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A novel thermoresponsive hydrogel matrix based on poly(N-ethoxypropylacrylamide)

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Summary

A new temperature sensitive hydrogel matrix, poly(N-ethoxypropylacrylamide), PNEPAM, was obtained by the bulk polymerization of N-ethoxypropylacrylamide (NEPAM). The monomer, NEPAM was synthesized by the nucleophilic substitution reaction of 3-ethoxypropylamine and acryloyl chloride. The polymerization was performed at +4 ^{o}C, by using N,N-methylenebisacrylamide (MBAM) as crosslinker, polyethyleneglycol (PEG) 4000 as diluent, and potassium persulfate (KPS) and tetramethylethylenediamine (TEMED) as the initiator and accelerator, respectively. PNEPAM gel matrices exhibited a thermosensitive bahaviour reasonably similar to poly(N-isopropylacrylamide), PNIPAM gels. The equilibrium swelling ratio at constant temperature increased with increasing initiator concentration and decreasing monomer concentration. The use of PEG 4000 as a diluent in the gel synthesis resulted in a significant enhacement in the thermosensitivity of gel matrix. The equilibrium swelling ratios up to 60 g water/g dry gel were observed in the low-temperature region. The results indicated that PNEPAM gel is a new alternative thermosensitive material to the NIPAM based gels.

Introduction

Temperature sensitive polymers is a recent research area attracting a great interest due to the wide applicability in biotechnological and biomedical area [1-12]. The main characteristics of these polymers is that they have a lower critical solution temperature (LCST) [13-15]. Temperature-sensitive polymers have been usually obtained by the polymerization of N-alkylacrylamide monomers [16-32]. Different N-alkylacrylamide monomers like isopropylmethacrylamide, diethylacrylamide, cyclopropylacrylamide, ethylpropylacrylamide, and ethoxyethylacrylamide were used in the synthesis of thermally sensitive polymeric structures [16-32]. These monomers are usually synthesized by nucleophylic substitution reaction of acryloyl chloride with the

appropriate amine. N-isopropylacrylamide (NIPAM) is the well-known monomer used for the production of thermally reversible linear polymers and crosslinked hydrogels [33-38]. NIPAM based thermally sensitive copolymers with different functionalities were also synthesized and used in the temperature controlled isolation of various biomolecules like proteins, nucleotides, DNA and RNA [39-45].
In our previous study, linear poly(ethoxypropylacrylamide) (PNEPAM) was proposed as a new thermosensitive polymer. The phase transition behaviour of soluble PNEPAM against temperature was extensively investigated in the aqueous media [46]. In this study, PNEPAM based crosslinked hydrogels are first prepared and their thermoresponsive behaviour is defined. Here, we wish to report the effects of production conditions (i.e. the initiator, crosslinking agent, monomer and diluent concentrations) on the thermoreversible swelling behaviour of new hydrogels.

Experimental

Materials

The monomer, N-(3-ethoxypropyl)acrylamide (NEPAM) was obtained by starting from acryloyl chloride (Aldrich) and 3-ethoxypropylamine (Aldrich). Potassium persulfate (KPS), (BDH) and tetramethylethylenediamine (TEMED) (Sigma) were used as initiator and the accelerator, respectively. N,N-methylenebisacrylamide (MBAM) (Sigma) was the crosslinking agent. Polyethyleneglycol (PEG-4000, Average molecular weight:4000 g/mol, BDH) was used as the polymeric porogen. All polymerizations and gel characterization experiments were performed in distilled-deionized water.

