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Synthesis and characterization of thermoresponsive and biocompatible core–shell microgels based on *N*-isopropylacrylamide and carboxymethyl chitosan

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ABSTRACT

The objective of the study is to report thermally sensitive and biocompatible capability core–shell microgels. These core–shell microgels composed of a chemically crosslinked poly(*N*-isopropylmethacrylamide) (PNIPAM) core and a carboxymethyl chitosan (CMCS) shell were designed and synthesized by seed emulsion polymerization in this work. Due to the excellent biocompatible nature of CMCS, these PNIPAM/CMCS hybrid microgels have good biocompatibility. The chemical component of the PNIPAM/CMCS particles was confirmed by comparing the Fourier-transform infrared spectra (FTIR) of PNIPAM and PNIPAM/CMCS particles. Transmission electron microscopy (TEM) picture shows the core–shell structure of the resulted PNIPAM/CMCS nanocomposite particles. Thermally induced deswelling of PNIPAM/CMCS water dispersions which occurred in the increase of the temperature in the region of the lower critical solution (LCST) was investigated by means of dynamic light scattering (DLS). The thermal study by using differential scanning calorimetry (DSC) revealed that volume-phase transition temperatures of the physical microgel increased first with an increase in CMCS and slightly increased thereafter with a further increase in CMCS.

1. Introduction

In recent years, environmentally responsive hydrogels that can bring an abrupt change in volume in response to external stimuli have attracted increasing attention due to their potential applications in many fields such as drug delivery systems, tissue engineering and biosensors (Akdemir & Kayaman-Apohan, 2007; Brahim, Narinesingh, & Guiseppi-Elie, 2002; Madhumathi et al., 2009; Wang, Chen, Yu, & Li, 2007; Wang, Zhao, Yang, Xu, & Yang, 2007). Among these materials, a widely studied responsive polymer is thermosensitive PNIPAM, which exhibits a volume-phase transition around LCST (approximately 32 °C) close to that of human body surface (Huang & Lowe, 2005; Sugiura, Imano, Takagi, Sakai, & Kanamori, 2009; Zhang, Cheng, Huang, & Zhuo, 2003). When the environmental temperature is below the LCST, the PNIPAM hydrogel adsorbs more water because of the strong hydrogen bonding between the water molecules and the amide groups of the PNIPAM. When the temperature increases, it will disrupt hydrogen bonds and favor hydrophobic interactions, and the hydrogel will demonstrate a sharp volume shrinkage and turn into the collapsed state (Cheng, Chu, Zhang, Zhou, & Xie, 2008; Ying, Kang, & Neoh, 2005; Zhang, Yang, Chung, & Ma, 2001).

Early studies have been mainly limited to the macroscopic hydrogels that need long equilibration time with respect to swelling and deswelling. However, a fast response is necessary for their practical usage (Hellweg, Dewhurst, Eimer, & Kratz, 2004; Zhang et al., 2008) in potential applications. While its size is much smaller than that of macrogels, the microgels respond to the external stimuli more quickly than that of macrogels and could be widely used in practical applications compared to macroscopic hydrogels (Matsumura & Iwai, 2005; Pelton, 2000).

PNIPAM microgel particles were first synthesized from *N*-isopropylacrylamide (NIPAM) and *N*,*N*′-methylenebisacrylamide (MBA) as chemical cross-linker in 1986 (Pelton & Chibante, 1986). Since then, PNIPAM microgel particles have attracted much attention and been extensively studied for drug delivery systems (Chung et al., 1999; Demanuele & Dinarvand, 1995). However, these microgels are not biocompatible because of their synthetic nature, which

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Table 1
Experimental conditions for core–shell microgel syntheses.

Sample ID	NIPAM (g)	CMCS (g)	MBA (mg)	H_2O (ml)
PNIPAM	1	0	20	30
PNCM01	1	0.5	20	30
PNCM02	1	1	20	30

may restrict their applications in drug release under physiological conditions (Liu, Huang, Liu, & Hu, 2007). For the purpose of biomedical application, there has been a growing interest in introducing the monomers with biological characteristics into PNIPAM to improve its biocompatibility and broaden its applications.

