ELSEVIER

Polymer 40 (1999) 6307–6312

polymer

Drying methods affecting the particle sizes, phase transition, deswelling/reswelling processes and morphology of poly(*N*-isopropylacrylamide) microgel beads

Shan-Yang Lin[a,*], Ko-Shao Chen[b], Liang Run-Chu[b]

[a]*Biopharmaceutics Laboratory, Department of Medical Research and Education, Veterans General Hospital-Taipei, No. 201, Sec 2, Shih-Pai Road, Taipei 11217, Taiwan*

[b]*Tatung Institute of Technology, Taipei, Taiwan*

Received 5 October 1998; received in revised form 24 November 1998; accepted 16 December 1998

Abstract

The influence of drying methods on the micromeritics, phase transition, deswelling/reswelling process and surface topography of poly (*N*-isopropyl-acrylamide) (PNIPAAM) microgel beads was investigated. Three different drying methods (quick-freezing, slow-freezing and oven-drying) were applied to prepare the dried PNIPAAM microgel beads. Undried PNIPAAM microgel beads were used as control. The results indicate that although different drying methods significantly influenced the particle size distribution, deswelling/reswelling volume, surface topography and morphology of PNIPAAM microgel beads, it did not seem to affect the lower critical solution temperature (LCST) of 32°C–34°C and molecular interaction in PNIPAAM microgel beads. According to ATR/FT-IR/DSC microscopic study, above the LCST, the free form of non-hydrogen bonded C=O band and intra-molecular hydrogen bonding played a dominant role in the molecular structure of PNIPAAM microgel beads, which was contrary to our previous study in which the non-hydrogen bonding contributed less to the molecular structure of PNIPAAM aqueous solution without a cross-linking agent. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Poly (*N*-isopropyl-acrylamide) (PNIPAAM); Microgel bead; Drying method

1. Introduction

Polymer gels with phase transitions and critical phenomena in response to external stimuli have received much attention [1–3]. The utilization of temperature changes as a signal for thermal-sensitive gels to modulate drug release or to take advantage in cell culture and separation process has attracted a lot of interest recently [3–8]. Poly (*N*-isopropyl-acrylamide) (PNIPAAM) is one of the well-known thermal-dependent water-soluble polymers with a lower critical solution temperature (LCST) at 32°C–35°C and its network reveals unique thermal volume transitions near LCST [1–3]. Our previous study has shown that inter-molecular interactions might occur mainly between PNIPAAM molecules and water, when temperature was below the LCST, but when the temperature was above the LCST, PNIPAAM molecules may aggregate in water as a result of both the intra-molecular interactions within PNIPAAM molecules and the hydrophobic interactions in the system [9].

Although the behavior of PNIPAAM gel in aqueous solutions has been extensively investigated [1–7], only a few studies have been carried out on its behavior in the solid state. It is of particular interest whether the drying method can alter the physico-chemical properties of the dried state of PNIPAAM gel to influence its thermal-dependent behavior. In this study, PNIPAAM microgel beads were prepared by an inverse suspension polymerization with a cross-linking agent [10] to investigate the effect of drying methods on the physico-chemical properties such as particle size, phase transition, deswelling/reswelling process or volume, morphology and surface topography of these dried PNIPAAM microgel beads. Undried PNIPAAM microgel beads were also studied as a reference system.

2. Experimental

2.1. Polymerization

N-isopropylacrylamide (NIPAAM, Eastman Kodak, USA) was recrystallized from *n*-hexane. NIPAAM (3 g)

* Corresponding author.
E-mail address: sylin@vghtpe.gov.tw (S.-Y. Lin)

0032-3861/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0032-3861(98)00872-6

