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Crosslinking of poly(vinyl alcohol)-graft-*N*-isopropylacrylamide copolymer membranes with glutaraldehyde and permeation of solutes through the membranes

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The phase transition behaviour of poly(vinyl alcohol)-graft-*N*-isopropylacrylamide copolymer membranes has been studied by measuring their steady-state fluorescence spectra and swelling ratios. Crosslinking with glutaraldehyde increased the tensile strength of the copolymer membrane and also resulted in a considerable decrease in the swelling ratio of the membrane. Well defined thermo-control of permeation through the membrane was achieved by crosslinking of the copolymer membrane with glutaraldehyde.

(Keywords: *N*-isopropylacrylamide; poly(vinyl alcohol); glutaraldehyde)

INTRODUCTION

It is well known that poly(*N*-isopropylacrylamide) (poly(NIPAAm)) hydrogels exhibit a lower critical solution temperature (*LCST*) at around 33°C in aqueous solutions, and that the hydrogels swell and shrink at temperatures below and above the *LCST*, respectively. Poly(NIPAAm) hydrogels have become of great interest, both from fundamental and practical points of view. In particular, many studies have been focused on the field of controlled drug delivery[1,2], regulation of the activity of enzymes[3,4], and thermo-controlled chromatography[5]. We have previously reported the preparation of hydrogel membranes by the graft copolymerization of NIPAAm on poly(vinyl alcohol) (PVA-graft-NIPAAm, PGN) and the temperature dependence of the permeation of solutes through these PGN membranes[6]. The membranes were annealed prior to use in the permeation experiments, because unannealed membranes were very soft and weak. The annealing treatment at 120°C for 10 h was required to give insoluble materials and also strengthen the PGN membranes. However, the membranes became brownish in colour and fragile when they were annealed at 140°C. It seems to be difficult to control exactly the properties of the PGN membranes by annealing treatment. On the other hand, PVA is well known to be insoluble in water as a result of the reaction with aldehydes via the formation of acetal bonds[7,8]. Therefore, we treated the PGN membranes with glutaraldehyde (GA) in order to strengthen the membranes. The PGN membranes treated with glutaraldehyde were found to be strong and suitable for use as thermo-sensitive polymer membranes. In this paper, we examine the effect of treatment with glutaraldehyde on the permeation of solutes through the PGN membranes and the phase transition behaviour of the NIPAAm segments by using fluorescence spectroscopy.

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EXPERIMENTAL

Materials

NIPAAm was kindly provided by Kohjin Co. and purified by recrystallization using hexane and benzene. PVA (degree of polymerization = 2600; degree of saponification > 99%) was kindly supplied by Nippon Gosei Co. Other chemical compounds were of reagent grade and were used as received.

N-[2-[[[5-Dimethylamino)-1-naphthalenyl]sulfonyl]-amino]ethyl]-2-acrylamide (DAN monomer), used as a fluorescent probe in this study, was synthesized according to the method described previously[9].

Preparation of the PGN copolymer with DAN

The PGN copolymer with DAN (PGN–DAN copolymer) was synthesized by the graft copolymerization of 0.5 g of PVA, 5.0 g of *N*-isopropylacrylamide, and 10 mg of the DAN monomer, using 10 mg of potassium peroxodisulfate in 20 ml of dimethyl sulfoxide (DMSO). The copolymerization reaction was carried out at 40°C for 20 h. After the reaction was complete, the mixture was poured into acetone and washed several times with the same solvent to remove the monomers and poly(NIPAAm). The amount of DAN chromophore incorporated in the PGN–DAN copolymer was determined by spectroscopy.

Preparation of membranes

PGN–DAN membranes were prepared by evaporating DMSO from 5 wt% solutions of the PGN–DAN copolymer in DMSO at 50°C for 96 h. The membranes were immersed in a glutaraldehyde aqueous solution (2 wt%) for 30 s (or 90 min), and then heated at 50°C for 150 s (or 10 min) to give the PGN–DAN–GA1 (or PGN–DAN–GA2) membrane.

Synthesis of linear poly(NIPAAm) with DAN

A linear poly(NIPAAm) with DAN (poly(NIPAAm–DAN)) was synthesized by the copolymerization of 1.0 g of NIPAAm and 2 mg of the DAN monomer, using 20 mg of 2,2′-azobisisobutyronitrile as an initiator, in 20 ml of benzene at 50°C for 25 h. The solvent was evaporated *in vacuo* after polymerization, and the polymer isolated by precipitation from a chroloform solution into n-hexane.

