

Structure and Protein Separation Efficiency of Poly(*N*-isopropylacrylamide) Gels: Effect of Synthesis Conditions

NILHAN KAYAMAN,¹ DILEK KAZAN,¹ ALTAN ERARSLAN,^{1,2} OĞUZ OKAY,^{1,2} BAHATTIN M. BAYSAL^{1,3}

¹ TUBITAK Marmara Research Center, Research Institutes for Basic Sciences and Genetic Engineering & Biotechnology, P.O. Box 21, 41470 Gebze, Kocaeli, Turkey

² Kocaeli University, Faculty of Arts and Sciences, Department of Chemistry, 41300 Izmit, Kocaeli, Turkey

³ Boğaziçi University, Department of Chemical Engineering, 80815 Bebek, Istanbul, Turkey

Received 12 December 1996; accepted 31 May 1997

ABSTRACT: Crosslinked poly(*N*-isopropylacrylamide) (PNIPA) gels with different crosslink densities in the form of rods and beads were prepared by free-radical crosslinking copolymerization. Solution and inverse suspension polymerization techniques were used for the gel synthesis. The gels were utilized to concentrate dilute aqueous solutions of penicillin G acylase (PGA), bovine serum albumin (BSA), and 6-aminopenicillanic acid (6-APA). The discontinuous volume transition at 34°C observed in the gel swelling was used as the basis of concentrating dilute aqueous protein solutions. PNIPA gels formed below 18°C were homogeneous, whereas those formed at higher temperatures exhibited heterogeneous structures. The water absorption capacity of PNIPA gels in the form of beads was much higher, and their rate of swelling was much faster than the rod-shaped PNIPA gels. It was also found that the polymerization techniques used significantly affect the properties of PNIPA gels. The separation efficiency decreased when the protein molecules PGA or BSA in the external solution were replaced with small-molecular-weight compounds, such as 6-APA. The protein separation efficiency by the gel beads increased to 100% after coating the bead surfaces with BSA. © 1998 John Wiley & Sons, Inc. *J Appl Polym Sci* **67**: 805–814, 1998

Key words: poly(*N*-isopropylacrylamide); temperature-sensitive gels; gel swelling; protein separation efficiency; gel extraction

INTRODUCTION

Hydrophilic gels called hydrogels are important materials of both fundamental and technological interest.¹ The swelling behavior of hydrogels in low-molecular-weight solvents has been investigated repeatedly in the last four decades. In recent years, attention has turned to the swelling

and collapse phenomena that are observed when a hydrogel network is brought into contact with a solvent. The possibility of a first-order phase transition has been predicted theoretically^{2–6} and proven experimentally on hydrolyzed polyacrylamide (PAAm) gels swollen in acetone–water mixtures.^{7–11} In such a transition, a change in a variable like pH, solvent composition, ionic strength, or temperature can induce a discontinuous change in the volume of the swollen gel.^{12–14}

In 1984, Hirokawa and Tanaka reported that poly(*N*-isopropylacrylamide) (PNIPA) gels exhibit discrete and reversible volume change in re-

Correspondence to: O. Okay.

Journal of Applied Polymer Science, Vol. 67, 805–814 (1998)
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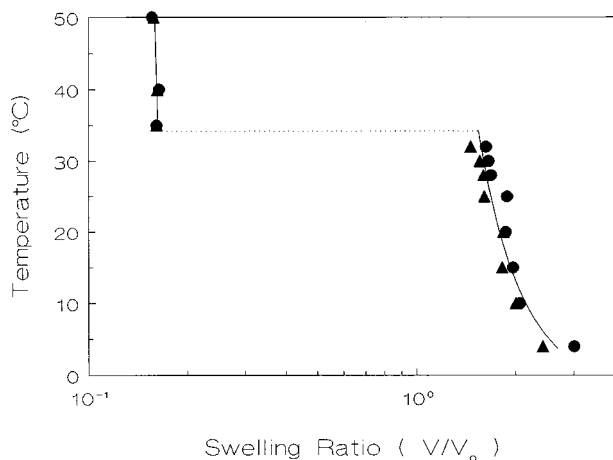


Figure 1 Variation of the equilibrium swelling ratio V/V_0 of PNIPA gels in water with the swelling temperature. The gel was synthesized as described in the text. 1.6 BAAM: (●) and (▲) 2.5 wt %.

response to an infinitesimal change in temperature.¹⁵ Such polymer gels that are sensitive to temperature have been suggested for use in a variety of applications, including controlled drug delivery,¹⁶ immobilized-enzyme reactors,^{17–19} and separation processes.^{20–26} For example, when a shrunken PNIPA gel is added to a dilute protein solution, the gel swells and absorbs water and other small molecules; while macromolecules, such as proteins, are not absorbed. After recovering the swollen gel and warming, it shrinks drastically and becomes ready for reuse in the concentration process. In this way, the excluded proteins are recovered in a concentrated solution. Thus, the solvent (water) and the main solute (protein) are separated by the PNIPA gel, functioning as an extraction solvent. Compared to the gel extraction process, the alternative process, namely the ultrafiltration process, is very expensive due to the high cost of membranes and the high application pressure necessary to force the solvent and other small molecules through the membrane.

