# Thermosensitive Properties of Amphiphilic *N,N*-Dimethylacrylamide Gel Grafted with Thermosensitive Oligo(*N*-isopropylacrylamide)

**Atsushi Takahashi,[1,2] Shuji Sakohara[1]**

[1]*Department of Chemical Engineering, Graduate School of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan*
[2]*Yokohama Corporate Research Laboratories, Mitsubishi Rayon Co., Ltd., 10-1 Daikoku-cho, Tsurumi-ku, Yokohama 230-0053, Japan*

Received 11 April 2011; accepted 13 June 2011
DOI 10.1002/app.35086
Published online 12 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

**ABSTRACT:** In this study, thermosensitive oligo(*N*-isopropylacrylamide) [oligo(NIPAM)] chains were grafted to amphiphilic *N,N*-dimethylacrylamide (DMAA) gel networks to form DMAA-*graft*-oligo(NIPAM) gels. The gels were prepared by copolymerizing oligo(NIPAM) macromonomers with DMAA. On heating, the gel caused structural changes in the gel network without causing a large volume change. The effects of the molecular weights and copolymerization ratios of oligo(NIPAM) on the temperature dependence of the swelling properties of the gel were investigated. Furthermore, to confirm the structural changes in the gel network, the temperature dependence of the diffusivity of rhodamine B through the gel membrane was investigated. Oligo(NIPAM) monomers with different molecular weights ($M_n$) of 2300, 7000, and 13,000 were prepared. The transition from hydrophilic to hydrophobic for oligo(NIPAM) of $M_n$ 7000 and 13,000 was observed at about 32°C, while that of $M_n$ 2300 was observed between 32 and 45°C. The gels grafted with oligo(NIPAM)s of $M_n$ 7000 and 13,000 shrank above 32°C; in other words, thermosensitivity was clearly observed. On the other hand, the swelling behavior of the gel grafted with oligo(NIPAM) of $M_n$ 2300 was similar to that of the DMAA gel; that is, negligible shrinkage of the gel was observed on heating. The diffusivity of rhodamine B through the gel membrane grafted with oligo(NIPAM) of $M_n$ 2300 increased stepwise between 40 and 45°C. These results suggest that by selecting the molecular weights and copolymerization ratios of oligo(NIPAM), structural changes in the gel network occur without an overall volume change. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 1208–1216, 2012

**Key words:** oligo(NIPAM)-grafted DMAA gel; oligo(NIPAM) macromonomer; thermosensitivity; structural change; diffusivity

## INTRODUCTION

Recently, the application of polymeric hydrogels to biochips such as DNA chips and protein chips has attracted special attention in the field of biotechnology.[1–5] In hydrogel-type biochips, capture probes are immobilized on the gel network and target probes diffuse through the gel network and react with the immobilized capture probes. The hydrogel-type biochip offers the advantage of a larger immobilization capacity than that of conventional biochips, in which capture probes are immobilized two-dimensionally on a flat plate. Furthermore, in the hydrogel-type biochip, the gel network serves as a matrix that holds water, therefore preventing the chips from drying out.

Hydrogel-type biochips are required to have the following features:

a. High diffusivity or permeability of the capture probes such as DNA through the gel network in the reaction process.
b. High transparency of the gel at room temperature. The hybridization reaction is usually detected by means of a fluorescence technique, and the detection is carried out at room temperature. The target probes are previously labeled with a fluorescent material. Hence, the target probes, on reaction with the capture probes in the gel, emit fluorescence, which signifies the occurrence of hybridization. Therefore, transparency of the gel at room temperature is a requisite for easy detection of the fluorescence.

The existing hydrogel-type biochips satisfy requirement (b). However, the diffusivity or permeability of the target probes through the gel network is low, and thus, a long analysis time is required. The hybridization reaction is carried out at a relatively high temperature, that is, between 50 and

*Correspondence to:* A. Takahashi (takahashi_at@mrc.co.jp).