Monomer synthesis

The synthesis protocol of ethoxypropylacrylamide (NEPAM) was given elsewhere [46]. Briefly, 3-ethoxypropylamine (0.11 mol) and p-benzoquinone (10 mg) were dissolved in a solution of triethylamine (0.12 mol) in dichloromethane (200 mL) cooled to 0^{o}C. Acryloyl chloride (0.10 mol) - dichloromethane (40 mL) solution was added dropwise into this solution within 2 h. The resulting solution was stirred magnetically for 24 hours at +4 ^{o}C. The organic phase extracted with cold water and dried over $MgSO_4$ was evaporated in vacuo for the isolation of NEPAM. The monomer was characterized by H-NMR and FTIR spectroscopy. H-NMR characteristics are as follows: ($CDCl_3$, 400 MHz): δ=1.19 (t, 3H, CH_3, J=7.01 Hz), 1.78-1.84 (m, 2H, CH_2), 3.40-3.53 (m, 6H, CH_2) 5.57-5.60 (m, 1H, vinyl-H), 6.13-6.25 (m, 2H, vinyl-H), 6.83 (bs, 1H, NH). FTIR characteristics: 3283 (N-H, amide), 3078-2802 (alkyl), 1659 (C=C), 1552 (amide II) [46].

Polymerization

The temperature-sensitive crosslinked polymeric hydrogels were prepared by bulk polymerization. Typically, NEPAM (0.125 g) was dissolved in water (0.70 mL) in a pyrex tube (7 mm i.d. and 100 mm length) in an ultrasonic bath. An aqueous solution of crosslinker, MBAM was then added (0.25 mL, 30 mg/mL) and the tube was placed in a water–ice bath, and it was allowed to cool to +4 ^{o}C. Onto this cooled solution, an aqueous KPS solution (0.15 mL, 50 mg/mL) cooled in an ice-bath and an aqueous

solution of TEMED (0.15 mL, 11.6 % w/w) were added. The polymerization medium was homogenized by mixing and purged with nitrogen for 5 minutes. Then, the tube was sealed by a stopper plug. The tube was placed in a water bath at +4°C, and gel formation occurs within 24 hours. The gel was taken into a distilled water medium at +4 °C (100 mL). Distilled water was replaced once at each 2 hours. By this way, the polymer gel was rinsed and possible unconverted monomers and the initiator system components were removed from the medium. After completion of rinsing, the gel was kept in distilled water at +4 °C. Similar procedures were also applied by changing initiator, monomer, diluent and crosslinker concentrations in order to obtain crosslinked gel structures with different thermoresponsive properties.

Determination of temperature-sensitivity

The equilibrium water contents of crosslinked PNEPAM gels obtained with different production conditions were determined against temperature in 0.05 M phosphate buffer at pH 7 in a water bath equipped with a heating-cooling system. For this purpose, a sample of rinsed gel having an approximate dry weight of 0.1 g (8 mm in diameter and 16 mm in length under production conditions) was kept in phosphate buffer for 24 hours at +4 °C. Then the weight of the swollen gel was determined. The temperature of the water bath was fixed to a higher value (i.e. 12°C) and the gel was kept at this temperature for 6 hours and its weight was again determined. By following this method, the gel was kept at different temperatures up 70°C for 3 h and its weight at each temperature was determined. Finally, the gel sample was dried in vacuum at 50°C for 48 hours. The dry weight of the gel was then determined. The equilibrium swelling ratio at each temperature (Q, (g swollen gel/g dry gel)x100) was calculated.
The step effect was applied on temperature in two opposite directions in order to examine the dynamic swelling and shrinking behaviors of PNEPAM gel. For the observation of dynamic shrinking, the gel was kept at +4 °C for 24 hours until they reached their equilibrium swelling and transferred into the distilled water at 70 °C. This moment was taken as the time of zero, and the gel weight was determined against time by periodical weighing. When the gel reached its equilibrium water content at 70 °C, it was transferred into distilled water at +4 °C. By taking this moment as time of zero, the change in equilibrium water content with the time was determined by weighing the gel sample in definite time intervals.

Results and discussion

The monomer, NEPAM was synthesized by the nucleophilic subsitution reaction between acryloyl chloride and 3-ethoxypropylamine given in Figure 1. The effects of polymerization conditions on the thermoresponsivity of crosslinked hydrogels is given below:

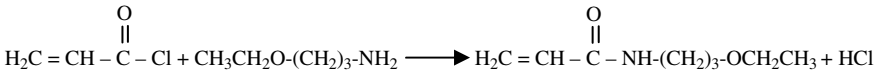

Figure 1. Nucleophilic substitution reaction for the synthesis of N-ethoxypropylacrylamide.