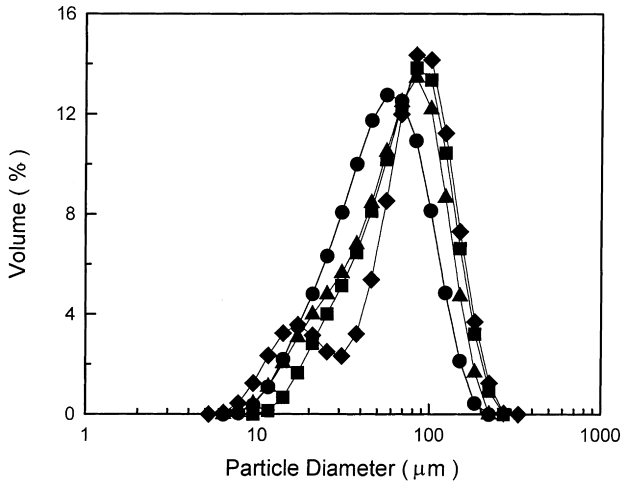

Fig. 1. Effect of drying method on the particle size distribution of PNIPAAM microgel beads. Key: ●, undried; ▲, quick-freezing method; ■, slow-freezing method; ◆, oven-drying method.

was dissolved in 30 ml of deionized, distilled water containing 0.14 g *N,N′*-methylenebisacrylamide (Sigma, USA) and 0.6 g of ammonium persulfate (Nacalai Tesque, Kyoto, Japan), and bubbled with nitrogen to remove the dissolved oxygen. This solution was poured into 150 ml of paraffin oil containing 1% of pluronic L-61 (Asahi Denka KoGyo K.K., Tokyo, Japan), which was previously purged with nitrogen. The agitation speed was 500 rpm in a three-necked reaction bottle, and nitrogen was continuously supplied in the course of the study. After aqueous droplets in the oil phase were formed, 3 ml of *N,N,N′,N′*-tetraethyl-methylenediamine (Nacalai Tesque, Kyoto, Japan) was added to the continuous phase to initiate redox polymerization, which was then performed at room temperature for 3 h [10]. After polymerization, the beads were separated out by excess deionized water, washed several times with a mixture of acetone and deionized water (1 : 1) to remove the monomer, and then centrifuged to obtain PNIPAAM microgel beads.

2.2. Different drying methods

Three drying methods were used to prepare the dried state of PNIPAAM microgel beads: (1) Quick-freezing method: the centrifuged PNIPAAM microgel beads were frozen rapidly to −70°C with liquid nitrogen and freeze dried. (2) Slow-freezing method: the centrifuged PNIPAAM microgel beads were frozen slowly to −20°C in the −20°C refrigerator and freeze dried. (3) Oven-drying method: the centrifuged PNIPAAM microgel beads were dried directly at 100°C oven for 30 min. The undried PNIPAAM microgel beads were also used as control.

2.3. Evaluation of the physico-chemical properties of the PNIPAAM microgel beads

2.3.1. Micromeritics

The dried PNIPAAM microgel beads prepared by different drying methods were pre-equilibrated in distilled water for 24 h at 25°C, and then their particle size distributions were determined by a particle size analyzer (Master-Sizer X, Malvern Instruments, USA).

2.3.2. Thermal analysis

The dried PNIPAAM microgel beads prepared by different drying methods were pre-equilibrated in distilled water for 24 h at 25°C, and then their phase transitions were thermally analyzed by using a differential scanning calorimeter (DSC-910, TA Instruments, USA). The heating rate was 1°C/min, with an open pan system in an N_2 gas flow. The instrument was calibrated with indium. Polymer-free distilled water was placed in the reference cell [9].

2.3.3. Deswelling/reswelling processes as a function of temperature

Certain amounts of PNIPAAM microgel beads prepared by different drying methods were pre-equilibrated in distilled water, and its equilibrium volume was measured in a temperature range from 10°C to 45°C or from 45°C to 10°C. The equilibrium volume at 10°C was set as 100%. Five measurements were performed with different samples, and mean and standard derivation (SD) were obtained.

2.3.4. Thermal micro-ATR/FT-IR spectroscopic study

The inter- and/or intra-molecular interactions between PNIPAAM molecules and water of the 24 h-water equilibrated PNIPAAM microgel beads prepared by different drying methods were determined by using an ATR/FT-IR/DSC microscopic system, as described in our previous study [9,11–15]. The component of amide I in the IR spectrum of PNIPAAM microgel beads at each specific temperature was estimated quantitatively by a curve-fitting program [16–18]. The proportion of a component was computed to be the fractional area of the corresponding peak, divided by the sum of the areas of peaks.