Therefore, the main objective of the present work is to improve PNIPAM with biocompatible components. Carboxymethylchitosan (CMCS), an important water-soluble chitosan derivative, contains –COOH groups and $–NH_2$ groups in the molecule. It has many attractive chemical, physical and biological properties such as low toxicity and excellent biocompatibility, all of which make it a promising biomaterial. Due to its unique properties, particularly its biocompatibility, CMCS has been extensively used in a variety of applications in the biomedical field (Guo & Gao, 2007; Lu et al., 2007; Wang, Chen et al., 2007; Xue, Li, & He, 2009).

Numerous reports have described different structures of PNIPAM-based core–shell particles with hydrophobic polymer as core and PNIPAM as shell or NIPAM copolymer as core and PNIPAM as shell (Lin, Chiu, & Lee, 2005; Santos, Elaissari, Martinho, & Pichot, 2005; Yi & Zheng, 2009). Particles with core–shell structures have been used to ensure materials with at least two kinds of properties, i.e., one endowed by the core and the other by the shell (Cui, Zhong, Xu, & Wang, 2007). However, as far as we know, little work has been done regarding particles with PNIPAM as the core and other polymer as the shell. Since the biomaterial surface first comes into contact with the living tissue when the biomaterial is planted in the body, the initial response of the body to the biomaterial depends on its surface properties. So, a study for modifying the surface of PNIPAM particle that has excellent biocompatibility is significant and helpful to the research on drug delivery (Zhao, Chen, Li, Su, & Zhang, 2006).

In this paper, we prepared PNIPAM particles coated by CMCS shell via the emulsifier-free seeded emulsion polymerization to improve biocompatible properties of PNIPAM. The chemical structure and morphology of the PNIPAM/CMCS microgel were determined by FTIR and TEM as well. The temperature sensitivity of the microgels was measured by DSC and DLS.

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAM) (Acros, Belgium) was recrystallized from a 65:35 (v/v) mixture of hexane and benzene and dried in vacuum. Carboxymethylchitosan (CMCS) was obtained from Nantong Xincheng Biological Industrial Co., Ltd. (Nantong, China). *N*,*N*′-Methylenebisacrylamide (MBA) (Acros, Belgium), ammonium persulfate (APS) and sodium pyrosulfite (SPS) are analytical reagents from Aldrich. All other reagents and solvents were of analytical grade and used without further purification.

All reactions were carried out in a four-neck, round-bottom 250 mL flask that was equipped with a stirrer, a reflux condenser, a thermometer and a nitrogen entrance, and heated in a water bath. The reflux condenser also acted as the nitrogen exit. The reaction conditions are listed in Table 1. The initiator concentration was varied in some experiments.

2.2. Preparation of PNIPAM/CMCS core–shell nanoparticles

The PNIPAM seed latex was prepared by emulsifier-free polymerization at 60 °C NIPAM and deionized water were charged into the flask and stirred extensively to make sure that NIPAM was dissolved completely. Then MBA was added and the solution was bubbled with nitrogen more than 30 min under slow stirring to remove the oxygen in the solution. When the reaction temperature was reached, the solution of APS and SPS as a pair of redox initiators dissolved in 2 ml H_2O was injected, respectively. The reaction continued for 3 h and then finished.

After the first stage of the reaction was completed, the latex was quenched to room temperature. In the second stage, CMCS was added quantitatively into the seed latex emulsion system and stirred continuously under the atmosphere of nitrogen. At the moment the monomer was swelled into the seed latexes owing to the stirring process and the seed was swollen for 24 h at room temperature. Then the reaction system was heated in a water bath. The aqueous solution of APS and SPS were added into the reaction system to begin the reaction of the second stage of soapless seeded emulsion polymerization. The final mixture was reacted for 3 h. The deionized water was used to wash the cooled latex repeatedly to remove unreactive monomers and other impurities at room temperature by the method of centrifugation.

In the second stage, the monomer and initiator solution were added at a low rate in order to avoid a high concentration of monomer in the outer layers of the seed particles. It would lead to particle agglomeration when the particle surface becomes very sticky at monomer concentrations between 40% and 60% (Goncalves, Asua, de Araujo, & Machado, 2008).

