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SHORT COMMUNICATION

Effect of freeze-drying and rehydrating treatment on the thermo-responsive characteristics of poly(*N*-isopropylacrylamide) microspheres

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Abstract Investigations on the effect of freeze-drying and rehydrating treatment on equilibrium volume changes and on the thermo-response rate of poly(*N*-isopropylacrylamide) (PNIPAM) microspheres were carried out. The experimental results showed that freeze-drying and rehydrating treatment had nearly no effect on the low critical solution temperature and equilibrium volume changes of PNIPAM microspheres. Furthermore, when the PNIPAM microspheres were frozen in only liquid nitrogen through rapid cooling, the response rate of PNIPAM microspheres to environmental temperature change was nearly not affected by the treatment, which was surprisingly different from the macroscopic hydrogel. The dimension effect was responsible for this phenomenon. The micron-sized PNIPAM microsphere itself has a much quicker response rate compared with the bulky hydrogel because the characteristic time of gel deswelling is proportional to the square of a linear dimension of the hydrogel.

Keywords Thermo-sensitive microspheres · SPG membrane emulsification · Colloidal drug carriers · Thermo-response rate · Freeze drying

Introduction

Poly(*N*-isopropylacrylamide) (PNIPAM) is one of the well-known thermo-sensitive polymers, which can have abrupt volume transition near the low critical solution temperature (LCST) [1, 2]. When the environmental temperature is below the LCST, PNIPAM adsorbs much water and exhibits a swollen and hydrophilic state, while above the LCST, it demonstrates abrupt volume shrinkage and becomes hydrophobic due to expelling of free water inside the polymer network. Because of their unique properties, PNIPAM hydrogels have found numerous potential applications in various biomedical and biotechnological fields, including controlled drug delivery systems [3–7], artificial organs [8, 9], "on–off" switches[10, 11], and so on.

A fast response to ambient temperature is one of the most important factors for those applications mentioned above, but the conventional hydrogels cannot fully satisfy the requirements because it usually costs several hours or days to arrive at their volume equilibrium states at above the LCST owing to a dense dehydrated skin layer around the outer surface of the hydrogels [12]. Up to now, many strategies have been proposed to promote the response rate of bulky PNIPAM hydrogels [13–27]. Among all the methods, the freeze-drying treatment is one of the simplest and most effective means to dramatically enhance the response rate of the bulky hydrogels by forming many macropores [25–27], which correspondingly results in an increase of the area of water diffusion and a decrease of the thickness of the surface layer of hydrogels after freeze-drying and rehydrating treatment. This is because the deswelling rate of hydrogel depends on the diffusion velocity of water through the pores and the skin layer [26].

From the application point of view, a small dimension is usually necessary for the stimuli-responsive hydrogels as drug delivery systems to transverse certain organs, and the small size of which minimizes any latent irritant reaction at the injection site [28]. Moreover, hydrogels with a smaller size are more superior to the bulky hydrogels in that they

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have a faster thermo-response rate and because the response time of hydrogel swelling/deswelling is proportional to a linear dimension of hydrogels [29, 30]. However, to the best of our knowledge, an investigation on the effect of freeze-drying and rehydrating treatment on the thermo-responsive characteristics of micron-sized hydrogel microspheres is still lacking. To date, only Lin et al. [31] have investigated the influence of the different drying methods on the static equilibrium volume change of micron-sized PNIPAM microgel beads. Unfortunately, nearly no work on the thermo-responsive deswelling rate has been performed.

