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Effect of thermal cycling on the properties of thermoresponsive poly(*N*-isopropylacrylamide) hydrogels

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Abstract

Crosslinked poly(*N*-isopropylacrylamide) hydrogels have been widely studied for a variety of thermoresponsive applications, including chromatography, affinity precipitation, controlled biocatalysis, viable cell immobilisation, biomimetic actuators and, in particular, modulated drug delivery. The exploitation of crosslinked poly(*N*-isopropylacrylamide) hydrogels in all these applications relies on the well-known temperature-sensitive swelling properties of these hydrogels. The purpose of the current study was to determine the effects of repeated thermal cycling on the thermoresponsive swelling behaviour of crosslinked poly(*N*-isopropylacrylamide) hydrogels. The results show that repeated thermal cycling leads to the formation of cracks on the surface of the hydrogels. Repeated thermal cycling also results in a decreased degree of swelling in the crosslinked poly(*N*-isopropylacrylamide) hydrogels at temperatures below the volume phase transition temperature (VPTT).

Keywords: Poly(*N*-isopropylacrylamide); Hydrogels; Effect of thermal cycling; Thermoresponsive polymers; Responsive drug delivery

1. Introduction

Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) and its copolymers have been extensively studied in the development of thermoresponsive drug delivery systems (Bae et al., 1987; Okano et al., 1990; Yoshida et al., 1993; Dinarvand and D'Emanuele, 1995; Brazel and Peppas, 1995; Baudys et al., 1997; Li and D'Emanuele, 2001). Crosslinked poly(NIPAAm) hydrogel exhibits a volume phase transition temperature (VPTT) at approximately 32 °C in aqueous media due to the hydrophilic–hydrophobic balance of its constituent polymer chains and directly related to the lower critical solution temperature phenomenon exhibited by linear poly(NIPAAm) in aqueous solution.

Thermally sensitive hydrogel delivery systems can exhibit both negative controlled release, in which drug delivery is halted at temperatures above the VPTT, and positive controlled drug delivery, in which the release rate of a drug increases at temperatures above the VPTT. In the lifetime of a thermoresponsive poly(NIPAAm) hydrogel drug delivery system, it is likely that the hydrogel component will undergo numerous repeated volume phase transitions in its manufacture and in the process of modulating drug release. There is a paucity of data on the effects of repeated collapse and swelling of hydrogels on their physicochemical properties. Such changes in the physicochemical properties would be important as they could have an impact on the behaviour on a hydrogel-based delivery system. Poly(NIPAAm)

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hydrogel exhibits dramatic volume phase transitions with small changes in temperature (Okano et al., 1990; Yoshida et al., 1993; Park and Hoffman, 1994) and it is possible that repeated collapse and swelling of poly(NIPAAm) hydrogel could influence properties such as the degree of swelling, shape and surface skin layer formation.

In the present study, the effects of thermal cycling (repeated swelling and collapse of the hydrogel) on the properties of crosslinked poly(NIPAAm) homopolymer hydrogel discs is investigated.

2. Materials

N-isopropylacrylamide (monomer (99%)) was obtained from ACROS Organics (New Jersey, USA) and used without further purification. *N*,*N*′-methylene-*bis*-acrylamide (crosslinker (99%)) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (accelerator (99%)) were obtained from Sigma–Aldrich Co. Ltd. (Gillingham, UK). Ammonium persulphate (initiator (98%+)) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, USA). Phosphorus pentoxide (drying agent (98%)) was obtained from Lancaster Synthesis Ltd. (Morecambe, UK). Water used was freshly distilled and degassed. Other chemicals used were of analytical reagent grade.

3. Methods

3.1. Preparation of crosslinked poly(NIPAAm) hydrogel discs

A free radical polymerisation method was used for the preparation of poly(NIPAAm) homopolymer hydrogel matrices in the shape of discs. Four batches of poly(NIPAAm) homopolymer hydrogel discs with varying crosslinker content were prepared.