The effect of initiator concentration on the temperature responsivity of crosslinked PNEPAM hydrogel is shown in Figure 2. The polymerization conditions are given in the legend. As seen here, the variation of swelling ratio with temperature was very similar to that of poly(N-isopropylacrylamide), (PNIPAM) gel [3,4,6,47,48]. In our previous study, PNEPAM linear homopolymer exhibited a concentration dependent cloud point behaviour in the aqueous medium [46]. Based on this behaviour, cloud point of linear PNEPAM decreased from 42 to 32°C by increasing PNEPAM homopolymer concentration from 0.1 to 5 % w/w. In Figure 2, fully shrunken state was obtained by starting from 50°C. Hence, crosslinked PNEPAM hydrogel exhibited higher phase transition temperature with respect to PNIPAM based hydrogels. Here the phase transition temperature was defined as the temperature corresponding to the beginning of fully shrunken state. It was found by the addition of 10 percent of total swelling ratio change onto the swelling ratio at 80°C. On the other hand, higher swelling ratios were obtained with the PNEPAM hydrogel produced with higher initiator concentration. The sulphur contents of PNEPAM gels produced with the KPS concentrations of 6 and 12 mg/mL were determined by the elemental analysis, as 0.9 and 1.6 % (w/w), respectively. Hence the swelling behaviour should be explained by the higher ionisation ability of hydrogel originated from the sulphate groups formed by the decomposition of persulfate type initiator and covalently linked to the hydrogel structure.

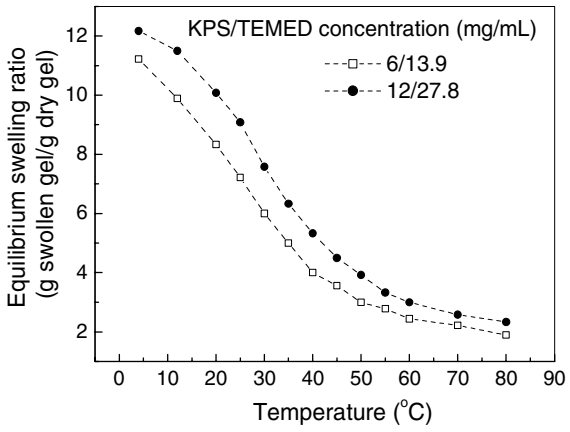

Figure 2. The effect of initiator concentration on the variation of equilibrium swelling ratio with the temperature. Gel synthesis :NEPAM:100 mg/mL, MBAM: 6 mg/mL, +4°C, 24 h.

The effect of NEPAM concentration on the thermoresponsivity of crosslinked PNEPAM hydrogel is shown in Figure 3. As seen here, higher PNEPAM concentration resulted in lower equilibrium swelling ratio at constant temperature. The volume of aqueous fluid-filled pores in the hydrated polymer chain network should be higher for the gel produced with lower PNEPAM concentration [49]. On the other hand, NEPAM concentration was not an effective variable on the phase transition temperature of PNEPAM hydrogels. The hydrogels obtained with various NEPAM concentrations passed to fully shrunken state by starting from 50°C.

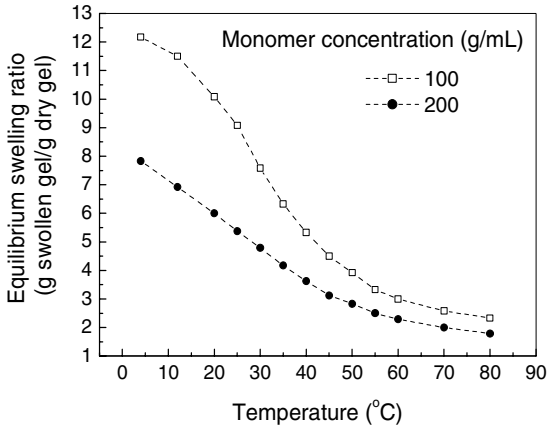

Figure 3. The effect of monomer concentration on the variation of equilibrium swelling ratio with the temperature. Gel synthesis conditions. KPS:12 mg/mL, TEMED: 27.8 mg/mL, MBAM: 6 mg/mL, +4°C, 24 h.

