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SHORT COMMUNICATION

Xian-Zheng Zhang
Chih-Chang Chu

A responsive poly(*N*-isopropylacrylamide)/poly(ethylene glycol) diacrylate hydrogel microsphere

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X.-Z. Zhang · C.-C. Chu (✉)
Department of Textiles and Apparel &
Biomedical Engineering Program,
Cornell University, Ithaca,
NY 14853-4401, USA
E-mail: cc62@cornell.edu

Abstract A responsive hydrogel microsphere, which is constituted by poly(*N*-isopropylacrylamide)/poly (ethylene glycol) diacrylate, was fabricated in an aqueous two-phase system based on the polymer–polymer immiscibility. Characteristics of the hydrogel microsphere, such as the particle size and the morphology of freeze-dried or hydrated natural microspheres in water, tetrahydrofuran (THF)/H_2O (1:1 in volume) or acetone/H_2O (1:1 in volume), were investigated. The results showed that the swelling ratio and the particle size of the hydrogel microspheres were highly dependent on solvent composition. In addition, these characteristics were dramatically reduced when THF or acetone was added into the aqueous media. Scanning electron microscopy and environmental scanning electron microscopy micrographs also visually demonstrated that the regular spherical shape of the microspheres in water turned to irregular in shape when the microspheres were immersed in THF/H_2O or acetone/H_2O mixtures instead of pure water.

Keywords Responsive · Hydrogel microsphere · Aqueous two-phase solution

Introduction

A hydrogel is a hydrophilic network that swells and absorbs a significant amount of water in aqueous media, but continues to maintain its three-dimensional structure owing to cross-linkages among polymeric chains. Owing to the structural similarity in high water content and surface properties to natural living tissues [1, 2], hydrogels have found various applications in fields of cell culture, tissue engineering, and controlled drug release. Among hydrogels, the responsive hydrogel has attracted much interest because of its unique property to undergo abrupt changes in structure and physical properties in response to external stimuli changes [3, 4, 5, 6]. For example, Hirokawa and Tanaka [6] reported that a poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel in water, which was cross-linked by *N*,*N*′-methylenebisacrylamide, may exhibit a volume-phase transition in response to external temperature change as well as the solvent composition changes [6].

One disadvantage of conventional macroscopic PNIPAAm hydrogels is the prolonged response time [7, 8]. Fast response to external stimuli, however, is a prerequisite for many potential applications. Decreasing the size of PNIPAAm hydrogels was found to be effective in solving this problem. Hydrogel microspheres are cross-linked latex particles and PNIPAAm hydrogel microspheres may exhibit a much faster response rate without losing similar characteristics as macroscopic hydrogels, such as the transition temperature and the swelling capacity of the volume-phase transition. Therefore, the attempts to improve the technique to prepare colloidal hydrogel microspheres have been intensified recently and many investigations are focused on PNIPAAm hydrogel microspheres or microgels [9, 10, 11, 12].

To date, a considerable number of strategies were developed to obtain microspheres. For example, emulsion/dispersion, inverse emulsion or multiple emulsion polymerization methods are widely used to fabricate microspheres [13, 14, 15, 16, 17]. Generally, an organic solvent is needed for the emulsion methods, which can limit the wide applications of microspheres especially in the biomedical field of controlled release of protein drugs [18, 19, 20]. Very recently, a technique excluding the use of an organic solvent was proposed and the aqueous two-phase technique is a promising method to avoid using any organic solvents [21, 22]. Such a technique is based on the polymer–polymer incompatibility because some aqueous solutions with a certain concentration will separate from each other.

In this work, a new PNIPAAm/poly(ethylene glycol) diacrylate (PEG-DA) hydrogel microsphere was fabricated in an aqueous two-phase system. PNIPAAm hydrogels are usually formed through covalent cross-linking by using a commercial cross-linking agent, such as *N*,*N*′-methylenebisacrylamide [6]. Instead of the conventional cross-linker, PEG-DA was synthesized and used as a new cross-linker. It was known that PEG has good biocompatibility and PEG based polymers have been proven to be very successful in preventing microclimate-induced instability reactions of proteins [23, 24, 25, 26, 27]. The temperature responsive properties of PNIPAAm-based microspheres have been studied extensively [28, 29, 30, 31]. In addition to the temperature-induced responsive behavior, Crowther and coworkers [32, 33] investigated the effect of alcohols on the swelling behavior of responsive hydrogel microspheres in alcohol/water mixtures. This paper presented the preliminary characteristics of the response of PNIPAAm/PEG-DA hydrogel microspheres concerning particle size as well as shape, morphology changes in different solvent compositions, i.e., water or a tetrahydrofuran (THF)/H_2O mixture (1:1 in volume) or an acetone/H_2O mixture (1:1 in volume).