In Figure 1, the equilibrium volume swelling ratio of PNIPA gels prepared in our laboratory is shown as a function of the temperature of the swelling agent water. The gels were prepared at room temperature ($23^\circ\text{C} \pm 1$) and at a monomer concentration of 8% (w/v) in water by using 1.6 and 2.5 wt % crosslinker (*N,N'*-methylenebisacrylamide), as described in the experimental section. The monomer *N*-isopropylacrylamide (NIPA) used in the gel synthesis was purchased from Eastman Kodak Co. (Rochester, NY) and

used without further purification. As seen in Figure 1, at low temperatures the PNIPA gel is swollen; at high temperatures, the gel is collapsed. At 34°C , marked with the horizontal dotted line, both a swollen and a collapsed state can be observed but never an intermediate state, indicating a first-order volume transition in the gel. It is worth noting, however, that the reported nature of the volume transition in nonionic PNIPA gels is conflicting, depending on who the researchers were. The temperature induced volume transition of this gel has been reported as both continuous^{27–30} and discontinuous.^{15,20} Moreover, it was also shown that a reproducible synthesis of PNIPA gels is difficult to achieve.^{26,31} This problem may be caused by the lower critical solution temperature (LCST) of PNIPA chains (approximately 32°C), which is close to the usual polymerization temperatures. One may expect that the kinetic events of free-radical crosslinking copolymerization, such as the gel (Trommsdorf) effect, may cause a local or overall temperature rise in the reaction system above LCST, resulting in a phase separation and formation of heterogeneous structures. Thus, depending on the heat transfer characteristics of the synthesis molds, PNIPA gels with various structures may be obtained from the same starting monomer solution.³¹ Furthermore, the presence of impurities in NIPA monomer purchased from the supplier may also affect the properties of the gels obtained.³⁰

In the present study, a series of PNIPA gels in the form of rods and beads with different crosslink densities were prepared by solution and inverse suspension polymerization techniques. The swelling capacities and the swelling rates of this gels, as well as their separation efficiencies in concentrating penicillin G acylase (PGA), bovine serum albumin (BSA), and 6-aminopenicillanic acid (6-APA) solutions, were examined. The effects of various synthesis parameters, such as the gel preparation temperature, the crosslink density of the gel, and the polymerization technique used, as well as the extraction temperature on the efficiency of the concentration process, were studied. It is known that the distribution of proteins between the gel and its surroundings as well as the swelling rate and the swelling capacity of the gel are important factors in protein concentration applications. Ideal polymer gels for use in concentrating protein solutions should exhibit high swelling rates and swelling capacities, and the gel should exclude all the protein molecules; that is, the separation efficiency should be 100%. How-

ever, PNIPA gels used in the previous studies exhibited swelling capacities and swelling rates, which are inadequate for an efficient extraction process. Moreover, protein molecules adsorbed on the PNIPA gel surfaces decreased the efficiency of the concentration process appreciably. In the present article, we also show that the coating of PNIPA gel beads by protein molecules prior to the separation experiment significantly increases their separation efficiencies.

EXPERIMENTAL

Materials

The monomer *N*-isopropylacrylamide (NIPA, Eastman Kodak), the crosslinker *N,N'*-methylenebisacrylamide (BAAm, Fluka), the initiator ammonium persulfate (APS, Aldrich), and the accelerator *N,N,N',N'*-tetraethylmethylenediamine (TEMED, Aldrich) were used as received. Bovine serum albumin (BSA; 66,000 g mol MW) was obtained from Sigma. 6-aminopenicillanic acid (6-APA; 218.26 g mol MW) was provided from Unifar Pharmaceutical Company as a gift. Penicillin G acylase (PGA; EC 3.5.1.11; 90,000 g mol MW; 14.1 U mg specific activity) was purified in Enzyme and Fermentation Technology Laboratory of TUBITAK Marmara Research Center, as described previously.³²

Polymerization Procedure

Poly(*N*-isopropylacrylamide) (PNIPA) gels were prepared by free radical crosslinking copolymerization of the monomer NIPA with a small amount of the crosslinker BAAm in aqueous solutions. The gels were prepared by solution and inverse suspension polymerization techniques as follows.