The objective of this study is to investigate whether freeze drying could enhance the thermo-responsive response rate of PNIPAM microspheres. In this work, we synthesized uniform PNIPAM microspheres through a two-step process as shown in Fig. 1. Monodisperse monomer-contained water-in-oil (W/O) emulsions were first prepared by employing the Shirasu porous glass (SPG) membrane emulsification method reported in our previous papers [32, 33] (as shown in Fig. 1 a and b). The SPG membrane emulsification is based on injecting a disperse phase through a porous membrane, with the resulting droplets forming at the end of pores on the membrane surface after coming into contact with the continuous phase. Thus, using this technique, it is easier to control the droplet size and size distribution [32–36]. Then, PNIPAM microspheres were synthesized by free-radical polymerization initiated with UV irradiation at 20 °C [37–39] (Fig. 1 c and d). The effect of freeze-drying and rehydrating treatment on the thermo-responsive volume-change rate of PNIPAM hydrogel microspheres was then investigated.

Experimental

Materials

N-isopropylacrylamide (NIPAM) was kindly provided by Kohjin Co., Ltd., Japan and used after purification by recrystallization with hexane and acetone. *N*, *N*′-methylenebisacrylamide (MBA), ammonium persulfate (APS), isopropyl alcohol, and kerosene were purchased from Chengdu Sitong Chemicals, China. Polyglycerol polyricinoleate (PGPR 90) was obtained from Danisco Co., Ltd., Denmark. Polymethylsilsesquioxane (GRT-350) was purchased from Chenguang Research Institute of Chemical Industry, China. Shirasu porous glass (SPG) membranes (D_P=4.8 μm) were bought from SPG Technology Co., Ltd., Miyazaki, Japan. Deionized water (18.2 MΩ, Millipore, Milli-Q) was used in the experiments.

Preparation of monodisperse PNIPAM microspheres

SPG membrane emulsification

A 10-mL aqueous solution containing 1.13 g (1.0 mol/L) of monomer NIPAM, 0.015–0.077 g (0.01–0.05 mol/L) of crosslinker MBA and 20 mg (8.8 mmol/L) of initiator APS was used as the disperse phase (water phase). A 160-mL kerosene solution containing surfactant PGPR (5 wt.%) was used as the continuous phase (oil phase). The SPG membrane emulsification module (Kiyomoto Iron Works Co. Ltd., Miyazaki, Japan) was used to prepare monodisperse monomer-contained W/O emulsions. Before membrane emulsification, the SPG membrane had been

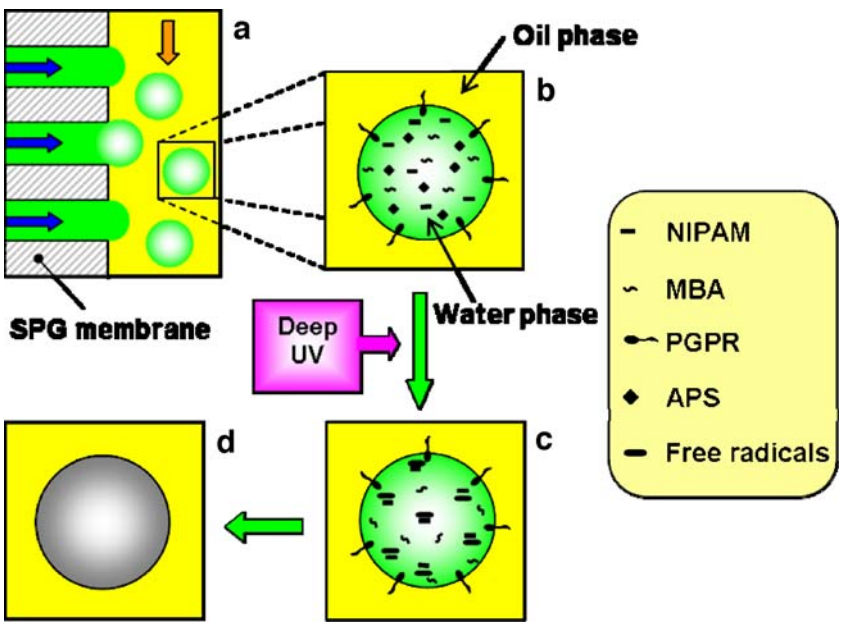

Fig. 1 *a–d* Schematic illustration for the synthesis of monodisperse PNIPAM microspheres by SPG membrane emulsification and UV irradiation polymerization