2.3.5. Surface topography

The surface topography of the dried PNIPAAM microgel beads prepared by quick- and slow-freezing methods was observed by using a scanning electron microscopy (S-2300, Hitachi, Tokyo, Japan). In order to maintain the shape and morphology of the undried PNIPAAM microgel beads, a critical-point drying method for scanning electron microscopy was used [19]. Another swelling process of the dried PNIPAAM microgel beads prepared by the quick-freezing method in dye aqueous solution at 25°C was continuously observed by optical microscopy (Olympus BH-2, Tokyo, Japan).

3. Results and discussion

Fig. 1 shows the particle size distributions of the 24 h swollen PNIPAAM microgel beads prepared by different

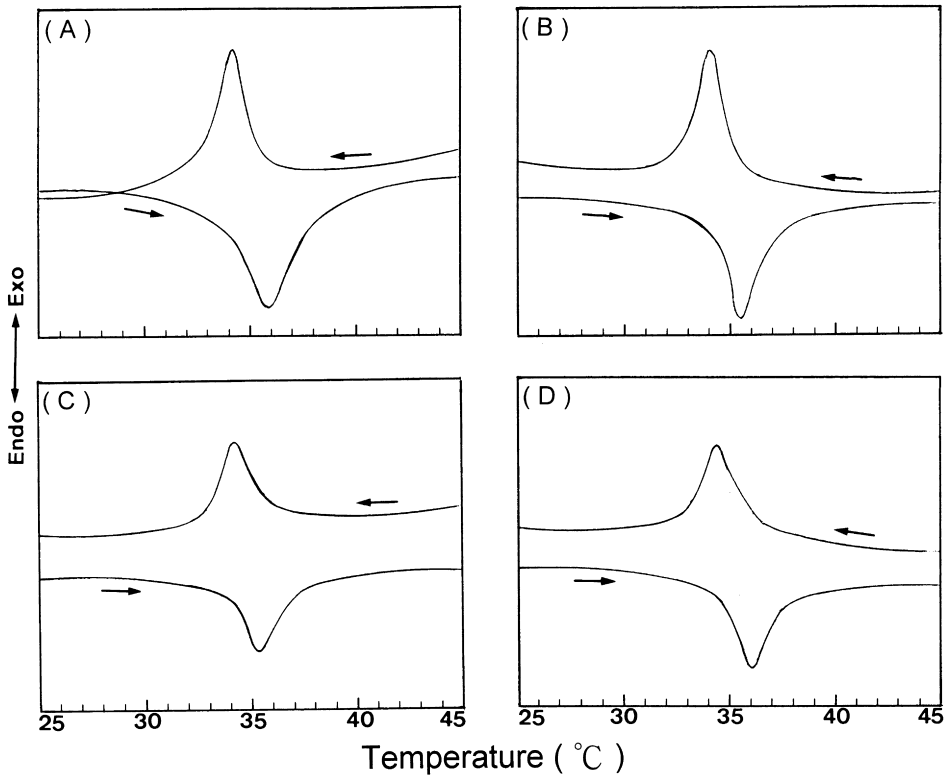

Fig. 2. DSC thermograms of PNIPAAM microgel beads prepared by different drying methods. Key: A, undried; B, quick-freezing method; C, slow-freezing method; D, oven-drying method.

drying methods. As the dried PNIPAAM microgel beads may swell in water or other solvents, their intact particle size could be more easily obtained by pre-equilibrating in water for 24 h at 25°C than by direct determination. The distribution of particle size thus obtained seemed to shift to a higher range than that of the PNIPAAM microgel beads without drying. The mean particle size turned to be 72.02, 81.29 and 85.73 μm, respectively with quick-freezing, slow-freezing and oven-drying methods, but to be 57.71 μm for the swollen PNIPAAM microgel beads without drying. In other words, different drying processes may change the physico-chemical properties of PNIPAAM microgel beads to enlarge their particle size after 24 h-equilibration in water.