Phase transition temperature

The phase transition temperatures of the membranes were measured using differential scanning calorimetry (Seiko DSC100). The phase transition temperature of the linear poly(NIPAAm-DAN) was determined by the spectrophotometric detection of a change in turbidity of the poly(NIPAAm–DAN) aqueous solution.

Mechanical properties

Tensile testing of the membranes was performed with a Seiko TMA 100 instrument; a typical specimen was 5 mm in length, 3 mm in width and 0.2 mm in thickness, with measurements being carried out at 27°C.

Permeation of poly(ethylene glycol) (PEG)

Permeation experiments were carried out at various temperatures (with magnetic stirring) using a diaphragm cell consisting of two detachable parts (volume of each = 22 cm^3). Each membrane was immersed in water at the relevant temperature, prior to use in the permeation experiments. The swollen membrane was set in the middle of the two parts of the cell, which were clamped together and sealed tightly with silicone rubber packing; the effective membrane area in the cell was 3.14 cm^2. The right-hand (R) side of the cell contained an aqueous solution of PEG400 (average molecular weight = 400), while the left-hand (L) side contained deionized water. The initial concentration of PEG400 in the R side of the cell was 1.0 wt%, and the cumulative amount permeated on the L side was measured by a Shodex RI refractometer SE-11.

Swelling ratio

The membranes were immersed in water at various temperatures. After immersion for 24 h, the membranes were wiped with a filter paper to remove any residual water on the surface. The swelling ratios of the membranes at various temperatures were determined using the following relationship:

$$\text{Swelling ratio} = \frac{a' \times b' \times c'}{a \times b \times c} \quad (1)$$

where a, b and c represent the lengths of the two sides and the thickness, respectively, of the dry rectangular membranes, with a', b', and c' representing the corresponding dimensions of the swollen membranes in water. The length of the two sides of the swollen membrane, namely a' and b', as well as the length of the two sides and the thickness of the dry rectangular membrane, i.e. a, b and c, could be measured easily with a ruler or micrometer. However, it was very difficult to measure c' exactly with a micrometer, because the membranes swelled considerably and became soft in water. It is assumed that the membranes swell almost uniformly in all directions. Therefore, the swelling ratio of the membranes could be rewritten as follows[6]:

$$\text{Swelling ratio} = \left(\frac{a' \times b'}{a \times b}\right)^{3.2} \quad (2)$$

i.e. the swelling ratio was estimated by measuring the length of the two sides of the rectangular membranes in both the dry and swollen states.

Fluorescence spectra

The u.v. absorption spectra of the PGN-DAN membranes in water were recorded with a Shimadzu UV-240 spectrophotometer. Steady-state emission spectra and excitation spectra (uncorrected) in water were measured on a Jasco FP-550A fluorescence spectrometer. The excitation wavelengths for the emission spectra were 335 nm for the PGN–DAN membrane (and the PGN–DAN–GA1 and PGN–DAN–GA2 membranes) and 330 nm for linear poly(NIPAAm–DAN), respectively.

The membranes were swollen in water at various temperatures for 12 h prior to measurement of the fluorescence spectra. The fluorescence spectra for the membranes were obtained by front-face excitation and front-face measurement of emission at various temperatures.

RESULTS AND DISCUSSION

Fluorescence spectroscopy is a powerful tool for investigating the microscopic environment of the fluorophores incorporated into the system investigated here, and several studies have been devoted to the solution properties of linear poly(NIPAAm) by means of fluorescence spectroscopic methods[10,11].

We chose the DAN chromophore as a fluorescent probe because its photophysical properties have been widely investigated, e.g. the emission maximum of the DAN chromophore shifts to shorter wavelengths with increasing hydrophobicity of the surroundings of the DAN chromophore[12,13]. Fluorescence spectroscopic studies by using the DAN chromophore have been reported on changes in the microscopic environments of polymers such as linear poly(NIPAAm)[14] and polyacrylamide gel[15] in relation to their volume phase-transition behaviour.