The effect of crosslinking agent (MBAM) concentration the thermoresponsivity of PNEPAM hydrogels is given in Figure 4. As seen here, MBAM concentration was an effective variable on the swelling behaviour particularly in the low temperature region. In other words, higher swelling ratio was obtained with the hydrogel produced with lower MBAM concentration. However, the equilibrium water content of all hydrogels with different MBAM concentrations were the same at the temperatures higher than 45°C. The crosslinking density is not a factor controlling the equilibrium water content of PNEPAM hydrogels in the shruken state since the gel behaviour is dominantly controlled by the hydrophobic interactions. The behaviour in Figure 4 is typical particularly for PNIPAM based temperature-sensitive gels. [6, 50-52].

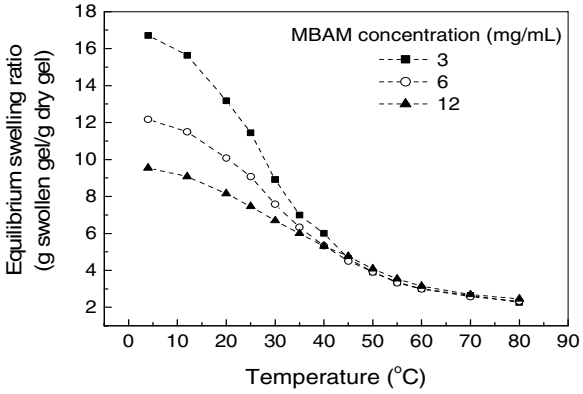

Figure 4. The effect of crosslinking agent concentration on the variation of equilibrium swelling ratio with the temperature. Gel synthesis conditions. KPS:12 mg/mL, TEMED:27.8 mg/mL, NEPAM:100 mg/mL, +4°C, 24 h.

In the synthesis of hydrogels, PEG-4000 was used as a polymeric porogen to generate porosity in the crosslinked hydrogels. PEG-4000 was previously used in NIPAM based hydrogels and provided an increase in the thermoresponsivity [6,53,54]. The effect of PEG-4000 concentration on the swelling behaviour of PNEPAM hydrogels is shown in Figure 5.

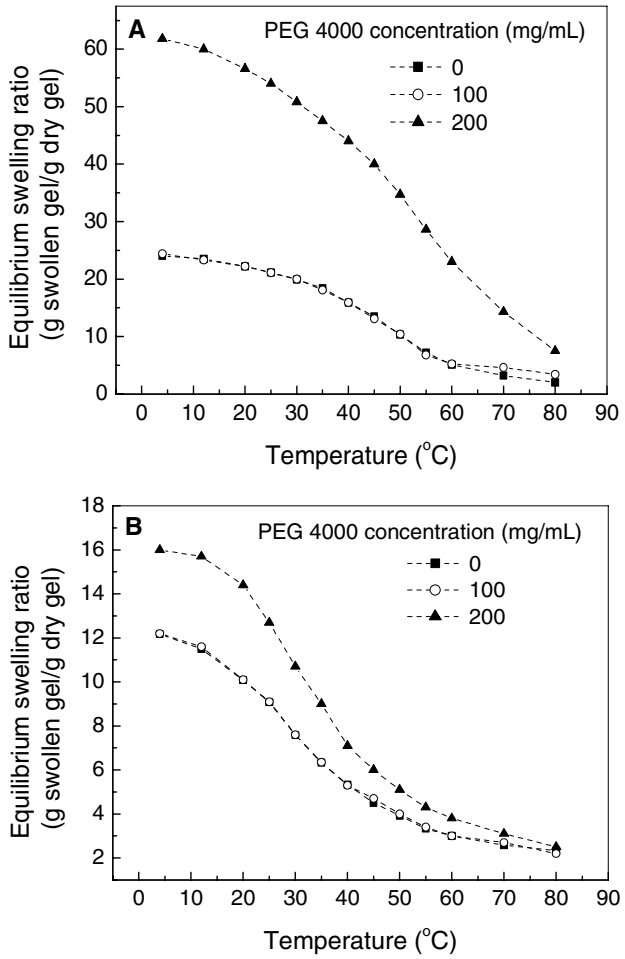

Figure 5. The effect of PEG 4000 concentration on the variation of equilibrium swelling ratio with the temperature. Medium: (A) Distilled water, (B) Phosphate buffer at pH 7, Gel synthesis: KPS:12 mg/mL, TEMED:27.8 mg/mL, NEPAM:100 mg/mL, MBAM: 6 mg/mL, +4°C, 24 h.