Experimental

Materials

All the reagents used were of analytical grade, unless otherwise stated. NIPAAm (from Aldrich Chemical Company, USA) was further purified by recrystallization in benzene/*n*-hexane. Dextran a the molecular weight of 66,000, PEG diol with a molecular weight of 8,000, acryloyl chloride, anhydrous magnesium sulfate, ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Sigma Chemical Company (St. Louis, MO).

Preparation of hydrogel microspheres

PED-DA 8000 was synthesized and purified according to the method in Ref. [34]. The typical hydrogel microsphere fabrication procedure is described as follows. In brief, the starting precursors PEG-DA (8,000, 0.35 g) and NIPAAm (0.75 g) were dissolved in 5.0 ml distilled water to form an aqueous phase and dextran (3.0 g) and $MgSO_4$ (3.0 g) were dissolved in 10 ml distilled water to form another aqueous phase. Here, the anhydrous $MgSO_4$ was used to salt-out the polymer in order to facilitate the formation of the aqueous two-phase system as mentioned earlier. The two aqueous solutions were vigorously mixed for 60 min at a stirring rate of 800 rpm. Phase separation takes place and the resulting water-in-water emulsion system was allowed to stabilize for 30 min without stirring. Subsequently, 0.5 ml APS solution (50 mg/ml) and 0.1 ml TEMED were added. The mixture was initiated for 12 h at room temperature to polymerize/cross-link the acryloyl moieties in the NIPAAm and PEG-DA. The cross-linked PNIPAAm/PEG-DA particles were purified by multiple centrifugation and washing steps with distilled water.

Characterization

The equilibrium swelling ratio (ESR) at room temperature was measured as follows. Accurate amounts of dried microsphere samples were measured in a cylindrical tube, which was covered to avoid liquid evaporation. Solvent, i.e., H_2O, THF/H_2O or acetone/H_2O, was added into the tube and the microspheres were allowed to swell at room temperature for at least 12 h to reach the equilibrium state. The equilibrated hydrogel microspheres were centrifuged and concentrated. After carefully removing the upper transparent liquid with a pipette, the microspheres collected were weighted and recorded rapidly. The average value among three equilibrium values for each sample was taken, and the swelling ratio was calculated as $(W_s - W_d)/W_d$, where W_s is the weight of swollen hydrogel microspheres and W_d is the weight of dry microspheres.

The particle size distribution was obtained using a particle size analyzer (particle size analyzer 2010, Brinkmann Instruments, NY, USA), which functions under the principle of laser diffraction. The samples were suspended in water or a THF/H_2O mixture (1:1 in volume) or an acetone/H_2O mixture (1:1 in volume) with about 5 wt % concentration respectively and the solvent in the coverlet was stirred gently.

Scanning electron microscope (SEM) investigation was carried out using a Hitachi S4500 SEM (Mountain View, CA, USA). The concentrated swollen microspheres were first freeze-dried in a Virtis freeze drier (Gardiner, NY, USA) under vacuum at −42 °C for at least 3 days

until all the solvents had sublimed. Before SEM observation, specimens of the hydrogel microspheres were fixed on aluminum stubs and coated with gold for 40 s.

A Philips ElectroScan 2020 environmental scanning electron microscope (ESEM) was used to obtain images of the hydrated hydrogel microspheres in their natural swollen state. The sample particles do not need the deposition of an electrically conducting coating (e.g., gold) and are prepared simply by casting a drop of the microsphere suspension onto a microscopy stub for direct observation at room temperature.

Results and discussion

Equilibrium swelling ratio

From our experimental observations, the microsphere solution turned opaque from the translucent aqueous solution when microspheres were transferred into the THF/H_2O or the acetone/H_2O mixture. This result indicated that the microspheres precipitated and underwent phase separation in both mixtures, which was similar to the conventional macroscopic PNIPAAm hydrogel. The studies of the ESR also demonstrated that the ESR of hydrogel microspheres depends on the properties of the solvents at room temperature. With the addition of THF or acetone, there is a depression in the ESR value. When THF or acetone was added into the aqueous solution, the ESR was reduced to approximately 14 or 12, respectively, from a high ESR in pure aqueous solution of approximately 20.