An electrophoresis gel caster system (SE 6015 dual gel caster, Hoefer Scientific Instruments, San Francisco, USA) was used as the hydrogel mould. NIPAAm, 4.50 g and 0.06 g of *N*,*N*′-methylene-*bis*-acrylamide (0.0682 mol%) were dissolved in an ice-jacketed beaker containing 30 ml of water. Nitrogen gas was then bubbled through the solution for 30 min. Fourty microlitres of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and 4 ml of a 1% w/v aqueous solution of ammonium sulphate were then added to the polymerisation solution. Nitrogen gas was bubbled through the polymerisation mixture for another minute. The resultant solution was then carefully poured between the two glass plates of the caster system, which were separated by 3 mm thick spacers. After 6 h, the clear hydrogel sheet was carefully removed from the glass plates and transferred to a tray containing distilled water. The hydrogel was left in distilled water for 5 days and the water was changed daily. The resulting sheet of hydrogel was removed from the tray of water and discs cut out using a metal borer (internal diameter 20 mm). These discs were dried to constant weight under vacuum at 55 °C in the presence of phosphorus pentoxide. The xerogel discs were stored in a dessicator until required.

The whole process was repeated using 0.12 g (0.136 mol%), 0.18 g (0.204 mol%) and 0.24 g (0.272 mol%) of *N*,*N*′-methylene-*bis*-acrylamide.

3.2. Thermal cycling

Hydrogel discs were allowed to swell in distilled water at 15 °C for 24 h (Julabo F10-HC, Julabo Labortechnik GmbH, Seelbach/Schwarzwald, Germany) and subjected to thermal cycling by placing them into 500 ml of distilled water heated to a constant temperature of 55 °C (Grant LTD6G/20, Grant Instruments Ltd., Cambridge, UK) and then transferring them into 500 ml of distilled water cooled to a constant temperature of 15 °C. Each thermal cycle consisted of 5 min in water heated to 55 °C followed by 5 min in water cooled to 15 °C.

Hydrogel discs were subjected to 30 thermal cycles, 15 thermal cycles or no thermal cycling. After being subjected to thermal cycling, the hydrogel discs were re-dried to constant weight under the same conditions stated above. The discs, which were not subjected to thermal cycling, were re-dried after the initial swelling in water at 15 °C.

3.3. Hydrogel characterisation

3.3.1. Equilibrium swelling ratio

The swelling behaviour of hydrogels was determined by measuring equilibrium swelling ratios as previously described (Dinarvand et al., 1995).

Equilibrium swelling ratio is defined as the ratio of the solvent-swollen weight of the hydrogel equilibrated at a given temperature to the weight of the hydrogel in its dry state, i.e. the xerogel. Hydrogel discs were weighed and allowed to reach their equilibrium swelling state in water at temperatures ranging between 10 and 65 °C. Using a top pan balance (Sartorius Research R200 D Electronic Semi-Microbalance, Sartorius GmbH, Goettingen, Germany), the weight of each hydrogel disc was measured daily once equilibrium swelling had been attained. The hydrogel discs were individually removed from the water each day and any excess water on the surfaces of the discs was removed by blotting with filter paper. The discs were then carefully wrapped in pre-weighed aluminium foil before weighing. This was done to prevent the evaporation of water from the hydrogel discs. Equilibrium swelling was defined to have been achieved when the weight of a hydrogel disc was recorded as being less than 0.5% different to that measured on the previous day. Measurements were performed in triplicate.

3.3.2. Thermal analysis

Differential scanning calorimetry (DSC) was used to determine the influence of crosslinker content and thermal cycling on the VPTTs of the hydrogels. A DSC220C differential scanning calorimeter (Seiko Instruments, Tokyo, Japan) was used to analyse hydrogel discs. The samples were prepared by first allowing the hydrogel discs to swell for 24 h in distilled water maintained at 15 °C. The discs were then removed from water and any excess water on the surfaces was removed by blotting with filter paper. Finally, the discs were coarsely crushed using a mortar and pestle and approximately 5–15 mg samples of hydrogel were weighed into aluminium pans, which were then sealed. The thermal analysis was carried out with a closed pan system in a nitrogen gas flow. Empty closed aluminium pans were used as the reference cell. The thermal analysis programme used raised the temperature from −10 to 90 °C at a rate of 10 °C per minute.