Fig. 2 reveals the DSC curves of the 24 h-water equilibrated PNIPAAM microgel beads prepared by different drying methods. The endothermic peaks appeared at 35.5°C (B), 35.2°C (C) or 35.9°C (D), respectively on the thermograms of these PNIPAAM microgel beads prepared by quick-freezing, slow-freezing or oven-drying method, as compared with the peak at 35.8°C for the undried PNIPAAM microgel bead (A). Moreover, the onset temperature was also found at 33°C–34°C for these microgel beads prepared by different drying methods, which might correspond to the phase transition and be defined as the LCST of PNIPAAM microgel beads [1–2]. However, the LCST of the undried PNIPAAM microgel beads was about 32°C, slightly lower than the LCST of the dried ones although of no significant difference. In the cooling process, one freezing exothermic peak at 34.2°C–34.7°C for all the samples was also observed. The smaller LCST value at 32°C–34°C for all the samples, as found in our study as well as others [1,2,20], suggested that the drying methods did not significantly alter the thermal behavior of PNIPAAM microgel beads.

An ATR/FT-IR/DSC microscopic system was used to investigate the inter- and/or intra-molecular interactions within PNIPAAM molecules or between PNIPAAM molecules and water of the 24 h-water equilibrated PNIPAAM microgel beads prepared by different drying methods. As amide chains are included in the PNIPAAM structure, they, like polypeptides, have amide bands in the IR spectrum. The principal contribution of the amide I band is its carbonyl stretching vibration. It is much more sensitive to change in the conformation and structure of the protein than the amide II [21]. The stretching vibration can be markedly influenced by hydrogen bonding. PNIPAAM, like nylon, may have its amide I regions assumedly composed of three distinct bands [16]: intra-molecular hydrogen bonded C=O band at 1631 cm^{-1}, inter-molecular hydrogen bonded C=O band at 1620 cm^{-1} and free form of non-hydrogen bonded C=O band at 1643 cm^{-1} [22,23]. The component in amide I of the IR spectrum at each specific temperature was estimated quantitatively by a curve-fitting program [16–18]. The proportion of each component was computed to be the fractional area of the corresponding peak, divided