2.3. Characterization of composite microspheres

2.3.1. Nanostructure characterization

TEM and DLS techniques were used to get information about the morphology and the size of the nanoparticles.

The purified nanoparticles were diluted in distilled water and a drop of suspension was placed on a Formvar-coated copper TEM grid and dried overnight at an ambient temperature. TEM micrographs were acquired by using a Hitachi H-7650 electron microscope when operating at 80 kV.

The size, the deswelling behavior and the polydispersities of microgel in distilled water were investigated by using temperature controlled DLS. Dynamic light scattering experiment was performed with an ALV (CGS-8F) laser light scattering spectrometer in pseudocross-correlation mode, which was equipped with an ALV-6010/160 mutiple Tau digital real time correlator. A He–Ne laser was used as the light source with a outpower of 25 mV and a maximum wavelength of 632.8 nm (λ). All the measurements were made at a 90 °C angle. Before DLS measurement, the latex was diluted with distilled water to an appropriate concentration and all dust was removed with a filter (1.2 μm). The average hydrodynamic diameter of the particles at different temperatures was measured after equilibration at each temperature for 10 min.

For FTIR measurements, the solid sample of the composite-latex was obtained by removing water in vacuum at room temperature. FTIR spectra were recorded on a Tensor 27 FTIR spectrometer with KBr pellets.

2.3.2. Thermal analysis

Thermal analysis was performed with a differential scanning calorimeter (Diamond, DSC). A certain amount of polymer sample was hermetically sealed in an aluminum pan. Heating scans were recorded in the range of 20–55 °C at a scan rate of 3 °C $\min^{-1}$. Deionized water was used as a blank reference.

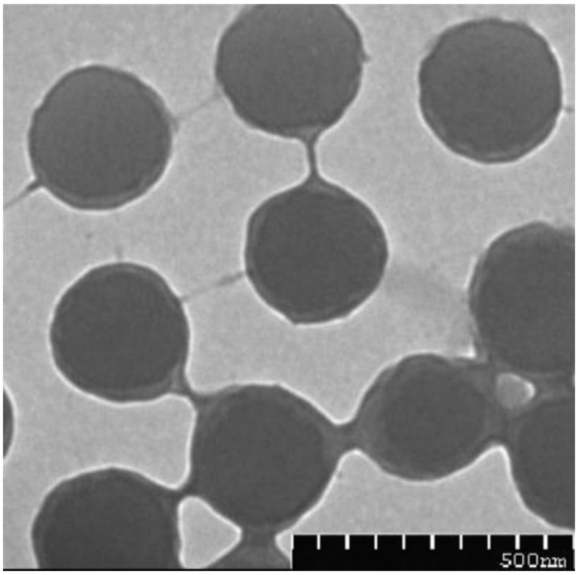

Fig. 1. The TEM image of PNCM01.

3. Results and discussion

3.1. Appearance and microstructures of the microgel

To investigate the microstructure of the PNIPAM/CMCS composite particles, TEM is performed. To further identify clearly the shapes of the PNIPAM/CMCS microgel particles, TEM image of PNCM01 particles is given in Fig. 1. It can be found that the microgels are spheres of a regular size. Moreover, the appearance indicates an obvious core–shell nanostructure with PNIPAM (the black region) as the core and CMCS (the pale region) as the shell. Furthermore, the size of PNCM01 particles is about 200 nm. It is worth noting that the nanoparticle sizes measured by this technique are somewhat inaccurate because the particles might have a trend to flatten and spread on the TEM grids during the sampling preparation. So the microgel gave a larger dispersity due to sample preparation (Ma, Huang, Zhu, Zhan, & Tang, 2005). The relatively lower dispersities of the synthesized microgels could be investigated by DLS.