hydrophobically modified with silicane resin GRT-350 [32, 33] and then wetted with the continuous phase by ultrasonically vibrating for several minutes. Then, the disperse phase stored in a pressure-tight vessel was pressed to permeate into the continuous phase through the SPG membrane under nitrogen pressure (i.e., transmembrane pressure) of 3.0 kPa. At the same time, the continuous phase was stirred with a magnetic bar at a stirring speed of 420 rpm to generate rotating flow so that the continuous phase could pass through the membrane surface continuously. Subsequently, uniform monomer-contained W/O emulsion droplets were then obtained and suspended in the continuous phase.

UV-induced polymerization

A 250-W UV lamp with an illuminance spectrum ranging from 250 to 450 nm (Institute of Beijing Light Lamp, China) was employed to initiate the monomer polymerization. After membrane emulsification, the obtained monomer-contained W/O emulsions were poured into a self-made cylindrical quartz vessel (6 cm in diameter and 9 cm in height) equipped with nitrogen inlet and outlet, and then bubbled with purified nitrogen for about 10 min to remove oxygen dissolved in the continuous phase. UV irradiation lasted for 40 min at a temperature of 20 °C under nitrogen atmosphere. During the polymerization process, the mixture in quartz vessel was gently stirred with a magnetic bar to prevent the coalescence of polymerized microspheres. After the polymerization, the resulting microspheres were separated from the oil phase by centrifugation (Biofuge Primo R, Sorvall, Germany) at 2,000 rpm for 10 min, and then washed more than five times by centrifugation (2,500 rpm, 20 min for each time) with isopropyl alcohol and pure water. Finally, the cleaned microspheres were redispersed in deionized water at room temperature for further characterization.

Morphological characterization of PNIPAM microspheres

Optical micrographs of PNIPAM microspheres immersed in deionized water were obtained by a microscope (BX 61, Olympus, Japan). The morphology of the air-dried and freeze-dried PNIPAM microspheres was observed by a scanning electron microscope (SEM, JSM-5900LV, Hitachi, Japan). The freeze-fried samples were prepared by immerging a polytetrafluoroethylene tube containing PNIPAM microsphere suspensions into liquid nitrogen for several minutes to let them freeze, and then the frozen microspheres were lyophilized by a freeze drier at −35 °C for about 48 h. Then, the powder of the dried microspheres was stuck to the SEM stubs using a carbon-impregnated double-sided adhesive tape. As for the preparation of air-dried microsphere samples, the microsphere suspensions were directly dropped into a cover slide fixed to the SEM stubs and were air-dried at room temperature. Finally, the freeze-dried and air-dried microsphere samples were sputter-coated prior to SEM observation.

Characterization of thermo-responsive properties of PNIPAM microspheres

Static equilibrium volume changes and dynamic volume changes of the resultant microspheres in deionized water were studied by an optical microscope equipped with a thermo-static stage system (TS 62, Instec, USA) and a charge-coupled device (CCD) camera. The temperature of the solution was verified and recorded by a noncontact thermometer (Raytek Corporation, USA) during the test. To study the thermo-responsive deswelling kinetics of the microspheres in deionized water responding to the environmental temperature, the microspheres were first equilibrated in deionized water at 20 °C, and then the surrounding temperature was suddenly increased to 40 and 50 °C, respectively. The time-dependent shrinking behavior of the PNIPAM microspheres was recorded by the CCD camera that was mounted to the optical microscope, and the mean diameter of the microspheres at each predetermined temperature or moment was obtained by measuring over 100 microspheres based on the captured optical micrographs of the microspheres. The measurements were carried out at least thrice to get the average value, and the data were in good agreement within a standard deviation of 3%.