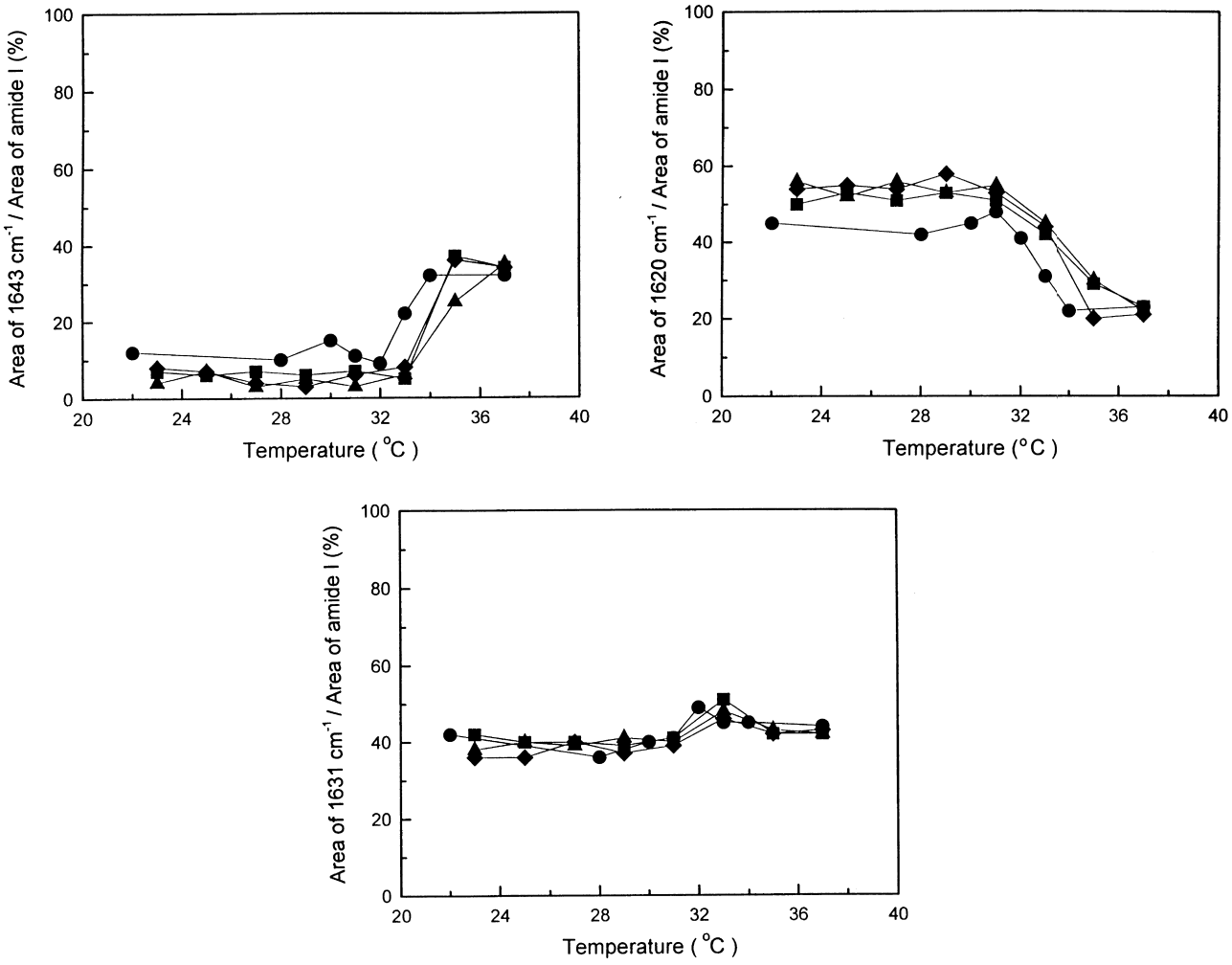

Fig. 3. Plot of the peak area ratios as a function of temperature for PNIPAAM microgel beads prepared by different drying methods. Key: ●, undried; ▲, quick-freezing method; ■, slow-freezing method; ◆, oven-drying method.

by the sum of the areas of peaks in the amides I band. As shown in Fig. 3, all the samples exhibited a similar thermal-dependent fashion, and near 32°C–33°C there was a marked change as a result of the existence of LCST of PNIPAAM microgel beads. When the temperature was below LCST both the inter- and intra-molecular hydrogen bonding played a dominant role in the molecular structure of PNIPAAM microgel beads (Fig. 3 B and C). Once the temperature went beyond the LCST, the composition of the free form of non-hydrogen bonded C=O band at 1643 cm^{-1} increased markedly but the inter-molecular hydrogen bonding at 1620 cm^{-1} between PNIPAAM molecules and water decreased dramatically (Fig.3 A and B). However, the mild increase in intra-molecular hydrogen bonded composition at 1631 cm^{-1} (Fig. 3C) suggests that above the LCST the free form of non-hydrogen bonded C=O band and intra-molecular hydrogen bonding may play a dominant role in the molecular structure of PNIPAAM microgel beads. This was contrary to our previous finding that the non-hydrogen bonding contributes less to the molecular structure of PNIPAAM aqueous solution without a cross-linking agent [9]. The different result might be related to whether the cross-linking agent was used or not, as it also plays a predominant role in the shrinkage/swelling process of PNIPAAM microgel beads.

The results of the deswelling or reswelling experiments of PNIPAAM microgel beads in water by raising or lowering the temperatures are shown in Fig. 4. It is evident that the phase transition temperature was observed at about 33°C for both deswelling or reswelling curves, which was consistent with the LCST result of DSC thermogram. It is interesting to note that during the deswelling process the undried PNIPAAM microgel beads or PNIPAAM microgel beads prepared by the quick-freezing method shrank to 90%, but those prepared by the slow-freezing or the oven-drying method only shrank to about 60%–70% even at higher temperatures. During the reswelling process the maximum swelling ratio of PNIPAAM microgel beads prepared by the quick-freezing, slow-freezing or oven-drying method were only swollen to 50%, 90% or 90%, respectively, as