3.2. FTIR spectrum of the microgel

The chemical compositions of the synthesized PNIPAM/CMCS and PNIPAM, CMCS, were confirmed by FTIR measurements. As shown in Fig. 2, the absorption bands at 1600, 1411, and 1066 cm^{-1} in the spectrum of CMCS were assigned to the anti-symmetrical, symmetrical stretching of COO$^-$ group and the C–O–C stretching band, respectively. The typical amide I and II bands at 1645 and 1545 cm^{-1}, and the bending vibrations of isopropyl groups at 1387 and 1367 cm^{-1} were observed respectively in the spectrum of PNIPAM. PNIPAM bands can also be seen clearly in the spectra of microgel. Since the PNIPAM bands overlapped the characteristic carbonyl band of CMCS, the skeletal peak of CMCS around 1066 cm^{-1} was used to detect its presence as shown in Fig. 2c.

3.3. Thermoresponsive properties

The calorimetrically obtained transition temperature of PNIPAM-based polymers is often defined as the onset of the DSC transition endotherm, T_{onset} (the intersection of the baseline and the leading edge of the endotherm). The temperature of the maximum heat capacity, T_{max}, is also used to describe the transition temperature (Chearuil & Corrigan, 2009). In the current work the phase transition temperature was defined as the temperature of onset.

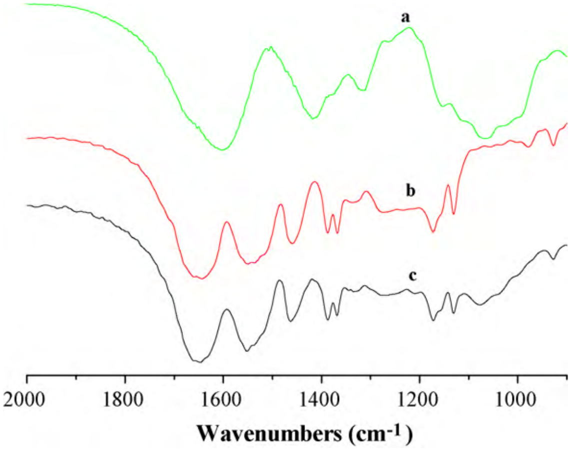

Fig. 2. FTIR spectra of (a) CMCS, (b) PNIPAM and (c) PNIPAM/CMCS.

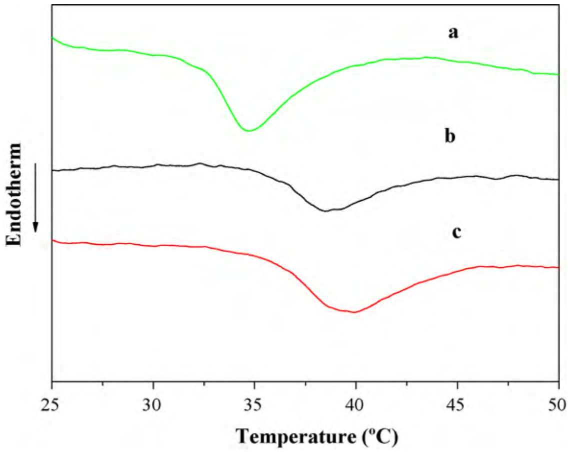

Fig. 3. DSC curves of (a) PNIPAM, (b) PNCM01 and (c) PNCM02 microgel dispersions.

Microcalorimetric endotherms for aqueous dispersions of PNIPAM, PNCM01 and PNCM02 are shown in Fig. 3 and some additional information and data is collected in Table 2. The endotherms are presented in this study as excess heat capacities vs. temperature. One can see that the endothermic enthalpies of the copolymers normalized to PNIPAM in Table 2 are all lower than that of PNIPAM homopolymer. This suggests that the existence of CMCS prevent the PNIPAM chains from collapsing completely (Liu et al., 2004).

Generally, the temperature at which the swelling degree of the sample decreases dramatically is regarded as the lower critical solution temperature (LCST) (Yan, Jing, Li, Zhai, & Wei, 2009). Thus, the LCST of PNIPAM/CMCS microgels is around 36 °C ($LCST_{PNCM01} = 36.22$ °C; $LCST_{PNCM02} = 36.44$ °C), which is slightly higher than that of pure PNIPAM microgel ($LCST_{PNIPAM} = 32.49$ °C)

Table 2
Transition temperature and transition enthalpies determined by DSC.