Results and discussion

Figure 2 shows the monodisperse PNIPAM microspheres prepared by SPG membrane emulsification and UV-induced polymerization. Figure 2a and b, respectively, shows the typical optical micrographs of PNIPAM microspheres before and after freeze drying, dispersed in deionized water, and Fig. 2c and d shows the typical SEM images of air-dried and freeze-dried PNIPAM microspheres, respectively. Figure 2e shows the typical SEM image of air-dried PNIPAM microspheres that underwent freeze-drying and rehydrating treatment before being air-dried at room temperature. Whether the PNIPAM microspheres underwent freeze-drying and rehydrating treatment, the air-dried PNIPAM microspheres always exhibit a satisfactory sphericity and a smooth and compact surface (Fig. 2c and e). However, the morphology and structure of the microspheres after the freeze-drying treatment showed a marked change as shown Fig. 2d. After freeze drying, the microspheres unexpectedly had a flower-like porous structure and produced a tip-shaped tail at one end of the microsphere, and a large number of micropores with sizes of several microns are generated throughout the

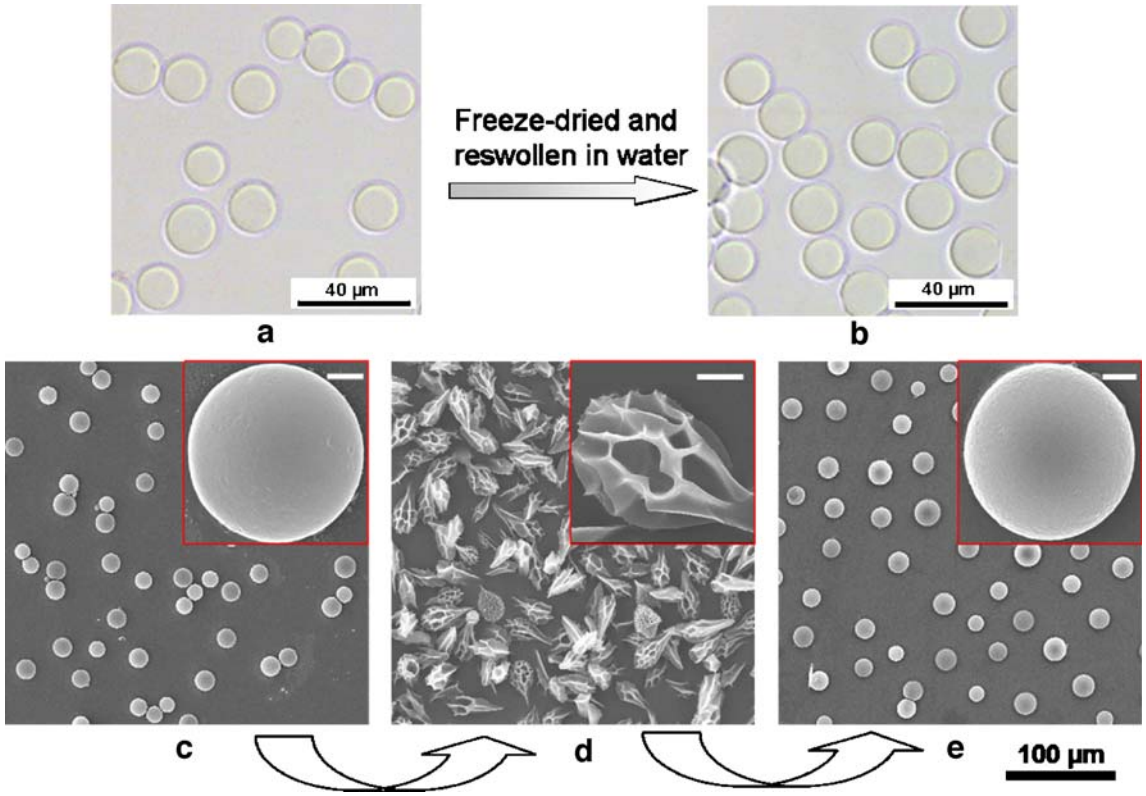

Fig. 2 **a** Optical micrograph of PNIPAM microspheres dispersed in deionized water at 20 °C. **b** Optical micrograph of PNIPAM microspheres that were firstly freeze-dried and then reswollen in deionized water at 20 °C. **c**, **d** SEM micrographs of the air-dried and freeze-dried PNIPAM microspheres; **e** SEM micrograph of air-dried PNIPAM microspheres that were firstly freeze-dried and then reswollen in deionized water. In the inserted pictures, scale bar (**c**, **e**) 2 μm; (**d**) 5 μm. $[C_{NIPAM}]$=1.0 mol/L, $[C_{MBA}]$=0.05 mol/L and $[C_{APS}]$= 8.8 mmol/L

microsphere. Lin et al. [31] also observed a similar phenomenon with freeze-dried PNIPAM microgel beads in their investigation.

The micropores within the microspheres after freeze drying were resulted from the ice crystals presented in the swollen PNIPAM microspheres acting as a template for pore generation [40]. The water inside the swollen microspheres was dispersed in an interconnected polymeric network, and allowed the polymer network to enlarge while swollen in water, and then the ice crystals rapidly formed within the network of microspheres upon immerging them into liquid nitrogen. The frozen microspheres were dried by sublimation of ice crystals under vacuum at a temperature below the ice freezing point. The direction of the micropores was resulted from the orientation of ice crystals [40]. When ice crystals were subjected to an external stress such as rapid cooling, for instance, immerged into liquid nitrogen in this