The structure of the PGN–DAN copolymer is shown in *Figure 1*. Its structure was confirmed by n.m.r., i.r., and absorption spectroscopy, and elemental analysis. The copolymer obtained was washed several times with acetone to remove the poly(NIPAAm) and the monomers. An average molecular weight for the poly(NIPAAm) segments grafted on to the PVA could not be determined exactly. On the other hand, a number-average molecular weight (determined by gel permeation

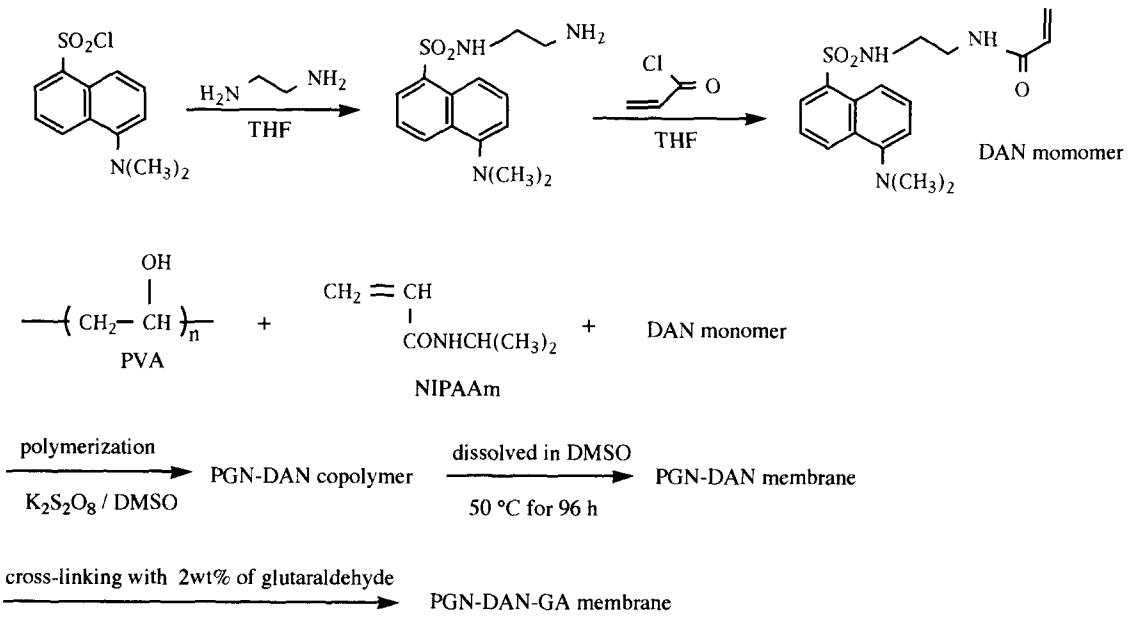

Figure 1 Synthesis of the PGN–DAN copolymer with fluorescent probe

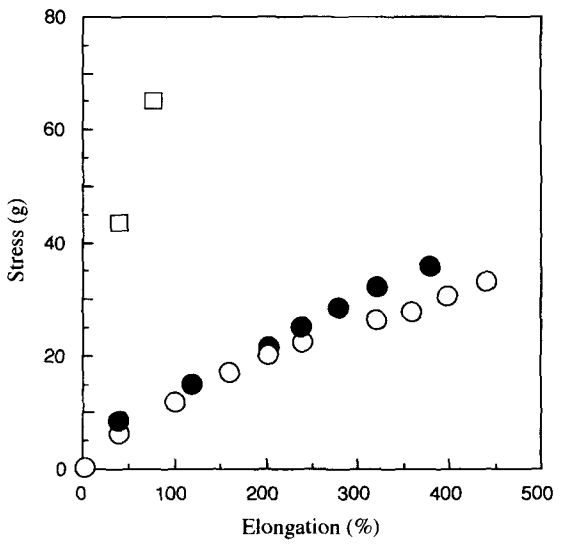

Figure 2 Elongation of three PGN membranes after various treatments: (○) PGN membrane; (●) PGN-A membrane; (□) PGN–GA

chromatography) for homopolymer of NIPAAm, which is obtained from the acetone washing solution, of 1×10^5 was measured. The conditions used for the grafting copolymerization of NIPAAm on PVA was similar to that employed for homopolymerization, e.g. solvent, temperature, concentration of monomer, and radical initiator. Therefore, the average molecular weight of the poly(NIPAAm) segments grafted on to the PVA is roughly estimated to be $\sim 1 \times 10^5$, based on the number-average molecular weight of the homopolymer of NIPAAm. The number of grafting points, based on the number-average molecular weight, is several multiples of ten in a PVA segment. The amount of DAN chromophore in the PGN–DAN copolymers, determined by absorption spectroscopy, was ~0.08 mol%.