Samples	Transition temperature (°C)		ΔH_{tr} (J g^{-1})
	T_{onset}	T_{max}	
PNIPAM	32.49	34.73	0.5865
PNCM01	36.22	38.47	0.3100
PNCM02	36.44	39.89	0.5163

and is closer to that of body temperature. This increase in temperature of onset suggests that CMCS increase the overall hydrophilicity of the systems as shown by the increase in the LCSTs. However, the thermal study revealed that the volume-phase transition temperatures of the physical microgel first increased with an increase in CMCS and slightly increased thereafter with a further increase in CMCS. Because CMCS is water-soluble, part of CMCS in the shell zone was washed out at the purge process and the residual part of CMCS was chemically bound up with PNIPAM. The result is that the shell was made thin and its thickness was inconsistent with the feeding ratio of PNIPAM/CMCS as shown in Fig. 1, and the LCST increased a little with the further increase in CMCS.

It is believed that the increase in the LCST from 100% PNIPAM is attributed to the increase of hydrophilic CMCS. In a pure PNIPAM microgel system, there exists a hydrophilic/hydrophobic balance in the NIPAM unit resulted from the hydrophilic (amide group) and hydrophobic (isopropyl group) regions of the PNIPAM. Generally, the main reason for this thermosensitive property of the PNIPAM microgel has been attributed to its uniquely rapid alteration in hydrophilicity and hydrophobicity (Khan, 2007).

At temperatures below the LCST, the strong H-bonding between the hydrophilic groups and water outweighs the unfavorable free energy related to the exposure of hydrophobic groups to water, leading to good solubility of the polymer in water (Herman, You, Jan, & Sung, 1993). However, as the external temperature increases to the LCST, the hydrogen bonds are overwhelmed by the hydrophobic interactions among the hydrophobic groups. As a result, entrapped water molecules are released from the gel via hydrogen bonds, and cause phase separation and shrinkage of the gel matrix (Zhang, Wu, & Chu, 2004). Similar to the PNIPAM microgel, the PNIPAM/CMCS microgels became swollen at temperature below the LCST, but underwent a deswelling process when the temperature was increased.

An incorporation of CMCS into PNIPAM would increase the LCST of the microgel. It can be explained that the carboxylic group (–COOH) of CMCS not only inhibits a hydrophobic interaction of isopropyl group, but also enhances an H-band between the –OH group of water and polymer chains. As a result, more energy would be required to break the interactions and thus the LCSTs were increased. From the point of application, a higher LCST of thermosensitive microgel is important since the LCST of the pure PNIPAM microgel is lower than that of body temperature. Thermosensitive microgels with their LCST near the body temperature would permit us to use this intelligent property for the biomedical applications.

3.4. *Dynamic light scattering*

Particle diameters were measured by dynamic light scattering in dilute dispersions. The hydrodynamic diameter of the particles, D_h, is the distance from the center of the particle to the shear plane at the surface, which is assumed to be that of a swollen particle (Duracher, Sauzedde, Elaissari, Pichot, & Nabzar, 1998).

Fig. 4 presents the plots of hydrodynamic diameter (D_h) of the PNIPAM/CMCS microgels as a function of temperature. It is clearly seen that the D_h decreases when the temperature is raised. Though the value of volume-phase transition temperature measured by DLS is lower than that measured by DSC, the changing trend is doubtless. However, in the temperature range (29–45 °C), particle size drastically decreases continuously with the increase of temperature, indicating that the prepared microgels are sensitive to temperature as a result of the reversible formation and breakage of hydrogen bonding between water molecules and hydrophilic groups, and the hydrophilic–hydrophobic balance between the hydrophilic and hydrophobic groups within the polymer chains.

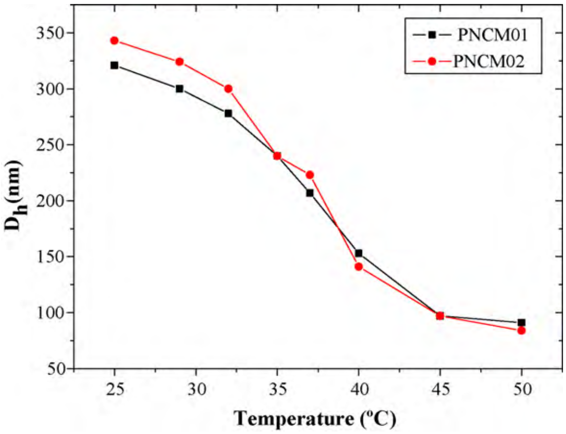

Fig. 4. Hydrodynamic diameters of the core–shell particles of PNCM01 and PNCM02 as a function of temperature.