3.3.3. Nuclear magnetic resonance (NMR) spectroscopy

The effect of thermal cycling on the chemical structure of crosslinked poly(NIPAAm) hydrogel was determined. Using ^{13}C NMR spectroscopy, the NMR spectra of hydrogel discs that were not subjected to thermal cycling and hydrogel discs that had been subjected to 30 thermal cycles were compared. The dry hydrogel discs were crushed into small flakes using a mortar and pestle. 0.25 g of hydrogel flakes and 4 ml of deuterium oxide (Goss Scientific Instruments Ltd., Great Baddow, Essex, UK) were measured into 10 mm diameter NMR tubes and left for 24 h at room temperature to allow the hydrogel to swell. ^{13}C NMR spectra were obtained at ambient temperature using a Varian Associates Unity 500 spectrometer operating at 125.8 MHz. About 10,000 transients were time-averaged using a pulse interval of 0.5 s and a flip-angle of 40°. Fourier transformation was carried out with a line broadening of 10 Hz.

4. Results and discussion

4.1. Thermal cycling

Hydrogel discs were prepared as described in Table 1. The letter (A, B, C or D) denotes the amount of *N*,*N*′-methylene-*bis*-acrylamide used in the preparation of the hydrogels and the number denotes the number of thermal cycles which the hydrogel discs were subjected to.

4.2. Morphology of hydrogel discs

It was found that the appearance of dry hydrogel discs changed with thermal cycling. The hydrogel discs that were not subjected to thermal cycling were colourless and transparent in the xerogel form. With thermal cycling the appearance of the hydrogel discs in their xerogel form was colourless and translucent. On inspection with a light microscope, it was found that this was due to small cracks, which covered the entire surface of the dry discs. These cracks were not observed in the hydrogel discs that were not subjected to thermal cycling.

The formation of cracks on the surfaces of the hydrogel discs was likely due to the stresses on the polymer from repeated swelling and collapse. During the thermal cycling process, transferring the hydrogel discs from a swelling medium cooled to a temperature below the VPTT (i.e. 15 °C) to a swelling medium heated to a temperature above the VPTT (i.e. 55 °C)

Table 1
Nomenclature used to describe hydrogel formulation and number of thermal cycles

Number of thermal cycles	Amount of crosslinker used in preparation of hydrogels (g)			
	0.06 (0.0682 mol%)	0.12 (0.136 mol%)	0.18 (0.204 mol%)	0.24 (0.272 mol%)
0	A0	B0	C0	D0
15	A15	B15	C15	D15
30	A30	B30	C30	D30

results in dense surface skin layer formation in the hydrogel discs. The hydrostatic pressure that results from the hindrance of the efflux of water from the hydrogel discs by the dense surface skin layer may be sufficient to cause disruption of the polymer matrix structure at the surface of the discs. After many thermal cycles, this could ultimately result in the formation of cracks on the surface of the hydrogel discs.

Repeated thermal cycling was found not to affect the overall shape of the discs. The shape of the hydrogel discs, which had been subjected to 15 or 30 thermal cycles, remained the same as prior to thermal cycling.

4.3. Equilibrium swelling ratio

Fig. 1 shows the swelling ratio profiles of the hydrogel discs, which were subjected to 15 thermal cycles. The figure shows that between 35 and 65 °C (i.e. above the VPTT of crosslinked homopolymer poly(NIPAAm) hydrogel) all the hydrogel discs are in a shrunken or deswollen state and exhibit a similar degree of swelling. At these temperatures the hydrogel discs were white and opaque, indicative of the phase separation between the polymer chains and the water molecules within the matrices. Compared to