Figure 2 shows the elongation of three PGN membranes, which were obtained without any treatment (PGN membrane), by annealing at 120°C for 10 h (PGN-A membrane), and by heating at 50°C for 150 s after immersion in glutaraldehyde aqueous solution (2 wt%) for 30 s (PGN–GA membrane). The tensile tests were carried out at 27°C i.e. the temperature at which the membranes were swollen. A slight increase in the tensile strength was observed by annealing at 120°C for 10 h. The glutaraldehyde treatment, in contrast to the annealing treatment, resulted in a pronounced increase in the tensile strength. The amount of glutaraldehyde incorporated in the PGN–GA membrane, estimated by an elemental analysis, was ~1.5 mol%. Although it is difficult to estimate the exact degree of crosslinking, the results obtained from tensile testing indicate intra- and intermolecular crosslinking via the formation of acetal bonds between the PVA and glutaraldehyde in the PGN–GA membranes[8]. We have previously reported the synthesis of the PGN membranes and the permeation of various solutes through such membranes[6]. The untreated PGN membranes were not suitable as polymer membranes for the permeation of solutes because they swelled considerably in water at room temperature and were very soft. In order to obtain strong membranes for permeation experiments, the membranes were annealed. The results from tensile testing clearly demonstrate that the treatment with glutaraldehyde provides a much higher mechanical strength to the PGN membranes as compared to the membranes obtained by annealing only.

The strong membranes in water composed of poly(NIPAAm) have been prepared by copolymerization of NIPAAm with other monomers such as butyl methacrylate (BMA)[2]. However, a change in the volume phase-transition temperature, as well as the swelling ratio of the membranes, was caused by copolymerization of NIPAAm with BMA due to the increase of hydrophobicity of the membranes. It is worthwhile here to study the influence of the crosslinking with glutaraldehyde on the phase-transition behaviour of the NIPAAm segments in the membranes.

Figure 3 shows the changes in the swelling ratio of

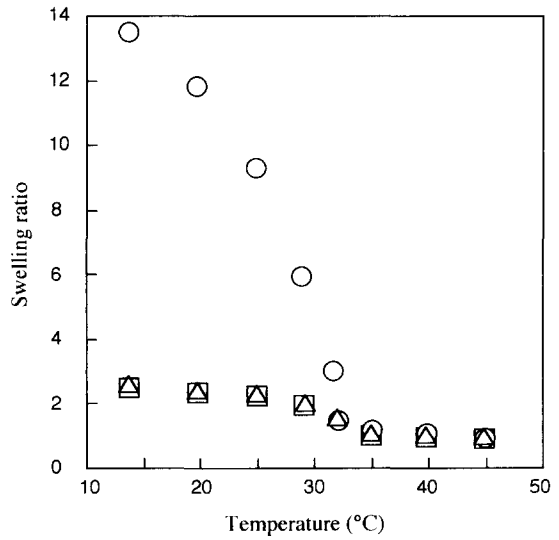

Figure 3 Temperature dependence of the swelling ratio of various membranes: (○) PGN–DAN; (□) PGN–DAN–GA1; (△) PGN–DAN–GA2

the PGN–DAN membranes with and without the glutaraldehyde treatment (PGN–DAN–GA1, PGN–DAN–GA2, and PGN–DAN membranes). A typical temperature dependence of the swelling ratio of the PGN membrane was observed for the PGN–DAN membrane[6], i.e. the swelling ratio decreased with increasing temperature up to 35°C. Although a similar temperature dependence of the swelling ratio was observed for both the PGN–DAN–GA1 and PGN–DAN–GA2 membranes, the swelling ratio was strongly depressed when compared to that of the PGN–DAN membrane below 35°C. The glutaraldehyde treatment was performed at 50°C, at which temperature the PGN–DAN membranes shrank. Thus, the spread and distance between the PVA chains during crosslinking strongly affected the swelling ratio below 30°C. However, no change in the swelling ratio was observed for any of the membranes above 35°C, at which temperature the membranes shrank completely. Therefore, the phase-transition temperatures of both the PGN–DAN–GA1 and PGN–DAN–GA2 membranes are almost the same as that of the PGN–DAN membrane.