Solution Polymerization

8.0 g of the monomer and the crosslinker mixture (NIPA and BAAm) and 80 mg of APS were dissolved in double distilled water to give a total volume of 100 mL. The solution was then purged with nitrogen gas for 10 min to eliminate dissolved oxygen in the system. After addition of 0.24 mL of TEMED, the solution was transferred to small tubes of 6 mm in diameter. After 2 h of polymerization at a predetermined temperature, the gels were cut into specimens of approximately 10 mm in length and immersed in a large excess

of water to wash out any unreacted monomers and initiators.

Inverse Suspension Polymerization

Copolymerization was conducted in a 500-mL round bottom, four-necked flask, fitted with a mechanical stirrer, nitrogen inlet, and pipette outlet. Clean PNIPA gel beads were prepared without using an external emulsifier in a water-insoluble continuous phase. Paraffin oil was used as the continuous phase as reported by Park and Hoffman.³³ 200 mL of paraffin oil were first introduced into the reactor and stirred at 100 rpm under nitrogen atmosphere. 4.03 g of the monomer mixture (NIPA and BAAm) and the initiator APS (40 mg) were dissolved in distilled water (20 mL), then, N₂ was bubbled through the solution for 10 min. This solution was transferred into the reactor, and, after 1 min, TEMED (0.12 mL) was added to the mixture to initiate the polymerization. The reaction was allowed to proceed for 3 h. The reactions were carried out at three different temperatures, namely, at 4, 10, and 25°C. After polymerization, the beads were separated from the oil phase and washed several times with acetone and water. The diameter of the hydrogel beads in the swollen state ranged from 0.25 to 2.8 mm. The particles were sieved using ASTM sieves, and those of selected fractions were used for further experiments.

Methods

Swelling Measurements

The PNIPA gel samples in the form of rods were immersed in vials filled with water or with aqueous protein solutions. The volume of the solution in the vial was much larger than the gel volume, so that the concentration of the solution was practically unchanged. The vials were set in a temperature-controlled bath of $\pm 0.1^\circ\text{C}$. In order to reach the equilibrium degree of swelling, the gels were immersed in solutions at least for 3 days. The diameter of the gel samples was measured by a calibrated digital compass. The equilibrium swelling ratio of the gels related to the network formation state, V/V_0 , where V and V_0 are the volumes of gel at equilibrium and after preparation, respectively, was calculated as

$$V/V_0 = (D/D_0)^3 \quad (1)$$

where D and D_0 are the diameter of the gels after

equilibrium swelling and after preparation, respectively. Since the diameter of PNIPA gels in the form of beads cannot be precisely measured by the digital compass, their swelling ratios were determined by immersing the beads in water or in aqueous protein solutions for at least one week. They were then weighed in the swollen and dry states. In order to dry the swollen gel beads, they were first collapsed in hot water and then in acetone; the collapsed samples finally dried in vacuum at 60°C. The equilibrium swelling ratios of PNIPA beads were given in terms of the water absorption capacity, which is defined as the volume of water in equilibrium swollen gel per gram of dry gel.

Protein Concentrating Experiments

Protein concentrating experiments were carried out in test tubes containing dilute protein solutions of concentration of less than 0.1 g/L. First, 0.5 g of PNIPA gels were added into the test tubes, and they were allowed to swell until about half of the volume of the external solution was absorbed by the gel. Then, the protein content in the outer solution as well as the enzyme activity were determined. Protein concentration in the solution phase was measured by coomassie blue binding method using bovine serum albumin as the standard.^{34,35} Enzyme (PGA) activity was measured by the *p*-dimethylamino benzaldehyde (PDAB) method.³⁶ One unit of enzyme activity is defined as the amount of enzyme required to produce 1 μ mol 6-APA per minute at 40°C and at pH 8.0 from 15 mM PGA in 50 mM phosphate buffer. Swollen gels after the experiments were collapsed at 55°C by incubating them for 60 min and then reused in the separation process.

The effectiveness of the protein separation can be quantified by an efficiency η , which is defined as the actual increase in protein concentration divided by the increase expected from the altered solution volume;²⁰ that is

$$\eta = \frac{c - c_0}{c_0 \left(\frac{v_0}{v} - 1 \right)} \times 100 \quad (2)$$

where c and v are the protein concentration and the volume of the solution phase respectively, and the subscript 0 denotes the initial values. For example, if half of the feed solution is absorbed by the gel (i.e., $v_0/v = 2$), and if the raffinate has

twice the feed concentration, then the efficiency η would be 100%.

