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Fluorine-containing thermo-sensitive microgels as carrier systems for biomacromolecules

Yuanyuan An[a], Lingfei Zhang[a], Shengdong Xiong[a], Shuilin Wu[a], Ming Xu[b,*], Zushun Xu[a,*]

[a] Ministry-of-Education Key Laboratory for the Green Preparation and Application of Functional Materials, Faculty of Materials Science and Engineering, Hubei University, Wuhan 430062, China
[b] Wuhan City Vocrtional College, Wuhan 430062, China

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ABSTRACT

A series of microgels with nanometer diameter were prepared from 2,2,3,4,4,4-hexafluorobutyl methacrylate (HFMA, FA for short) and N-isopropylacrylamide (NIPAAm). The composition and structure of microgels were studied by FTIR and ^{1}H NMR. The transmission electron microscope (TEM) results showed that the introduction of fluorine was very advantageous in improving the stability and monodisperity of NIPAAm microgel. The differential scanning calorimetry (DSC) and photon correlation spectroscopy (PCS) revealed that fluorinated NIPAAm microgels still displayed obvious temperature-responsiveness, and the lower critical solution temperature (LCST) of microgel particles decreased significantly. PCS tests also displayed the swelling and absorption behavior of microgels. Fluorescence spectrum showed that the microgel particles were able to combine with BSA, which would facilitate further development of fluorinated thermo-sensitive microgel in biomacromolecule carrier and delivery system.

1. Introduction

Thermo-sensitive hydrogel can sense the change of environment temperature, and respond by swelling or shrinking of volume [1]. These intelligent materials have been widely concerned and researched, because of their great application potential in drug delivery [2–5], cell encapsulation and delivery [6], cell culture [7], tissue engineering [8,9], biosensors [10] and other biomedical fields. With a lower critical solution temperature (LCST) at about 32 °C in pure water, N-isopropylacrylamide (NIPAAm)-based thermo-sensitive hydrogel becomes a focus [11].

Poly(N-isopropylacrylamide) (pNIPAAm) assumes a flexible, extended coil conformation in aqueous solutions below the LCST. At the LCST, it becomes hydrophobic and the polymer chains seem to collapse prior to clustering in globular structures. Copolymerization of NIPAAm with a hydrophilic monomer would result in an increase in LCST. Likewise, copolymerization with a hydrophobic monomer leads to a lower LCST than pure pNIPAAm. This may help to adjust the LCST of thermo-sensitive hydrogel [12].

Owing to the unique characteristics of fluoropolymers such as high hydrophobicity, high thermal and mechanical stability, low dielectric constant [13,14], oil- and water-repellency, and very interesting surface properties [15,16], attention has recently been paid to fluoropolymer. Studies from the perspective of biomedical applications of fluoropolymer [17], for instance, blood substitutes, gas carriers, bioconversion, extraction, and so forth are becoming hotspots. Here we use fluoride as hydrophobic monomer to modify NIPAAm thermo-sensitive hydrogel.

Conventional hydrogel has many defects such as low mechanical strength [18–20], slow respond speed [21–24] and nonbiodegradability [25]. To our knowledge, the response rate could be improved substantially by reducing the size of hydrogel [26]. In other words, being small makes microgel faster to respond to environmental changes. Meanwhile, unlike block hydrogel, microgel could be injected into and excreted from the body, without surgeries of embedding and removing. Thus, the small size is also helpful to avoid traditional poor mechanical strength and nonbiodegradability of bulk hydrogel when it is used in human bodies. Furthermore, core/shell morphology, which offers the possibility to control the distribution of the particles, make the microgel have particular physical and chemical properties [27,28]. Hence, it is a good choice to adopt core/shell structure to get microgel's high stability, small size, and good monodispersity.

Although some microgels based on NIPAAm had been reported [27,29,30], there are few reports on fluorinated NIPAAm microgels and its interaction with biomacromolecules. In this paper, stable and uniform fluorinated NIPAAm thermo-sensitive microgel

* Corresponding authors. Tel.: +86 27 61120608; fax: +86 27 88665610.
E-mail addresses: whxmyun2005@126.com (M. Xu), zushunxu@hubu.edu.cn (Z. Xu).

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was successfully synthesized under microwave radiation [31]. The structure of the microgel was characterized by FTIR and ^{1}H NMR, the thermo-sensitivity was measured by photon correlation spectroscopy (PCS) and differential scanning calorimetry (DSC) [32,33]. The dispersive stability of microgel particles in aqueous media was evaluated by PCS. In addition, the interaction between microgel and bovine serum albumin (BSA) [34] was analyzed by fluorescence and TEM. We have studied the adsorption of protein on fluorine-containing amphiphilic copolymers micelles [35]. Here we try to prepare a novel series of fluorine-containing thermo-sensitive copolymer microgels had promising potential applications as an intelligent biomacromolecule carrier and delivery system in biomedical field.