Fluorescence spectroscopy is well suited for the investigation of a microscopic environment in the system described above. To reveal the influence of the crosslinking on the phase-transition behaviour of the NIPAAm segments in the membranes, fluorescence spectra were measured at various temperatures. The emission and excitation spectra of the linear poly(NIPAAm–DAN) in water (2 g l^{-1}, with the concentration of DAN being $\sim 1.8 \times 10^{-5}$ M) are shown in *Figure 4*. The emission spectra of the linear poly(NIPAAm–DAN) in water exhibited peaks at 542 (curve a) and 506 (curve c) nm, at 11 and 35.5°C, respectively. The excitation spectra were similar to the absorption spectrum obtained for the linear poly(NIPAAm–DAN). Thus, the fluorescence spectra can be identified as the emission from the DAN chromophore incorporated in the poly(NIPAAm) segments. The change in the emission maxima reflects the microscopic hydrophobicity of the poly(NIPAAm) segments grafted on to the PVA. The phase-transition temperature of the poly(NIPAAm–DAN) in water, determined by spectroscopy, was ~32.5°C. Therefore, the blue shift of the emission maximum at 35.5°C resulted from the increase of the hydrophobicity of the poly(NIPAAm) segments due to aggregation of these segments. Similar emission and excitation spectra to those of the linear poly(NIPAAm–DAN) were observed

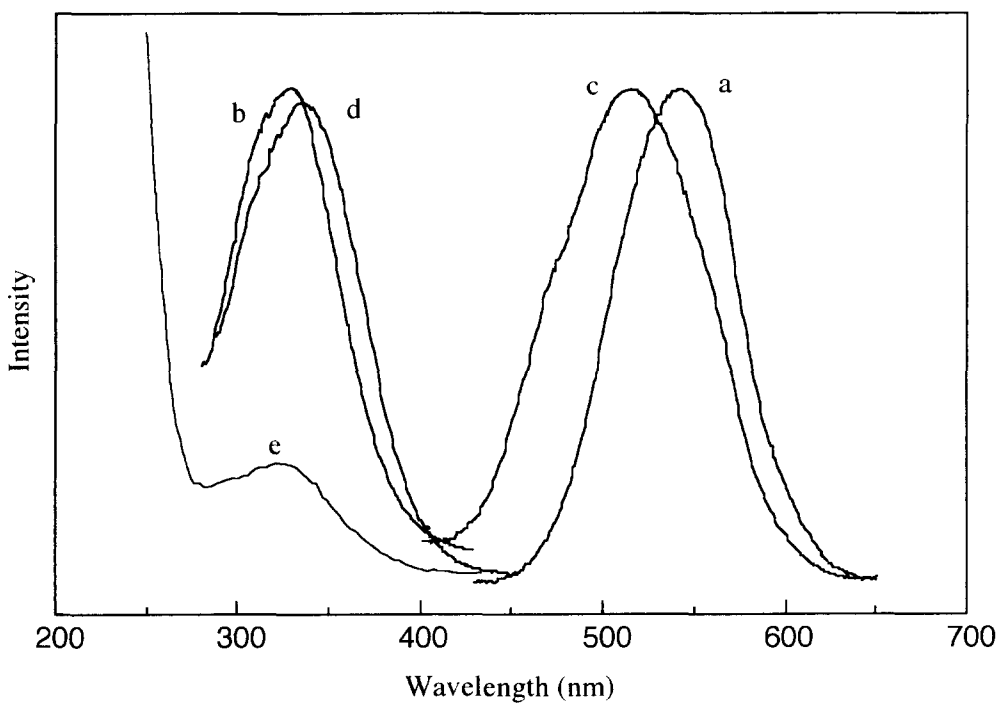

Figure 4 Fluorescence and absorption spectra of the linear poly(NIPAAm–DAN) in water: (a) fluorescence spectrum at 11°C; (b) excitation spectrum at 11°C; (c) fluorescence spectrum at 35.5°C; (d) excitation spectrum at 35.5°C; (e) absorption spectrum (the excitation wavelength was 330 nm)