RESULTS AND DISCUSSION

Solution Polymerization

PNIPA gels were prepared by free-radical cross-linking copolymerization of NIPA and BAAM monomers. Since both the monomers are solid at usual polymerization temperatures, it is necessary to carry out the reactions in an aqueous solution of the monomers. For the present study, the initial monomer concentration was taken to be constant at 8% (w/v), whereas the crosslinker (BAAM) content was varied from 1.0 to 2.5 wt %. As seen in Figure 1, at low temperatures, water is a relatively good solvent for PNIPA gels; whereas it becomes poor one as the temperature is raised. Therefore, one may expect that the PNIPA gels formed at low temperatures are homogeneous, whereas those formed at higher temperatures should exhibit heterogeneous structures. Indeed, visual observations showed that the gels prepared at 18°C or lower were transparent; whereas those formed at higher temperatures (i.e., at 25°C) were opaque. Since the turbidity of a gel is a direct result of light scattering from the spatial inhomogeneities of the gel refractive index, it also indicates the scattering of light by discrete water droplets expelled from the PNIPA network at high temperatures. Thus, at low temperatures, water remains in the gel phase throughout the polymerization, resulting in the formation of expanded homogeneous networks. At high temperatures, water separates out of the network phase due to the polymer-solvent incompatibilities (χ -induced syneresis³⁷) resulting in heterogeneous structures. It must be noted that, although a discrete volume transition in PNIPA gels occurs at 34°C (Fig. 1), the turbidity in the gel samples appears below this temperature (i.e., at 25°C). This may be related to a rise in temperature of the polymerization system due to the gel effect.³⁰

The effect of the gel preparation temperature on the equilibrium swelling ratio of the PNIPA gels, V/V_0 , is shown in Figure 2. The gel samples were prepared at 2 wt % BAAM concentration but at different temperatures between 4 and 34°C. The swelling measurements were carried out at room temperature (23°C \pm 1) in water. Each swelling ratio reported in this graph is an average

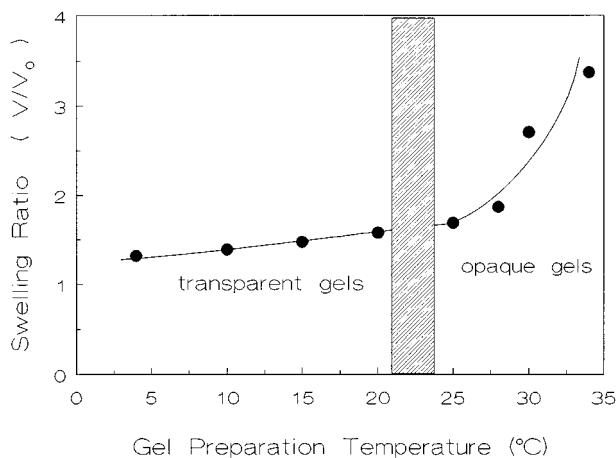


Figure 2 Variation of the equilibrium swelling ratio of the PNIPA gels, V/V_0 , with the polymerization temperature. BAAM: 2 wt %. Swelling temperature: $23^\circ\text{C} \pm 1$. The shaded area represents the transition region from homogeneous to heterogeneous gels.

of three separate measurements. For the transparent gels (i.e., those formed below 25°C), the equilibrium swelling ratio V/V_0 increases only slightly with increasing gel preparation temperature. However, for heterogeneous gels, V/V_0 strongly depends on the preparation temperature, and it drastically increases with increasing temperature of the polymerization mixture. Furthermore, the gels prepared at temperatures higher than 30°C exhibited a loose structure, indicating a very low degree of effective crosslinkages within these gels. These results can be explained as follows: If the temperature is below 25°C , the diluent water present during the network formation process remains in the gel throughout the polymerization. As the temperature is increased, the diluent starts to separate out of the network phase during the reactions. Thus, the polymerization system tends to separate into polymer-rich and polymer-poor regions. Due to the high concentration of pendant vinyl groups in polymer-rich regions, one may expect enhanced rates of crosslinking and multiple crosslinking reactions, which would lead to the formation of highly crosslinked, microgel-like regions in the final gels.³⁸⁻⁴¹ On the other hand, the polymer-poor regions become slightly crosslinked due to the high degree of dilution; thus, they constitute the interstices between the microgels in the final networks. According to this picture, the highly crosslinked regions may contribute to the rubber elasticity of the final gels as single junction points. Increasing temperature will increase the extent of phase separation and,

also, the concentration difference between the gel and diluent phases. As a result, the compactness of the microgels will increase, but the connections between the microgels become weaker and weaker as the temperature increases, which would lead to increased degree of swelling of heterogeneous PNIPA gels on raising temperature. One may expect that if the temperature is too high, water separates totally out of the network phase during the polymerization, and the phase separated compact particles cannot occupy the whole available volume because of the insufficient amounts of monomer in the diluent phase. Thus, the system becomes discontinuous and a solution of PNIPA microgels will result.⁴⁰