case, they will be oriented because of a temperature gradient, and subsequently lead to the formation of an ordered direction of the micropores. As for the formation of tip-shaped tails, a possible reason is that water within the top of the microsphere network was pressed to flow downwards during the freezing process of microsphere by a driving force. The top of the microsphere was first frozen, and the water streaming from the top of the microsphere was also gradually frozen. Finally, a tip-shaped tail was resulted. In view of this phenomenon, a conclusion can be drawn that such a freeze-drying treatment is ineffective for hydrogel microspheres to maintain their good spherical shapes.

It is particularly interesting to observe that the freeze-dried porous microspheres can recover the spherical morphology after reswelling in deionized water (Fig. 2b and e). This is mainly ascribed to the elastic and flexible characteristics of PNIPAM polymeric networks [26]. Stretched polymer networks can occur to contract, the pore regions are stepwise occupied by water when the porous microspheres were reswollen, and then the rehydrated microspheres can return to the surface morphology without the lyophilizing treatment. Furthermore, the size of the freeze-dried and reswollen microspheres was almost the same as that without freeze-drying treatment.

Figure 3 shows the temperature-dependent equilibrium volume change of monodisperse PNIPAM microspheres before and after freeze-drying treatment. The volume of both microspheres changed slowly when the environmental temperature was below 31 °C, and then suffered from a sharp decrease while the temperature was raised across 32 °C,

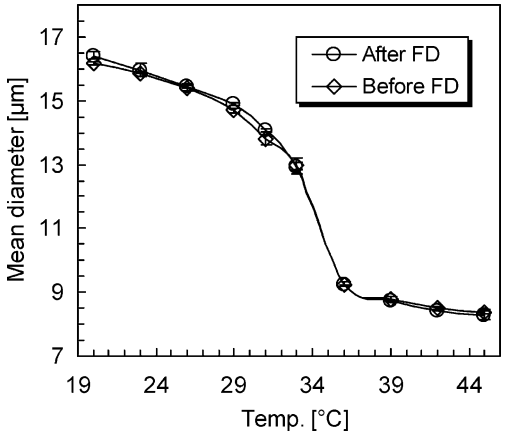

Fig. 3 Temperature-dependent equilibrium volume change of PNIPAM microspheres before and after freeze-drying treatment. [C_{NIPAM}]= 1.0 mol/L, [C_{MBA}]=0.05 mol/L, and [C_{APS}]=8.8 mmol/L

and finally kept almost constant upon heating above 36 °C, which was in good agreement with the bulky PNIPAM hydrogel. The results showed that the freeze-drying treatment nearly had no effect on the LCST and the equilibrium volume changes of the PNIPAM microspheres. The volume of the microspheres nearly did not change after the lyophilizing treatment, except that the size of PNIPAM microspheres after freeze-drying and reswelling with deionized water at 20 °C was slightly larger than that without freeze drying (Fig. 3). Kato et al. [25] also observed a similar phenomenon when bulky PNIPAM gel was submitted to the freeze-drying and rehydrating treatment, and believed that the glide between the polymer chains inside the PNIPAM gel had occurred beyond the elastic limit and expanded the polymer network.