The resultant hydrogel microspheres are composed of PNIPAAm and PEG-DA, and have responsive properties owing to the PNIPAAm moiety. It is well known that the traditional PNIPAAm hydrogel exhibits changes in its shape, volume and physical properties in response to the solvent composition. Linear PNIPAAm chains collapse and phase separate in mixtures of two solvents, although PNIPAAm dissolves well in each of them separately. Generally, PNIPAAm chains dissolve in water or polar organic solvents (e.g., THF, acetone) and the hydrogel exists in a swollen state at room temperature. However, the solubility of the PNIPAAm chains is greatly reduced if an organic solvent is added into the water to form a mixed solvent. In other words, PNIPAAm exhibits cononsolvency in the mixed aqueous solution [35, 36, 37, 38, 39] and the PNIPAAm-based hydrogel microspheres exhibit reduction or shrinkage in volume owing to the collapsed polymeric chains.

Particle size distribution

The evolution of the particle size distribution of hydrogel microspheres in water or mixtures is shown in Fig. 1. The same particle concentration was used in these experiments, only with different solvents. There is a clear shift of size distributions towards smaller size because of the addition of THF or acetone. As shown in Fig. 1a, when immersed in water, nearly 60% of the swollen microspheres exhibit a diameter at around 50 μm and around 30% of the swollen microspheres have a diameter in the range between 3 and 10 μm. However, when immersed in the THF/H_2O mixture, nearly 70% of the microspheres are around 20 μm in diameter and around 20% of the microspheres have a diameter below 1 μm (Fig. 1b). Obviously, the microspheres tended to be small in size when transferred from water to the THF/H_2O mixture. In order to further investigate the effects of organic solvent on the size, microspheres were immersed in the acetone/H_2O mixture and it was found that the microspheres tended to be smaller (below 15 μm in diameter, Fig. 1c), but with a wide distribution in size. The change of the size as well as the size distribution of the microspheres in different solvents is attributed to the solvent-sensitive nature of the PNIPAAm-based hydrogel.

Hydrogels swell in a good solvent and collapse in a poor solvent. If the solvent is somehow changed from good to poor, a phase separation would take place. As mentioned earlier, PNIPAAm dissolves well in water and the PNIPAAm hydrogel exhibits a swollen state at room temperature. However, the solubility of PNIPAAm is reduced dramatically when a polar organic solvent, such as THF or acetone, is added. The organic solvent exists as a cononsolvent for PNIPAAm chains in water [35, 36]. Owing to this special cononsolvency phenomenon, the PNIPAAm-based hydrogel exhibits a reentrant phase transition, where hydrogels collapse once and reswell as the ratio of the solvents in the mixture is varied monotonically [40]. In reality, the anomalous aggregation of the PNIPAAm hydrogel has been reported in a water/acetone mixture [41]. When THF or acetone was added into the aqueous solution, PNIPAAm chains precipitated, which caused the volume to collapse to a compact form of the hydrogel network. Thus, the PNIPAAm/PEG-DA hydrogel microspheres tended to be small. However, at the present stage, it is still an open question why the particle size in the acetone/H_2O mixture was so randomly distributed, and was different from the particle sizes in water or the THF/H_2O mixture.

SEM morphology

The SEM micrographs in Fig. 2 show the shapes and morphologies of the microspheres in different solvents, which highly influenced the shape as well as the morphology of the PNIPAAm/PEG-DA hydrogel microspheres. In addition, when the microspheres were