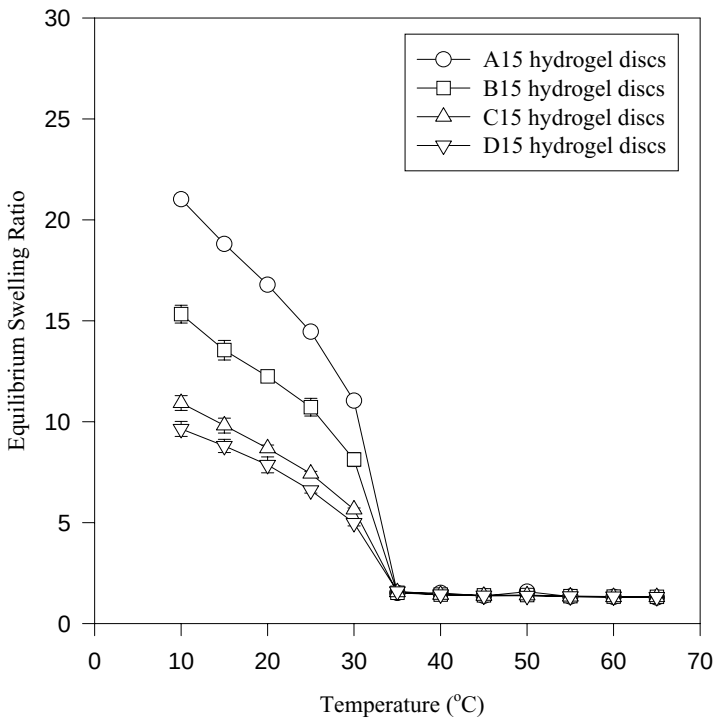

Fig. 1. Equilibrium swelling ratio profiles of A15, B15, C15 and D15 hydrogel discs. Each point on the graph represents a mean swelling ratio (±S.D.).

the swollen hydrogel discs at temperatures below the VPTT, the deswollen discs are significantly less fragile and do not break or split easily with handling. Similar findings were observed with the hydrogel discs that had been subjected to no thermal cycling and 30 thermal cycles. The swelling ratio of all the hydrogel discs increase significantly at temperatures below 35 °C. In all the hydrogel discs, the degree of swelling increased with decreasing temperature from 35 to 10 °C. The degree of swelling is related to the amount of crosslinker used in the preparation of the hydrogel discs, with swelling ratio increasing as crosslinker content is reduced. The same trends were seen with the hydrogel discs that had been subjected to no thermal cycling and 30 thermal cycles.

Fig. 2 shows the swelling ratio profile of type A hydrogel discs. No significant difference was observed in the degrees of swelling between all the hydrogel discs, regardless of crosslinker content and the number of thermal cycles to which the discs had been subjected, at temperatures between 35 and 65 °C. At temperatures between 10 and 35 °C, the hydrogel discs that were not subjected to thermal cycling exhibited higher degrees of swelling than the hydrogels that were subjected to 15 or 30 thermal cycles. The results show that there was no significant difference in the degrees of swelling of hydrogel discs subjected to 15 thermal cycles and those subjected to 30 cycles at temperatures between 10 and 35 °C.

The difference in the swelling behaviour between the hydrogel discs that had been subjected to thermal cycling and those that had not may be a consequence of the cracks formed on the surface of the hydrogel during thermal cycling. The volume of water that the hydrogel discs were capable of holding at temperatures below the VPTT may be significantly reduced by the presence of cracks on the surface of the discs. This, therefore, results in the lower swelling ratios exhibited by hydrogels discs that had been subjected to thermal cycling.