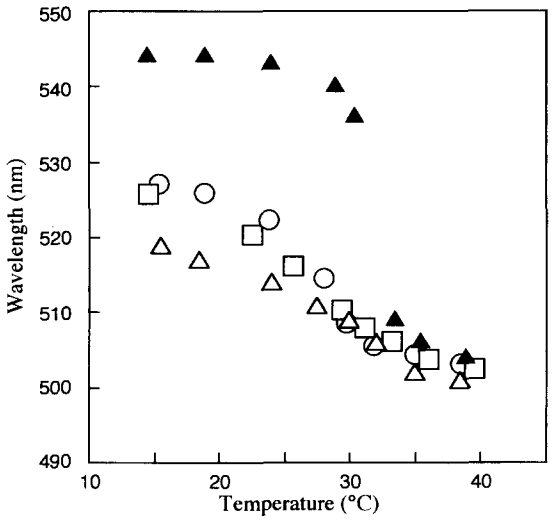

Figure 5 Temperature dependence of the maximum wavelength of the emission spectra of: (○) the PGN–DAN membrane; (□) the PGN–DAN–GA1 membrane; (△) the PGN–DAN–GA2 membrane; (▲) the linear poly(NIPAAm–DAN)

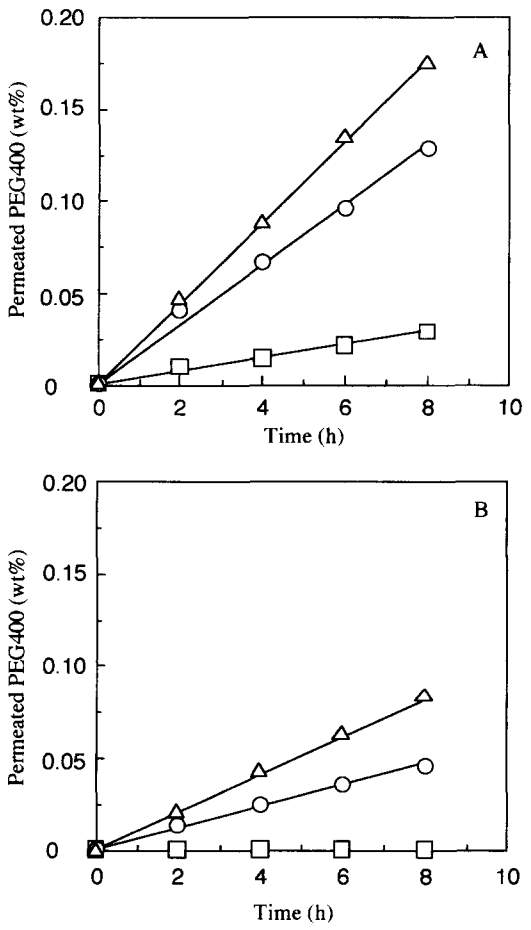

Figure 6 Changes in concentration of PEG400 as a function of time on the L side of the PGN–DAN (A) and PGN–DAN–GA1 (B) membranes at various temperatures: (△) 23; (○) 32; (□) 40°C

for the PGN–DAN–GA1, PGN–DAN–GA2, and PGN–DAN membranes.

Figure 5 shows the temperature dependence of the emission maximum wavelength of the linear poly-

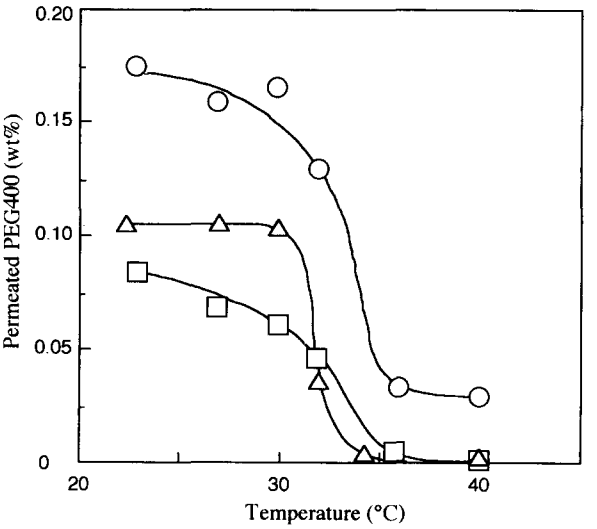

Figure 7 Changes in the cumulative amounts of PEG400 permeated for 8 h through the various membranes: (○) PGN–DAN; (□) PGN–DAN–GA1; (△) PGN–DAN–GA2