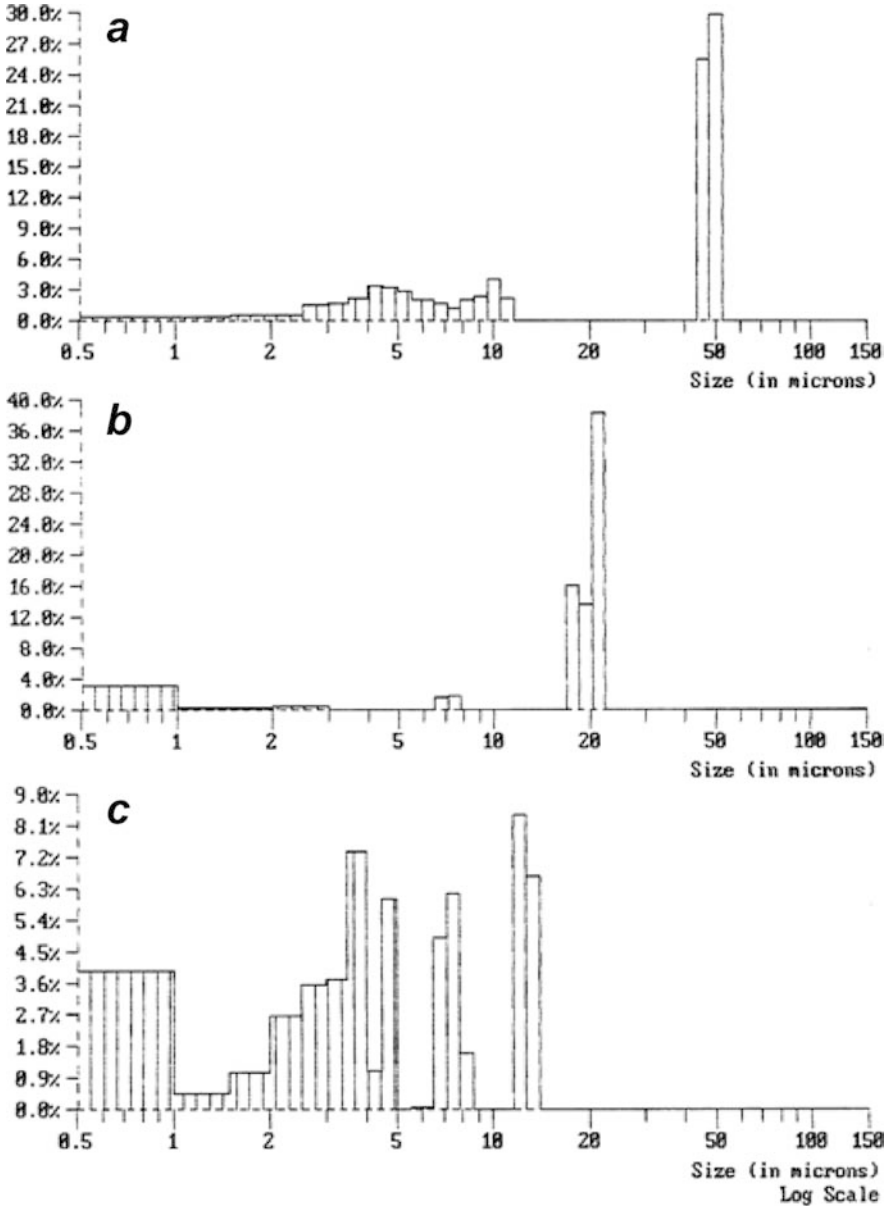
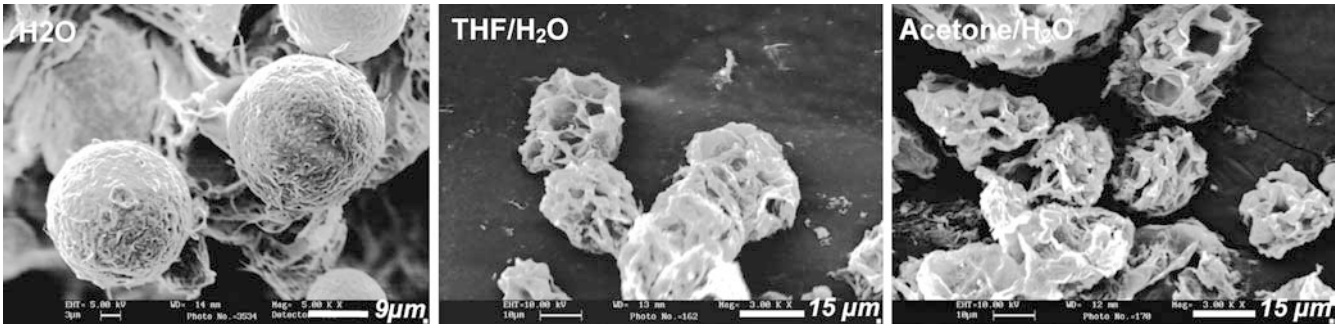