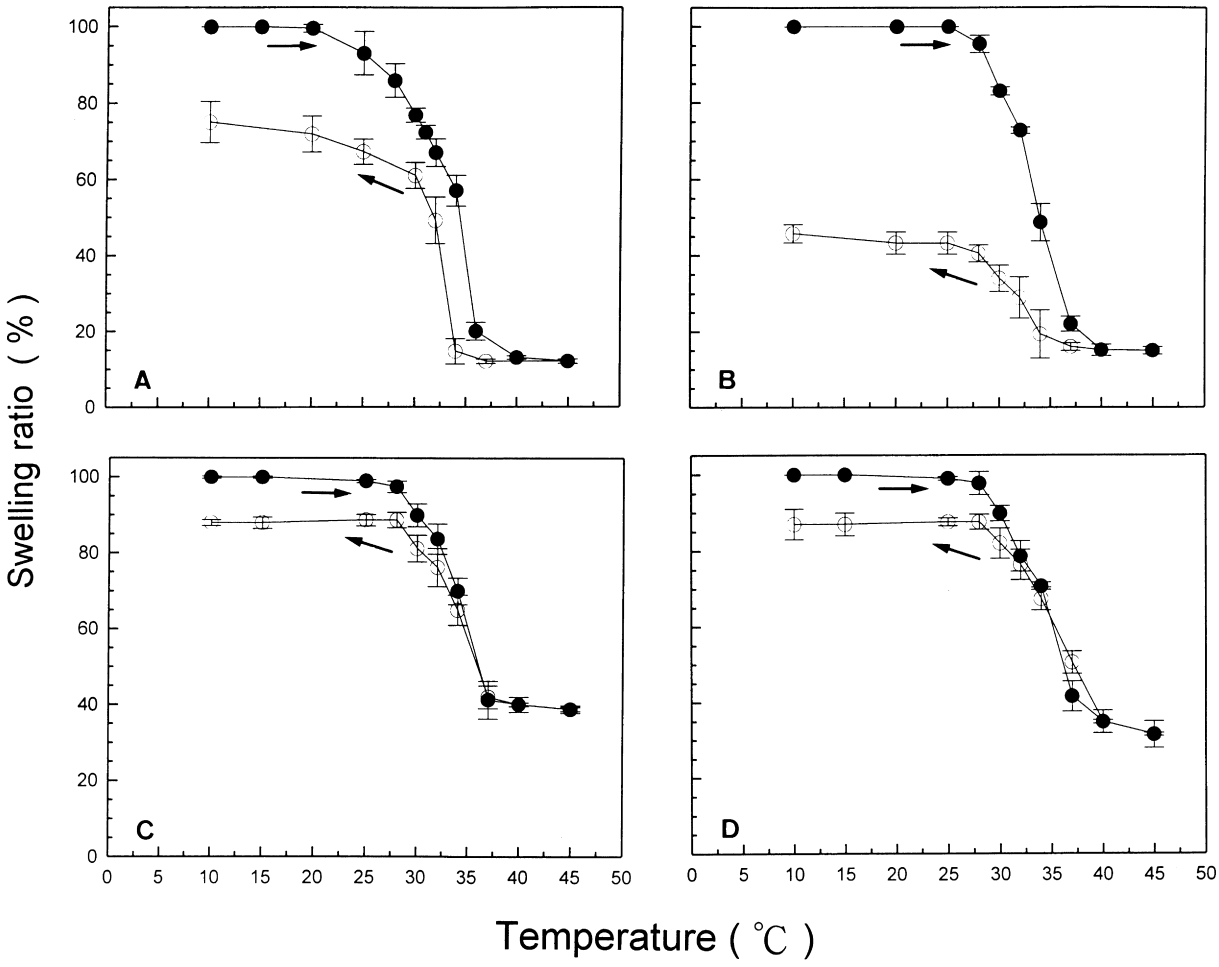

Fig. 4. Deswelling/reswelling processes of PNIPAAM microgel beads as a function of temperature. Key: ●, deswelling process; ○, swelling process. Different drying methods A, undried; B, quick-freezing method; C, slow-freezing method; D, oven-drying method. Vertical bars represents the standard deviations of five measurements.

compared with 75% of the swelling ratio of the undried PNIPAAM microgel beads. In particular, the reswelling volume of PNIPAAM microgel beads prepared by the quick-freezing method only reached to one-half of the original volume. This strongly indicates that the drying method may influence the deswelling or reswelling behavior of PNIPAAM microgel beads, although their phase transition temperature did not change. The reason is unclear, and needs further study in the future.