Another factor determining the equilibrium swelling ratio of the gels is the amount of the crosslinker BAAM used in their synthesis. In Figure 3, the equilibrium swelling ratio of PNIPA gels, V/V_0 , is plotted against the BAAM concentration. Here, the gel preparation temperature was fixed at 25°C . The filled and empty circles represent the results of swelling measurements carried out at 4°C and at room temperature ($23^\circ\text{C} \pm 1$), respectively. The equilibrium swelling ratio decreases as the BAAM content increases due to the increasing crosslinking density of the networks (i.e., due to the decrease of the molecular weight of the network chain). Furthermore, the gel swells much more at low temperatures due to the decrease of the polymer-solvent interaction parameter χ of PNIPA-water system. From Figures 2 and 3, we can conclude that high swelling ratios can be achieved at low swelling tempera-

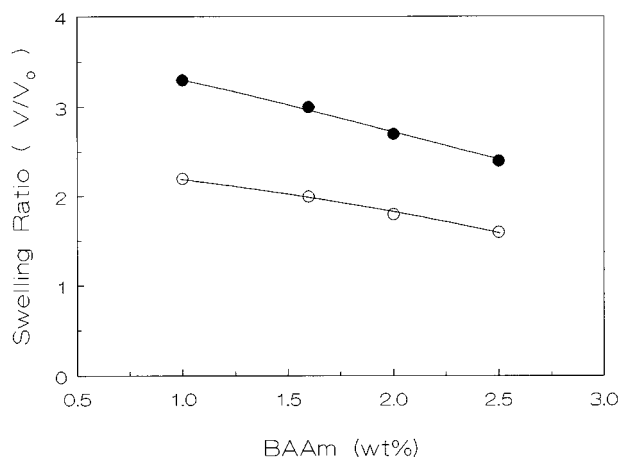


Figure 3 Variation of the equilibrium swelling ratio of PNIPA gels, V/V_0 , with the crosslinker (BAAM) content. Swelling measurements were performed at (●) 4°C and (○) $23^\circ\text{C} \pm 1$. The gel preparation temperature: 25°C .

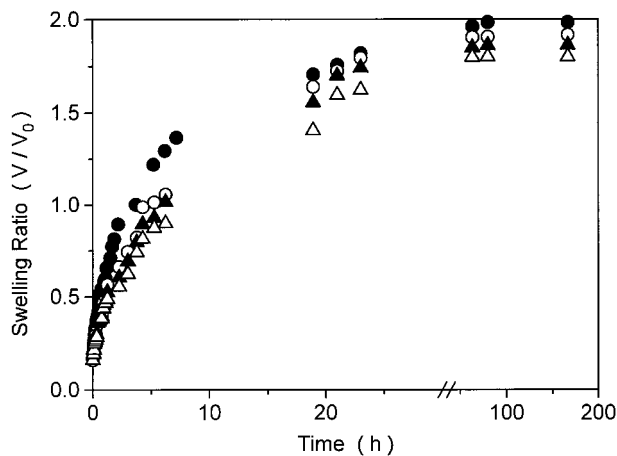


Figure 4 Variation of the swelling ratio V/V_0 of PNIPA gels immersed in BSA solutions with the swelling time. BSA concentration in the external solution: (●) 0, (○) 0.125, (▲) 0.50, and (△) 1.0 mg per 10 mL. BAAM content of the gel: 1.6 wt %. Preparation temperature: $23^\circ\text{C} \pm 1$. Swelling temperature: 25°C . Prior to the swelling experiments, the equilibrium swollen gel samples in water after synthesis were collapsed at 55°C by incubating them for 60 min.

tures using loosely crosslinked PNIPA gels prepared at temperatures as high as 35°C .

Variation of the swelling ratio of PNIPA gels immersed in aqueous solutions of bovine serum albumin (BSA) with the swelling time is shown in Figure 4. Prior to the swelling experiments, the equilibrium swollen gels in water after synthesis were collapsed by incubating them for 60 min at 55°C . The BSA concentration in the external solution was varied between 0 and 0.1g/L. Swelling experiments were carried out at 25°C by using PNIPA gels with 1.6 wt % BAAM content prepared at $23^\circ\text{C} \pm 1$. The gel volume increases first abruptly up to 2 h and then slightly increases with increasing swelling time. The gel attains its equilibrium volume in water after about 70 h. The results indicate that the gel reaches 30% of its equilibrium volume within 2 h. This value is important for application of PNIPA gels as extraction solvent because it determines the time interval of the extraction process. Figure 4 also indicates that both the rate of swelling and the equilibrium volume of the gel phase slightly decrease with the addition of BSA in the external solution. This feature is better seen in Figure 5, which illustrates the dependence of the swelling ratio on the BSA concentration in the outer solution for fixed swelling times. The gel swells in BSA solutions less than in pure water. Increasing

BSA concentration up to 0.1 g/L slightly decreases the volume of the gel phase. As will be shown below, the BSA concentration inside the gel phase is much lower than in the external solution. The contraction of the gel in BSA solution is probable a result of this concentration difference between the inside and outside the gel phase, which creates an osmotic pressure compressing the network.