(NIPAAm–DAN), PGN–DAN membrane, PGN–DAN–GA1 membrane, and PGN–DAN–GA2 membrane. The emission maximum wavelength of the PGN–DAN membrane was found to shift to shorter wavelengths when compared to those of the linear poly(NIPAAm–DAN) below 30°C, namely, the hydrophobicity of the microscopic environment of the PGN–DAN membrane is higher than that of the linear poly(NIPAAm–DAN). The temperature dependence of the emission maximum wavelength of the PGN–DAN–GA1 and PGN–DAN–GA2 membranes was almost similar to that of the PGN–DAN membrane. However, a small blue shift of the emission maximum wavelength was observed for both the PGN–DAN–GA1 and PGN–DAN–GA2 membranes, relative to that of the PGN–DAN membrane. The amounts of glutaraldehyde incorporated in the membranes were ~1.5 and 1.7 mol% for the PGN–DAN–GA1 and PGN–DAN–GA2 membranes, respectively. The blue shift increased with increasing the amount of glutaraldehyde incorporated in the membranes. Consequently, the crosslinking increased slightly the hydrophobicity of the surroundings of the poly(NIPAAm) segments in the membranes.

Figure 6 shows the variation with time of the amount of PEG400 permeated through the PGN–DAN (A) and PGN–DAN–GA1 (B) membranes at various temperatures. It can be clearly seen that the amounts of PEG400 that are permeated increase with increasing permeation time and decreasing temperature. The cumulative amounts of PEG400 permeated for 8 h through the PGN–DAN–GA1, PGN–DAN–GA2 and PGN–DAN membranes were plotted as a function of temperature in *Figure 7*. The slope of the curve plotted for the PGN–DAN membrane decreased gradually with increasing temperature up to 30°C, and then decreased suddenly above this temperature. However, the permeation of a small amount of PEG400 through the PGN–DAN membrane was recognized even above 35°C, i.e. the temperature at which aggregation of the poly(NIPAAm) segments in the membrane took place. In contrast to the PGN–DAN membrane, permeation of PEG400 through the PGN–DAN–GA1 and PGN–DAN–GA2

membranes was hardly observable above 35°C. The results are closely related to the crosslinking with glutaraldehyde. The increase in the hydrophobicity as well as the decrease of the swelling ratio by crosslinking with glutaraldehyde resulted in not only a decrease in the permeation of PEG400, but also in no permeation through the membranes above a temperature of 35°C. In addition, the permeation of PEG400 through the PGN–DAN–GA2 membrane was greater than that through the PGN–DAN–GA1 membrane below 30°C. The permeation behaviour of solutes through polymer membranes is dependent on such properties of the membranes as hydrophobicity–hydrophilicity, size and size-distribution of micropores, structure of functional groups, etc. There was no difference in the swelling ratio between the PGN–DAN–GA1 and PGN–DAN–GA2 membranes, as shown in *Figure 3*. On the other hand, steady-state fluorescence spectroscopy revealed that the surroundings of the poly(NIPAAm) segments in the PGN–DAN–GA2 membrane were more hydrophobic compared to those in the PGN–DAN–GA1 membrane. The increase in permeation of PEG400 cannot be explained as a result of the increase of hydrophobicity of the PGN–DAN–GA2 membrane. Therefore, it can be assumed that the micropore size of the PGN–DAN–GA2 membrane is larger than that of the PGN–DAN–GA1 membrane below 30°C. Although the slight increase of hydrophobicity may be related to the change in micropore size of the PGN–DAN membranes, more data are needed in order to clarify more accurately these results.

CONCLUSIONS

The effect of the crosslinking with glutaraldehyde on the phase-transition behaviour of the poly(NIPAAm) segments in PGN membranes was investigated by measuring the tensile strengths, swelling ratios, fluorescence spectra, and permeation of PEG400 through the PGN membranes. Crosslinking greatly improved the mechanical strength of the membranes. In addition, crosslinking hardly influenced the phase-transition temperature of the poly(NIPAAm) segments, but did increase slightly the hydrophobicity of the PGN–DAN–GA membranes. This increase in the hydrophobicity of the PGN–DAN–GA membranes made a contribution to the well defined thermo-sensitive permeation behaviour through the membranes.

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