The dramatic temperature-sensitive changes in the swelling of crosslinked poly(NIPAAm) hydrogel is

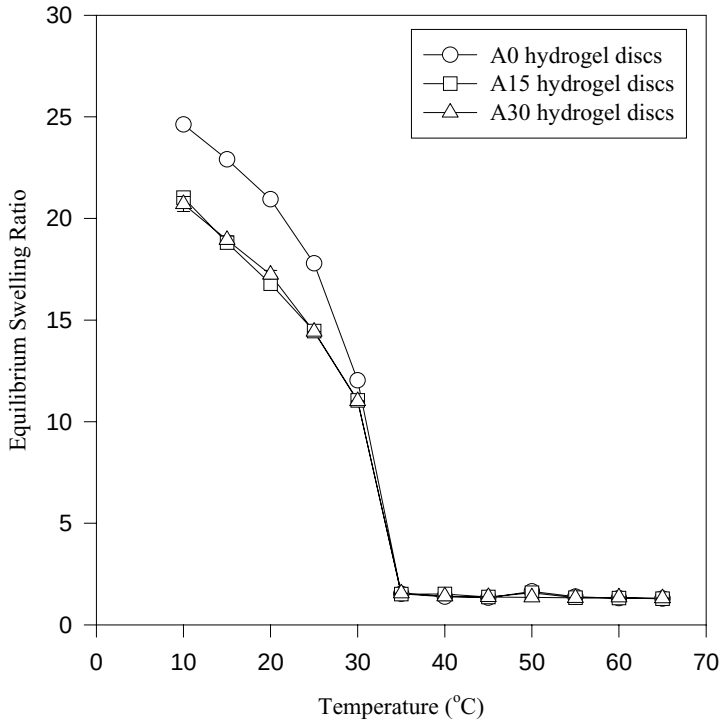

Fig. 2. Equilibrium swelling ratio profiles of A0, A15 and A30 hydrogel discs. Each point on the graph represents a mean swelling ratio (±S.D.).

discontinuous and a VPTT can be distinguished. The VPTT can be estimated as the point of inflection in the plot of swelling ratio versus temperature (Liu et al., 1999). Using this method, the VPTTs of all the hydrogel types was estimated to be $32.5 \pm 2.5\,^{\circ}C$.

4.4. Thermal analysis

An endothermic peak was observed in the thermograms for each hydrogel between 30.85 and 35.72 °C. The onset points of the endothermic peaks are known to correspond to the VPTTs of the poly(NIPAAm) hydrogels (Bae et al., 1990; Lin et al., 1999). The onset points of the peaks were determined by calculating the intersection point of the two tangents for the baseline and the downward slope of each individual peak. These fell between 27.65 and 31.38 °C.

Tables 2–5 show the temperatures at which the endothermic peaks and the onset points of the endothermic peaks appeared on the thermograms for all of the hydrogel discs analysed.

Although the VPTTs of the A, B, C and D hydrogel disc samples differ with the number of thermal cycles by a few degrees, no general trends in the change in VPTTs with the increasing number of thermal cycles could be deduced from the results. The swelling behaviour of crosslinked poly(NIPAAm) hydrogel is known to be highly sensitive to even small variations in the preparation conditions (Gehrke, 1993; Rathjen et al., 1995). Therefore, although the polymerisation reaction temperature, dissolved oxygen concentration and polymerisation time had been controlled, small fluctuations in these and other preparation conditions could have resulted in the small differences observed in the VPTTs between the different samples.

The VPTTs determined using the DSC thermograms were slightly higher than those determined using the equilibrium swelling ratio data. Small differences in the VPTTs of crosslinked poly(NIPAAm) hydrogels between the two methods used for their determination was also observed in a previous study (Dinarvand, 1993).

Table 2
Endothermic peaks and onset points determined from the thermograms for A0, A15 and A30 hydrogel discs

Type of hydrogel	A0 (°C)	A15 (°C)	A30 (°C)
Endothermic peak	34.10	33.86	33.04
Onset point	31.17	31.38	30.78

Table 3
Endothermic peaks and onset points determined from the thermograms for B0, B15 and B30 hydrogel discs

Type of hydrogel	B0 (°C)	B15 (°C)	B30 (°C)
Endothermic peak	31.70	33.86	33.06
Onset point	28.94	30.84	29.95

Table 4
Endothermic peaks and onset points determined from the thermograms for C0, C15 and C30 hydrogel discs

