution. In addition, CdS-*co*-PNIPAAm hydrogel microspheres had a higher diameter range, which was associated to isopropyl chains of the PNIPAAm that require an additional volume at the network interior to be accommodated in a stable and organized configuration.

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