Fig. 1 Particle size distribution of poly(*N*-isopropylacrylamide) (*PNIPAAm*)/poly(ethylene glycol) diacrylate (*PEG-DA*) hydrogel microspheres in **a** water, **b** tetrahydrofuran (*THF*)/H_2O (1:1 in volume) and **c** acetone/H_2O (1:1 in volume)

Fig. 2 Scanning electron microscope micrographs of PNIPAAm/PEG-DA hydrogel microspheres in water, THF/H_2O (1:1 in volume) and acetone/H_2O (1:1 in volume)

submerged in water, they had a spherical and regular morphology with a porous homogeneous surface. However, the morphologies of the microspheres in



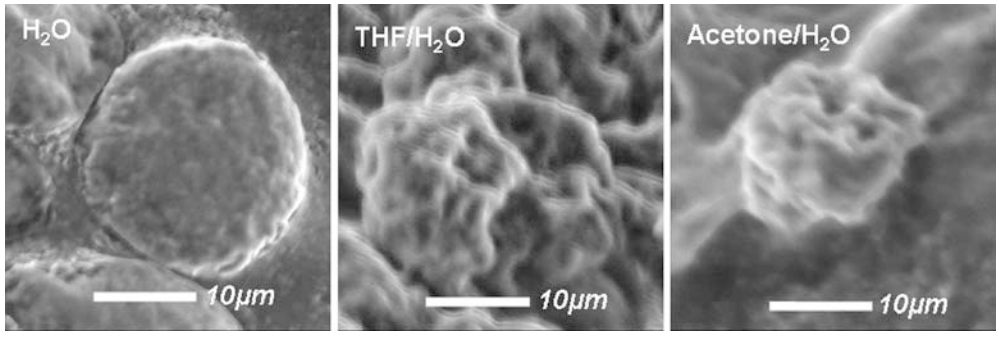


Fig. 3 environmental scanning electron microscope micrographs of PNIPAAm/PEG-DA hydrogel microspheres in water, THF/H_2O (1:1 in volume) and acetone/H_2O (1:1 in volume)

THF/H_2O and acetone/H_2O mixtures tended to be irregular. The spherical microspheres become irregular microparticles with a heterogeneously distributed matrix having several irregular but large pores. The different morphologies of the microspheres in different solvents can also be explained by the solvent sensitivity of PNIPAAm. As discussed earlier, PNIPAAm chains precipitate from the mixed solvents and hydrogel microspheres collapse with the addition of THF or acetone. The addition of THF or acetone may give irregular microparticles rather than regular microspheres. Although the particle size analysis in Fig. 2 exhibits a significant reduction in particle size with a polar organic solvent in the aqueous solution of PNIPAAm-based hydrogel microspheres, the difference in shape as well as morphology, which cannot be observed by particle size analysis, can be virtually seen clearly under SEM observation.

ESEM morphology

It was impossible to observe the hydrogel microspheres in their natural swollen state by using the traditional SEM technique because an extremely high vacuum is needed for SEM observation, which precludes the imaging of hydrated samples in their natural state. The new technique of ESEM was used to obtain images of the hydrated samples in their natural swollen state. This technique does not need any special treatment or modification of the sample, not even the deposition of an electrically conducting coating (e.g., gold). The ESEM micrographs in Fig. 3 show the shapes and morphologies of PNIPAAm/PEG-DA hydrogel microspheres in different solvents. The morphology and the surface of the hydrogel microspheres became irregular and heterogeneous with the appearance of big pores on the surface when they were immersed into the THF/H_2O or acetone/H_2O mixtures. These changes in the shape, morphology and surface in the mixtures also confirmed the SEM findings.

Conclusions

A PNIPAAm/PEG-DA hydrogel microspheres were fabricated in an aqueous two-phase system with the complete absence of any organic phase in the fabrication. The preliminary results showed that the properties, including the particle size as well as shape and the morphology of the hydrogel microspheres, were significantly affected by the addition of polar organic solvents into water. Soaking PNIPAAm/PEG-DA microspheres in THF/H_2O (1:1 in volume) or acetone/H_2O (1:1 in volume) mixtures caused the microspheres to become smaller and degrade from the regular spherical shape in water. The solvent composition responsive property of these hydrogel microspheres was attributed to the cononsolvency phenomenon of PNIPAAm chains in mixed solvents.

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