The effect of ionic strength on the swelling behaviour of PNEPAM hydrogel is shown in Figure 6. Similar to the PNIPAM based thermosensitive hydrogels, the equilibrium swelling ratio significantly decreased with increasing ionic strength at the temperatures lower than 40°C [6].

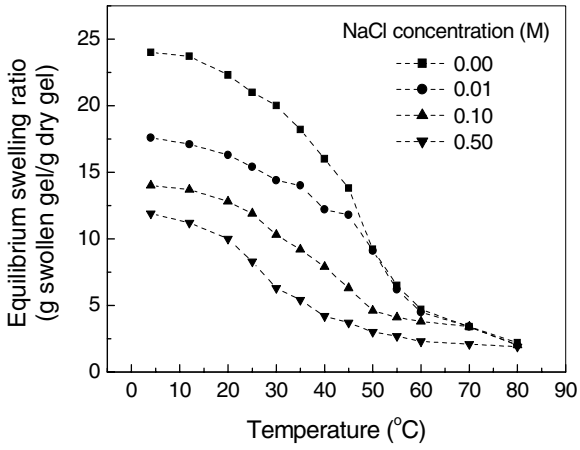

Figure 6. The effect of ionic strength on the variation of equilibrium swelling ratio with the temperature. Gel synthesis conditions. KPS:12 mg/mL, TEMED:27.8 mg/mL, NEPAM:100 mg/mL, MBAM: 6 mg/mL, +4°C, 24 h.

The effect of step change of temperature on the dynamic swelling and shrinking behaviours of PNEPAM gel is shown in Figure 7. As seen here, the PNEPAM gel passed from fully swollen form to the fully shrunken form in approximately 0.5 h by applying a step change for the temperature from +4 to 70°C. However, the swelling period was reasonably longer with respect to the shrinking period when the step change of the temperature was applied in the reverse direction (i.e. from 70 to +4°C) with the same magnitude.

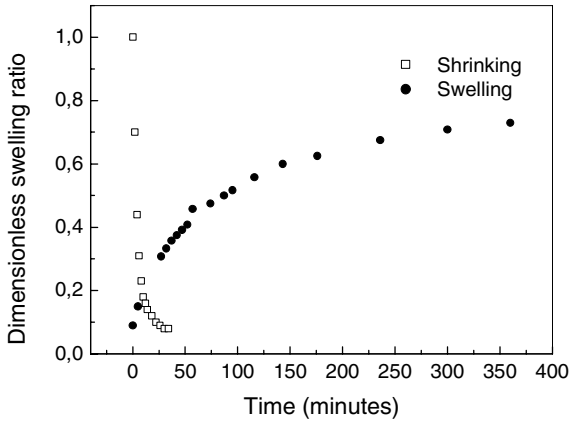

Figure 7. The variation of swelling ratio with the time for the shrinking induced by the step change of temperature from +4 to 70°C and for the swelling induced by the step change of temperature from 70 to +4°C. Gel synthesis conditions. KPS:12 mg/mL, TEMED:27.8 mg/mL, NEPAM:100 mg/mL, MBAM: 6 mg/mL, +4°C, 24 h. Gel dimensions: 8 mm in diameter and 16 mm in length under production conditions.

This behaviour was also similar to that observed with PNIPAM gels and should be explained by the convective transport of water induced by shrinking of crosslinked PNEPAM chains [6,7,43]. In this case, the internal hydrodynamic pressure inside the gel upon shrinking causes convective outflow of water from the gel interior [55].

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