Figure 4 shows the time-dependent diameter change and volume change of monodisperse PNIPAM microspheres with different crosslinkages before and after freeze-drying treatment when environmental temperature was suddenly increased from 20 to 40 and 50 °C, respectively. When other preparation conditions, such as the monomer NIPAM and initiator APS concentrations, and the transmembrane pressure were kept unvaried, the initial mean diameter of swollen PNIPAM microspheres in water (at 20 °C) increased with decreasing of the crosslinker MBA concentration in preparation. The lower the MBA concentration, the lower the crosslinkages of the polymer networks inside the microgels,

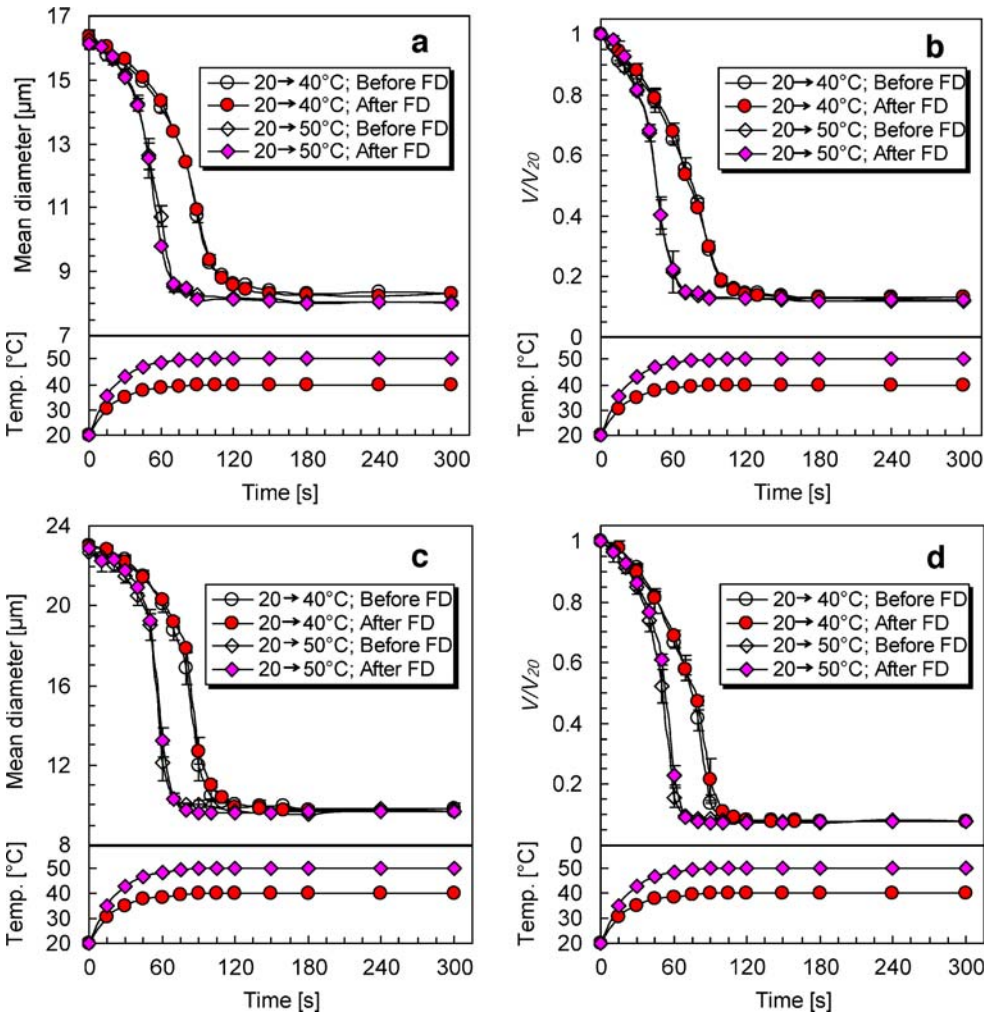

Fig. 4 Time-dependent diameter changes (**a**, **c**) and volume change rate (**b**, **d**) of monodisperse PNIPAM microspheres with different crosslinkages before and after freeze-drying treatment. [C_{NIPAM}]=1.0 mol/L and [C_{APS}]=8.8 mmol/L. **a**, **b** [C_{MBA}]=0.05 mol/L; **c**, **d** [C_{MBA}]=0.01 mol/L

i.e., the looser the polymer networks inside the PNIPAM microspheres; therefore, the larger the microspheres swelled in water at a temperature below the LCST of PNIPAM.

The deswelling rate of PNIPAM microspheres increases with increasing of the upper temperature limit. The higher the environmental temperature, the quicker the heat transferred into the PNIPAM microspheres; therefore, the hydrogen bonds between the polymer chains and water molecules were ruptured more rapidly upon heating, and as a result, the deswelling rate of microgels was faster. On the other hand, when the solution temperature was increased from 20 to 40 or to 50 °C (above the LCST of PNIPAM), the freeze-drying and rehydration treatment nearly did not affect the deswelling rate of PNIPAM microspheres no matter how the crosslinkage changed. Kato et al. [25, 26] found in their studies that the deswelling rate of bulky PNIPAM hydrogel could be markedly accelerated by the freeze-drying and rehydration treatment, and believed that an expansion of the diffusion area of water and a decrease of the thickness of the surface layer for a macroporous gel were responsible for the pronounced enhancements in the deswelling rate [25]. However, the deswelling rate of micron-sized PNIPAM microspheres with different crosslinkages was nearly not improved after freeze-drying and rehydrating treatment when the environmental temperature was increased from 20 to 40 or to 50 °C in this study.