2. Experimental

2.1. Materials

All the reagents were purchased from Sinopharm Group Chemical Reagent unless otherwise noted. N-isopropylacrylamide (NIPAAm), N,N′-methylenebisacrylamide (MBA) and anhydrous alcohol were used as received. The potassium persulfate (KPS) was recrystallized and dried prior to use. 2,2,3,4,4,4-hexafluorobutyl methacrylate (HFMA) was purchased from Harbin Xeogia Fluorine-silicon Material Co., Ltd., distilled under reduced pressure, and stored under dry environment with low temperature. Bovine serum albumin (BSA) was purchased from Biosharp. Deionized water, obtained with a Milli-Q system, was used for all synthesis reactions, purification and solution preparation.

2.2. Preparation of non-core/shell microgel

Solution of monomers and cross-linking agent was prepared by dissolving a certain amount of NIPAAm, MBA and FA in 35 ml mixed solvent of water and ethanol, stirring vigorously till the solution was clear while inputting nitrogen flow. Next, the reaction was performed under microwave in a 100 ml four-neck round-bottom flask which equipped with a magnetic stir bar, a reflux condenser, thermometer, and nitrogen inlet. As the reaction temperature reached 75 °C, KPS was added to the solution of monomers and cross-linker. After 3-h reaction, the poly[2,2,3,4,4,4-hexafluorobutyl methacrylate-co-N-isopropylacrylamide] [P(HFMA-co-NIPAAm)] microgel emulsion was reached.

The production was purified by dialysis (Dialysis membrane, MWCO14000) against water (two changes per day for two days at 25 °C). The control sample, pNIPAAm microgel emulsion was prepared in the same way.

2.3. Preparation of microgel with core/shell structure

In this work, both two types of microgels were prepared by seed emulsion polymerization under microwave. Microgel with fluorinated shell [C–S(F)] was synthesized at first. A certain amount of NIPAAm, and MBA were dissolved and decentralized in 30 ml water. Reactions were then conducted in four-necked, round-bottom flask equipped with a magnetic stirrer, thermometer, condenser and nitrogen inlet in the microwave reactor. It was heated to 75 °C with nitrogen bubbling and radical polymerization was then initiated with KPS. The stirring solution was allowed to react for a period of 30 min under nitrogen. After pre-emulsification, the shell monomer mixture (NIPAAm, MBA and FA) was added into the flask. The mixture was heated to 75 °C with nitrogen bubbling about 15 min. The secondary polymerization was finally initiated through the addition of KPS. Following synthesis, the microgel with fluorinate shell [C–S(F)] was purified by dialysis (Dialysis membrane, MWCO14000) against water (two changes per day for two days at 25 °C).

The same procedure was used to synthesize fluorinate core microgel, whose core was made of p(NIPAAm-co-FA) and shell NIPAAm alone. The production, microgel with fluorine-containing core [C(F)–S] was purified by dialysis (Dialysis membrane, MWCO14000) against water (two changes per day for two days at 25 °C).

2.4. Characterization of microgel

2.4.1. Composition and structure analysis

A Fourier transform infrared spectrometer (Spectrum One, Perkin–Elmer) was used to identify the structure. Solid samples after dialysis, purification, demulsification and drying were dissolved in tetrahydrofuran, and were coated on KBr disks.

The ^{1}H NMR spectra were measured by a VarianINOVA-600 NMR spectrometer at 150 MHz, using DMSO as solvent.

2.4.2. Surface tension measurement

The surface tension measurement was performed by the wilhelmy Type using a Krüss interface tension meter (KrüssGmbH, Hamburg, Germany) at 20 °C. The freshly prepared stock solutions were diluted to different concentrations for surface tension measurement. The same solution was used in the PCS and TEM studies. From the results of the surface tension measurements, the CMC of synthetic was extrapolated.

2.4.3. Transmission electron microscopy

The morphology of the microgel, polymer/BSA complexes was characterized with TEM (Tecnai G20, FEI Corp., USA). A drop of the sample was placed on a Formvar-coated copper grid which was dried in air. The TEM images were obtained at 25 °C at an electron acceleration voltage of 80 kV.

2.4.4. Photon correlation spectroscopy

The average hydrodynamic diameter and size distribution of the microgel were measured by photon correlation spectroscopy (PCS) (Autosize Loc-Fc-963, Malvern Instrument). The experiments were performed by changing temperature from 25 °C to 37 °C. The sample was directly poured into a cuvette, the cuvette was set inside a sample holder then. Measurements were made at an angle of 90° with 679 nm wavelength laser light. All the data were averaged from 3 to 5 parallel measurements.

2.4.5. Differential scanning calorimetry

Differential scanning calorimetry thermograms were obtained on a Perkin-Elmer DSC-7 differential scanning calorimeter. Samples were placed in aluminum pans and measurements were performed under nitrogen atmosphere by raising the temperature from 20 °C to 50 °C at a rate of 1 °C/min.

2.4.6. Fluorescence

The fluorescence properties of microgel in the absence and presence of BSA were studied on a RF-540 (Hitachi high-technologies corporation, Tokyo, Japan) spectrometer. The fluorescence emission spectra were recorded in the wavelength range 300–400 nm by exciting the samples at 280 nm and the excitation and emission slits were 10 nm.

3. Results and discussion

3.1. FTIR

FTIR studies were performed to confirm the nature of microgel. Fig. 1 showed the FTIR spectra of the pNIPAAm(A) and