The surface topography of the dried PNIPAAM microgel beads prepared by quick-freezing or slow-freezing method was observed by using scanning electron microscopy. The undried PNIPAAM microgel beads obtained with the critical-point drying method for scanning electron microscopic study was also carried out. It was first evidenced that the undried PNIPAAM microgel beads prepared by an inverse suspension polymerization exhibited a spherical shape and a smooth topography by using the critical-point drying method (Fig. 5 A1 and A2). Also, a critical-point method can be used to protect the shrinkage of the original samples during scanning electron microscopic study [19]. Once different drying methods were applied, the dried PNIPAAM microgel beads showed a different shape, morphology and surface topography. The leaf-like shape with more porosities was observed from the surface of dried PNIPAAM microgel beads prepared by the quick-freezing method (Fig. 5 B1 and B2). Perhaps, the morphology of quick-freezing samples seems to be related to the limitation of reswelling of the dried PNIPAAM microgel beads, as the porous shape still appeared on the surface of the swollen PNIPAAM microgel beads during the swelling process by determining with optical microscopy. When the slow-freezing method was used, the irregular, non-compact shape, collapsible and non-spherical shapes of PNIPAAM microgel beads were formed (Fig. 5 C1 and C2). Moreover, many broken pieces and fragments were also found in the quick- and slow-freezing samples. Different drying methods seemed to easily modify the surface topography and morphology of the PNIPAAM microgel beads.

The results reported here show that the drying methods can significantly influence the particle size distribution, deswelling/reswelling volume, surface topography and