Because the size of the microspheres in this study (micron-sized scale) is much smaller in comparison with that of bulky PNIPAM hydrogel (at least the millimeter scale), thus, the microsphere itself has a quick deswelling rate before freeze-drying treatment because the characteristic time of gel deswelling is proportional to the square of a linear dimension of the hydrogel [29]. The deswelling rate of a hydrogel depends mainly on the diffusion velocity of water passing through the polymer network and the skin layer. The thickness of the skin layer of micron-sized PNIPAM microspheres should be much thinner than that of bulky hydrogel, and the freeze-drying treatment for the microsphere did not distinctly reduce the thickness of the skin layer. Therefore, the accelerating extent resulting from freeze-drying treatment for the deswelling rate of microgels is negligible compared with that resulting from the effect of the small dimension of the microspheres. That is, when the microgel was frozen in only liquid nitrogen through rapid cooling, the freeze-drying treatment has a very little effect on the deswelling rate of micron-sized PNIPAM microspheres when the environmental temperature was increased from 20 to 40 or to 50 °C in this study.

Conclusions

In summary, although the freeze-drying and rehydrating treatment has been reported to be a simple and effective method to enhance the response rate of bulky hydrogels, the experimental results in this study showed that, when the microgel was frozen in only liquid nitrogen through rapid cooling, the freeze-drying and rehydrating treatment is nearly ineffective to improve the response rate of micron-sized PNIPAM hydrogel microspheres. The main reason is that the PNIPAM microsphere itself has a quick deswelling rate before freeze-drying treatment because of small size, and that the size effect is larger than the pore effect contributing to the response rate of the hydrogel.

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