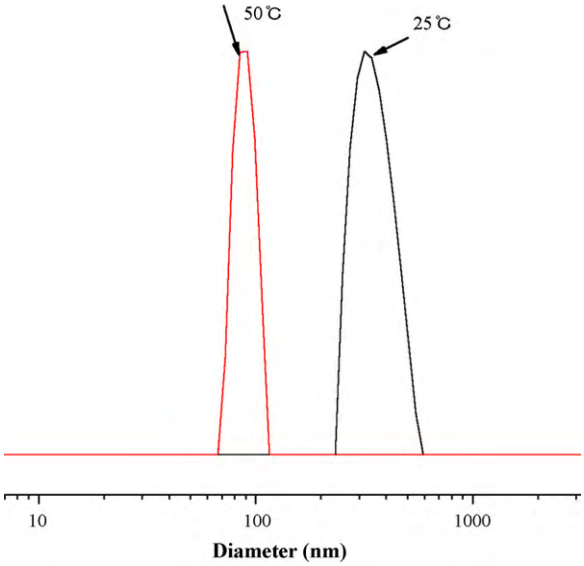

Fig. 5. Plot of size distribution of the PNCM01 microgel at 25 °C and 50 °C.

The particle size characterized by DLS is slightly different from that of the TEM image. Although there are some differences between the different methods because of the systematic errors caused by the instruments and the methods, the copolymers were in the nanometer size range which was the objective of the study.

The average diameter obtained by DLS at low temperature is larger than that at high temperature. It is understandable that the final particles exhibit an excellent swelling behavior in water below LCST and are in a shrunken form at temperature above the LCST.

Another important quantity for evaluating the swelling properties of microgel particles is the so called swelling (or deswelling) ratio (α) which can be calculated by using the hydrodynamic diameter in the swollen and shrunken states (Zhang, Zha, Ma, & Liang,

Table 3
Deswelling ratio of the microgels with various CMCS content.

Code	D_h (nm)		α
	25 °C	50 °C	
PNCM01	321	91	43.89
PNCM02	343	87	61.28

2007).

$$\alpha = \frac{V_{\text{swollen}}}{V_{\text{shrunken}}} = \left(\frac{D_h^{298.15\,\text{K}}}{D_0^{323.15\,\text{K}}}\right)^3$$

Here, the swelling ratios for the PNIPAM/CMCS microgels were calculated from the hydrodynamic diameters obtained at 25 °C and 50 °C. It is well-known that deswelling ratio (α) reflects to some extent the cross-linking density of PNIPAM/CMCS microgels. As summarized in Table 3, it is obvious that α value of the PNCM02 microgel is much larger than that of the PNCM01 microgel due to the hydrophilicity of CMCS.

Fig. 5 displays a typical size distribution plot of the PNCM01 microgel at 25 °C and 50 °C, respectively, from which it can be seen that size distribution of the PNCM01 microgel is narrow. Furthermore, with the increase of temperature, the size distribution becomes narrower, which can be ascribed to the globule-to-coil conformation transition when the temperature exceeds that of volume phase transition of microgels.

4. Conclusions

A novel microgel class consisting of biocompatible CMCS and temperature-sensitive PNIPAM was designed and synthesized by seeded emulsion polymerization. The resulted microgels would have combined unique properties of their component precursors. Due to the presence of PNIPAM in microgels, they acquired thermoresponsive capability to CMCS, while CMCS with the biological properties could improve biocompatibility of the microgels (Zhang, Li, Gong, Zhao, & Zhang, 2002). The properties of these microgels, such as swelling ratio and their LCSTs, were examined in terms of the composition ratio of CMCS to PNIPAM. Most importantly, the phase transition temperature of these intelligent microgels is nearer to that of the body compared with the pure PNIPAM, which is critical for some of their biomedical applications.

Acknowledgments

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