Type of hydrogel	C0 (°C)	C15 (°C)	C30 (°C)
Endothermic peak	30.85	35.47	33.85
Onset point	27.65	30.72	29.18

Table 5
Endothermic peaks and onset points determined from the thermograms for D0, D15 and D30 hydrogel discs

Type of hydrogel	D0 (°C)	D15 (°C)	D30 (°C)
Endothermic peak	35.19	35.72	33.33
Onset point	29.28	28.47	27.92

4.5. Nuclear magnetic resonance (NMR) spectroscopy

All the hydrogel disc samples exhibited identical spectra. All the carbon atoms in the NIPAAm units were identifiable on the spectra. However, the hydrogels contain very small proportions of *N*,*N*′-methylene-*bis*-acrylamide (the crosslinker). The amount of *N*,*N*′-methylene-*bis*-acrylamide in the hydrogels is so small in comparison to the amount of NIPAAm units present that peaks assigned to the carbon atoms that form *N*,*N*′-methylene-*bis*-acrylamide are lost in the background noise and are not identifiable on the spectra or are masked by the peaks assigned to the NIPAAm units.

No significant differences between the spectra of the samples of hydrogel discs that were subjected to 30 thermal cycles and the spectra of the samples of hydrogel discs that were not subjected to thermal cycling could be identified. This suggests that thermal cycling does not noticeably affect the chemical structure of the hydrogels. Although the hydrogels contain only a

small amount of *N*,*N*′-methylene-*bis*-acrylamide, hydrolysis of the crosslinks and the subsequent formation of carboxyl moieties would be associated with a change in the NMR spectrum. This is not apparent and therefore the results suggest that thermal cycling does not affects the integrity of the crosslinks.

5. Conclusions

It has been shown that thermal cycling significantly affects both the appearance and the swelling behaviour of crosslinked homopolymer poly(NIPAAm) hydrogels. It was found that thermal cycling in water resulted in the formation of cracks on the surface of the hydrogel discs. The formation of the cracks in the hydrogel may be due to repeated stress on the polymer network caused by the hydrostatic pressure resulting from the collapse of the hydrogel on increasing the temperature above the VPTT. Formation of a dense surface skin layer on increasing the temperature above the VPTT prevents the expulsion of water from the hydrogel and therefore leads to an increase in the hydrostatic pressure within the polymer network as the hydrogel continues to collapse. The cracks may affect the ability of the hydrogel to form a dense surface skin layer on increasing the temperature above the VPTT. This is important as the ability to form a dense surface skin layer is utilised as a method of controlling drug release in some thermoresponsive drug delivery systems (Okano et al., 1990, 1991; Okano and Yoshida, 1993; Yoshida et al., 1993).

At temperatures below the VPTT, the degree of swelling of the hydrogel discs varied depending on whether the hydrogel discs had been subjected to thermal cycling. At these temperatures, the hydrogel discs that were not subjected to thermal cycling exhibited higher degrees of swelling than the hydrogel discs that had been subjected to 15 or 30 thermal cycles. This difference in swelling behaviour may be due to the cracks observed in the hydrogel discs that had been subjected to thermal cycling. The cracks may hinder the ability of the hydrogel discs to retain water and lead to reduced degrees of swelling.

The hydrogel discs that had been subjected to 15 thermal cycles and those that had been subjected to 30 thermal cycles exhibited similar degrees of swelling at all the temperatures investigated. This suggests that the degree of swelling, at temperatures below the volume phase transition, decreases with increasing numbers of thermal cycles to which the hydrogels were subjected up to a critical number of thermal cycles, above which the degree of swelling no longer decreases. Thus, thermoresponsive drug delivery systems consisting of crosslinked poly(NIPAAm) hydrogel may benefit from being subjected to thermal cycling as part of their preparation process to ensure that the rates of drug release do not change with use. The ^{13}C NMR spectroscopy studies showed no evidence that thermal cycling affects the chemical structure of crosslinked poly(NIPAAm) hydrogel.

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