Figure 6 shows the separation efficiencies η of BSA, PGA, and 6-APA molecules by gel extraction with PNIPA gels plotted against the crosslinker content of the gels prepared at $23^\circ\text{C} \pm 1$. The filled and empty circles represent the results of experiments carried out using BSA and PGA solutions, respectively. The error bars indicate standard deviations of the results of at least three experiments with BSA solutions. It is seen that about 80% of both BSA and PGA molecules do not penetrate inside the network. The loss of 20% may be attributed to the adsorption of protein molecules on the surface of the gel samples. Experiments carried out using BSA solutions at two different temperatures, namely, at 4 and $23^\circ\text{C} \pm 1$, showed that the efficiency of the extraction process is largely insensitive to the extraction temperature. Compared to the protein molecules, the separation efficiency of 6-APA is zero, indicating that the concentration of 6-APA inside the gel phase is equal to that in the outer solution. Since the molecular weights of BSA and PGA are high (66,000 and 90,000 daltons, respectively), they cannot penetrate the gel phase due to the loss in entropy of the protein molecules, as well as due

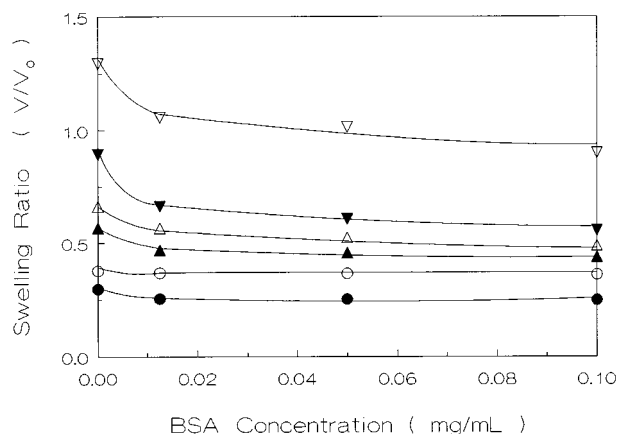


Figure 5 Variation of the swelling ratio V/V_0 of PNIPA gels with the BSA concentration in the outer solution for a fixed swelling time. Swelling time: (●) 9, (○) 20, (▲) 55, (△) 75, (▼) 135, and (▽) 500 min. See Figure 4 caption for the synthesis condition of the gel.

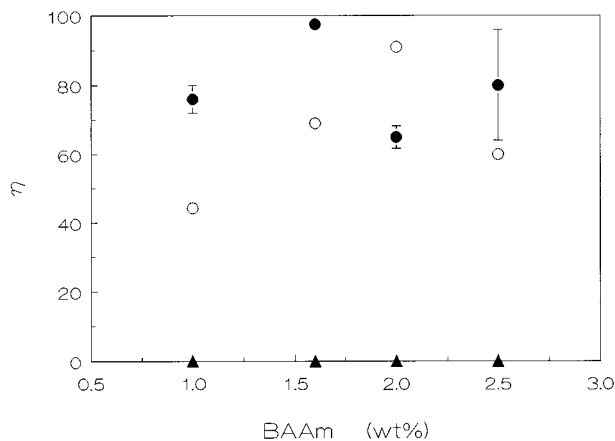


Figure 6 Effect of the crosslinker (BAAm) content of PNIPA gels on the separation efficiency η of (●) BSA, (○) PGA, and (▲) 6-APA from dilute solutions. Gel preparation temperature: $23^{\circ}\text{C} \pm 1$. The error bars indicate standard deviations of the results of at least three experiments carried out with BSA solutions. For 1.6 wt % BAAm content, the standard deviation is smaller than the symbol. Experiments with BSA solutions were performed both at room temperature ($23^{\circ}\text{C} \pm 1$) and at 4°C .

to the repulsion between network and protein chains. However, as the molecular weight of the solute decreases as in the case of 6-APA (218 g mol), the amount of molecules penetrating into the gel increases (i.e., the separation efficiency decreases). These results are in accord with our previous observation carried out with poly(acrylamide-*co*-acrylic acid) gels swollen in aqueous solutions of poly(ethylene glycol) of different molecular weights.^{42,43}

Figure 7 shows the variation of the separation efficiency η of PGA with the preparation temperature of PNIPA gels of 2 wt % BAAm content. η is about 80% for homogeneous gels (i.e., for gels prepared below 25°C). However, for heterogeneous gels formed at higher temperatures, η decreases abruptly due to the simultaneously increase of the gel's swelling ratio (Fig. 2), which facilitates the penetration of PGA molecules inside the gel phase.