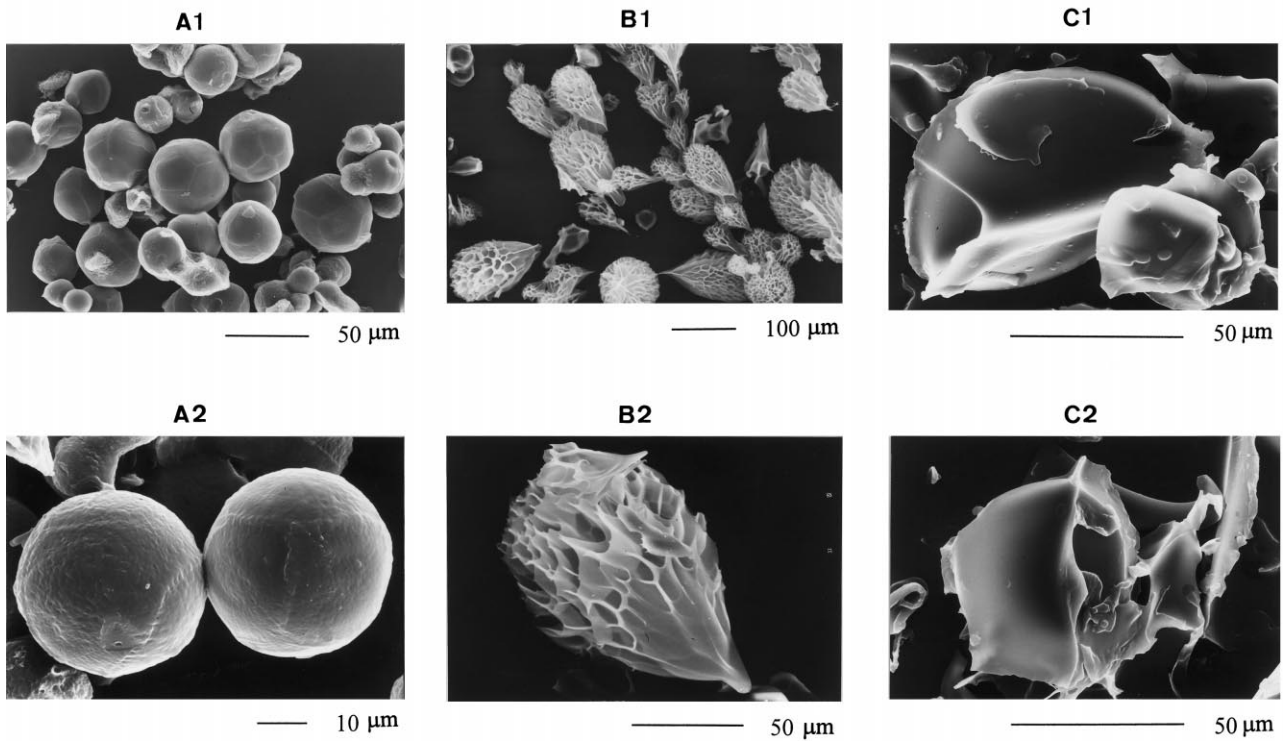

Fig. 5. Scanning electron micrographs of PNIPAAM microgel beads prepared by different drying methods. Key: A1, A2, critical-point drying method B1, B2, quick-freezing method; C1, C2, slow-freezing method.

morphology of PNIPAAM microgel beads, but do not seem to affect the phase transition of and molecular interaction in PNIPAAM microgel beads. We anticipate that this study will be important for the investigations of PNIPAAM microgel beads. Improper drying method may cause the defect of the intact PNIPAAM microgel beads to influence its physico-chemical properties.

References

[1] Tanaka T, Sato E, Hirokawa Y, Hirotsu S, Peetermans J. Phys Rev Lett 1985;55:2455.
[2] Schild HG. Prog Polym Sci 1992;117:163.
[3] Okano T, Bae YH, Jacobs H, Kim SW. J Control Rel 1990;11:255.
[4] Hoffman AS. J Control Rel 1987;6:297.
[5] Nguyen AL, Luong JHT. Enzyme Microb Technol 1990;12:663.
[6] Park TG, Hoffman AS. Appl Biochem Biotechnol 1988;19:1.
[7] Takezawa T, Mori Y, Yoshizato K. Biotechnology 1990;8:854.
[8] Yoshida R, Uchida K, Kaneko Y, Sakai K, Kikuchi A, Sakurai Y, Okano T. Nature 1995;374:240.
[9] Lin SY, Chen KS, Liang RC. Polymer 1999, in press.
[10] Park TG, Hoffman AS. Biotechnol Prog 1994;10:82.
[11] Lin SY, Liang RC, Lin TC. J Chin Chem Soc – Taipei 1994;41:425.
[12] Lin SY, Liao CM, Liang RC. Polym J 1995;27:201.
[13] Lin YY, Chen KS, Lin SY. J Chin Chem Soc – Taipei 1995;42:865.
[14] Lin SY, Liao CM, Hsiue GH. Polymer 1996;37:269.
[15] Lin SY, Duan KJ, Lin TC. Spectrosc Acta A 1996;52:1671.
[16] Skrovanek DJ, Painter PC, Coleman MM. Macromolecules 1986;19:699.
[17] Musto P, Karasz FE, MacKnight WJ. Macromolecules 1991;24:4762.
[18] Lin SY, Lee SM, Cheng CL, Liang RC. Biochem Biophys Res Commun 1995;216:183.
[19] Bray DF, Bagu I, Koegler P. Microsco Res Techniq 1993;26:489.
[20] Hirose Y, Amiya T, Hirokawwa Y, Tanaka T. Macromolecules 1987;20:1342.
[21] Harris PI, Chapman D. Methods in Mol Biol 1994;22:183.
[22] Pimentel GC, McClellan AL. The hydrogen bond, San Francisco: W.H. Freeman, 1960 p. 87.
[23] Pretsch E, Seibl J, Simon W, editors. Tables of spectral data for structure determination of organic compounds 2. Berlin: Springer-Verlag, 1989. p. 150.