The specific activity of the PGA solution used in the experiments was 14.1 U/mg. At this level of specific activity, the enzyme solution may contain some contaminant proteins, which may have higher or lower molecular weights than that of the PGA molecule. Consequently, besides the loss of the total amount of protein, the loss of the total amount of enzyme activity may also be observed after the extraction of enzyme solutions by PNIPA

gels. Indeed, it was found that the amount of protein removed from the solution is higher than that of the amount of PGA activity. This indicates the possible absorption of some contaminant protein molecules of much smaller molecular weights together with the PGA molecules by the gel phase.

Inverse Suspension Polymerization

In this section, PNIPA gel beads prepared by inverse suspension polymerization technique were used for swelling and separation experiments. Rigid, transparent, and spherical beads were obtained without using an external emulsifier; therefore, they are free from impurities affecting the separation process. The polymerization reactions were carried out at 100, 200, and 250 rpm stirring rates; at high stirring rates, more than 90% of the beads obtained were in the size range of 0.25–0.40 mm in the swollen state. At a stirring rate of 100 rpm, the inverse suspension polymerization technique gave spherical particles of sizes 0.125–1.8 mm. A representative optical micrograph of the particles is shown in Figure 8. Figure 9 shows a typical size distribution of PNIPA beads with 2 wt % BAAm content prepared at 100 rpm. The bar graph indicates that about 70% of the microspheres were in the range of 0.8–1.4 mm.

The influence of the preparation technique of PNIPA gels with 2 wt % BAAm content on their swelling properties is shown in Table I. Here, the equilibrium swelling ratios are given in terms of the water absorption capacity (volume of water in equilibrium swollen gel per gram of dry gel).

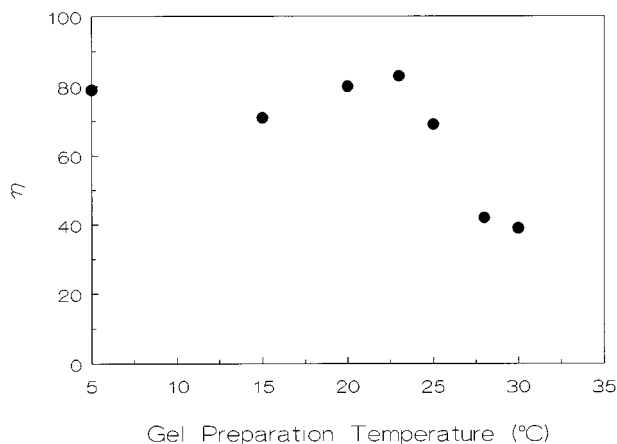


Figure 7 Variation of the separation efficiency η of PGA with the gel preparation temperature. Swelling experiments were performed at 4°C . BAAm content of the PNIPA gel: 2 wt %.

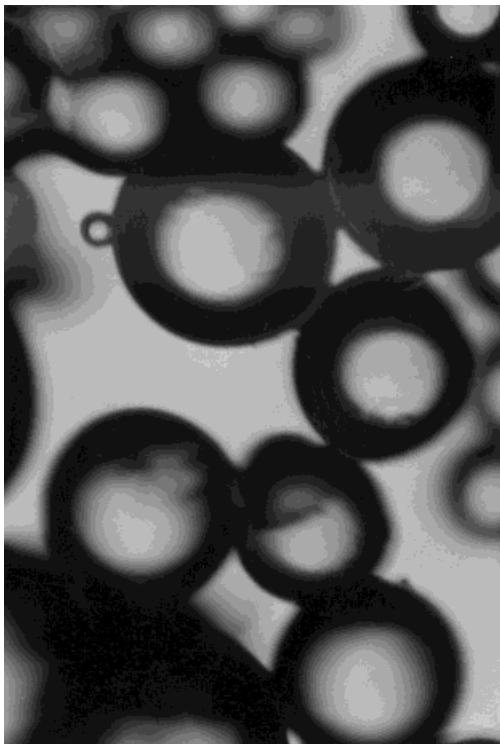


Figure 8 A representative optical micrograph of PNIPA gel beads obtained by inverse suspension polymerization technique. Magnification $\times 40,000$.

The gels were prepared under the same experimental conditions; the differences are only in the preparation technique used. The gels A were prepared by inverse suspension polymerization technique, whereas the gels B were obtained by solution polymerization. Table I clearly indicates that the gels A swell in water much more than the gels B. Furthermore, contrary to the gels B, which

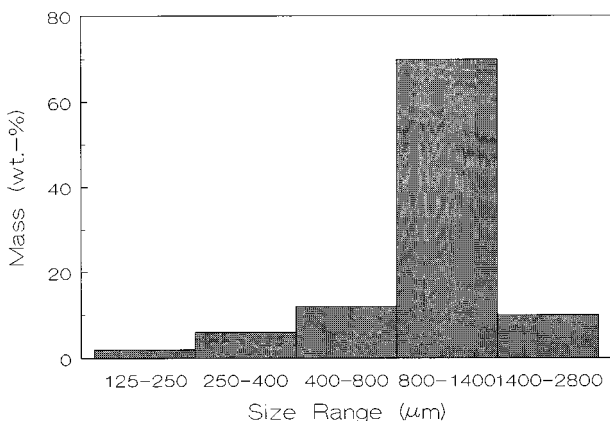


Figure 9 Size distribution of PNIPA beads with 2 wt % BAAM content.

Table I The Influence of the Preparation Technique of PNIPA Gels on Their Swelling Properties

Preparation Temperature (°C)	Water Absorption Capacity (mL of Water in Swollen Gel/g of Dry Gel)			
	at 4°C		at 23°C \pm 1	
	A	B	A	B
4	37	24	33	15.5
10	34	27	31	16.5
25	32	28	25	22

BAAM: 2 wt %. Gels A were prepared by inverse suspension polymerization technique, whereas gels B were obtained by solution polymerization.

exhibit increased swelling on raising the preparation temperature (cf. Fig. 2), the water absorption capacity of the gels A decreases as the gel preparation temperature increases. This inverse relation between the preparation temperature and equilibrium swelling ratio of the gel beads, as well as their higher swelling ratios compared to the gels formed in solution, can be accounted for the characteristics of inverse suspension polymerization technique. One possible explanation is the solubility of the monomers in the continuous phase, which decreases the monomer concentration in the droplets and so increases the number of elastically inactive links within the final gel beads.

When the swelling rates of the gels A and B were compared, polymer gels in the form of beads absorb water more rapidly than the gels prepared by solution polymerization. For example, 1 g of the gel beads in dried state absorbs 20 mL of water in 10 min; whereas the same amount of gel in the form of rods absorbs only 3 mL of water in the same time interval. The higher water absorption capacity and rapid swelling feature of PNIPA gel beads make them the first choice as a separation agent in extraction experiments. In Table II, the separation efficiency η of PGA by PNIPA gel beads of sizes 0.25–0.40 mm is given for various gel preparation temperatures. Table II shows that when the polymer beads were used in the separation processes, the protein separation efficiency is less than 17%. This lower efficiency is probably due to the increase of the surface area of the gel when it is in the form of spherical beads, which increases the amount of adsorbed solute molecules on the bead surfaces. To overcome this prob-

lem, the gel beads of sizes 0.25–0.40 mm were immersed in BSA solutions of concentration 1 mg BSA/mL, and they were left for 24 h prior to the separation experiments. In this way, their surfaces were coated by protein molecules prior to the experiments. Then, these gels were used in concentration experiments of PGA solutions. In the third column of Table II, η values are collected for coated PNIPA gel beads prepared at three different temperatures. It is seen that the separation efficiency η increases up to 74%. Thus, the protein adsorption on the gel surface was reduced significantly after the pretreatment of the gel beads. The effect of the particle size of the coated gel beads on the separation efficiency η is seen in Table III. η increases as the particle size of the coated beads increases. η approaches to 100%; that is, the loss of protein in the solution becomes practically negligible when the size of the PNIPA beads is larger than 0.8 mm.

CONCLUSIONS

From the results presented in this article, it can be concluded that the polymerization technique used significantly affects the properties of PNIPA gels. The solution polymerization technique at temperatures as high as 35°C leads to the formation of heterogeneous PNIPA gels with high swelling ratios in water. The swelling capacity of the gels further increases when they are prepared in the form of beads by using inverse suspension polymerization technique. The separation efficiency η of BSA and PGA by gel extraction with PNIPA gels formed in solution was found to be about 80%. If the same gels were prepared by in-

Table II PGA Separation Efficiency η at 4°C Using PNIPA Gel Beads of Sizes 0.25–0.40 mm

Gel Preparation Temperature (°C)	η	
	Uncoated Beads	Coated Beads
4	4 (5)	28 (28)
10	11 (14)	47 (47)
25	17 (37)	74 (74)

The values in parenthesis are η values calculated from the enzyme activities using eq. (2) after replacing concentration terms with enzyme activities in the feed and in the raffinate. BAAM: 2 wt %. Concentration of the feed solution: 0.1 mg/mL.

Table III PGA Separation Efficiency η at 4°C Using Coated PNIPA Gel Beads of Various Sizes

Bead Size (mm)	η
0.25–0.40	69
0.40–0.80	72
0.80–1.4	99
1.4–2.8	100

η values were calculated from the enzyme activities in the feed and in the raffinate. BAAM: 2 wt %. Gel preparation temperature: 23°C \pm 1. Specific activity of the feed solution: 14.1 U/mg.

verse suspension polymerization technique, η decreases up to 4–17%. However, after coating, the gel beads by protein molecules prior to the separation experiments, separation efficiency values of 100% were reached using gel beads of sizes larger than 0.8 mm.

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