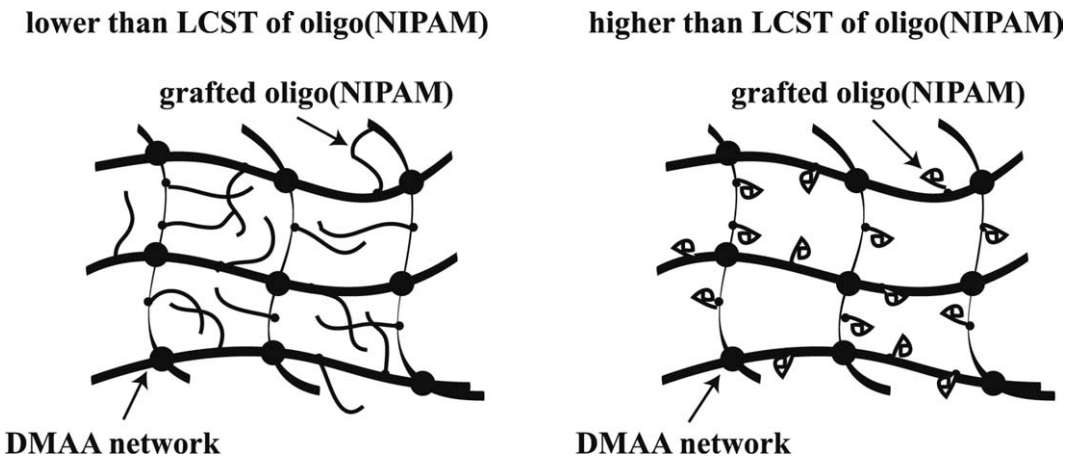


**Figure 1** Concept of structural changes in DMAA-*graft*-oligo(NIPAM) gel networks.

70°C. In the reaction process, the gels are not required to be transparent.

To improve the diffusivity of the target probes, in our previous article,[6] we proposed a semi-interpenetrating polymer network (semi-IPN) gel consisting of a cross-linked amphiphilic *N*,*N*-dimethylacrylamide (DMAA) gel and interpenetrating thermosensitive poly(*N*-isopropylacrylamide) [poly(NIPAM)]. The thermosensitive behavior of this semi-IPN gel in buffer solutions was investigated. The network structure of the semi-IPN gel changed from a homogeneous structure to an inhomogeneous structure composed of coarse and dense networks with the transition of poly(NIPAM), and the permeability of materials was enhanced through this coarse network.

There have been several reports on semi-IPN gels composed of a hydrophilic gel and an interpenetrating thermosensitive polymer; these reports state that the responsiveness, mechanical strength, and partition of the hydrophobic materials in these gels can be improved by controlling the temperature.[7–10] However, semi-IPN gels have several drawbacks. For instance, interpenetrating polymer molecules easily seep out of the gel over time. To overcome this drawback, in our previous study,[6] NIPAM was copolymerized in the DMAA gel network, thus enhancing the interaction between the interpenetrating poly(NIPAM) molecules and the gel network. Another drawback of the semi-IPN gel is that it shrinks with the collapse of the interpenetrating thermosensitive polymer; the degree of shrinkage increases on copolymerizing NIPAM in the gel network. This is unfavorable for biochips and complicates the use of semi-IPN gel for biochips.

To alleviate these drawbacks, we focused on gel networks grafted with thermosensitive oligomers as shown in Figure 1. To promote the permeation or diffusion of the target probes through the gel network, the effective pore size of the gel network should be large; in other words, the cross-linking density should be small. The mechanical strength of the gel, however, decreases with the cross-linking density. By grafting oligomers in the gel network, the mechanical strength is maintained, even if the cross-linking density is low. Although the permeation or diffusion of the target probes is hindered by the grafted oligomers at low temperatures, the thermosensitive oligomers collapse at high temperatures, thus enhancing the permeation or diffusion of the target probes. Usually, the hybridization reaction in biochips is performed at relatively high temperatures. Further, the gel is required to accommodate structural changes in its network without a change in volume.

In the literature, there have been reports on gels grafted with stimuli responsive oligomers or polymers.[11–15] These gels were designed to improve their response to shrinkage with the aim of using them in drug delivery systems (DDS) etc. Kubota et al.[14] prepared a gel network consisting of poly(acrylic acid) grafted with oligo(NIPAM) gel and investigated its thermosensitive properties in a pH range in which the volume change was negligible. They reported that the permeability of theopyline through the gel increased when the temperature was above the lower critical solution temperature (LCST) of oligo (NIPAM) because of the shrinkage of only the oligo (NIPAM) chains; there was no overall shrinkage in the gel network. However, acrylic acid gel easily undergoes a phase transition with change in pH. To understand these structural changes in the gel network, we investigated the effect of the molecular weights of the grafted oligomer on the structural changes in the gel network and on the temperature dependence of the permeability.

In this study, structural changes in the DMAA-*graft*-oligo(NIPAM) gel were investigated via the monitoring of the temperature dependence of the diffusivity of rhodamine B as a model material through the gel. The DMAA gel is amphiphilic, and its swelling degree is almost independent of the

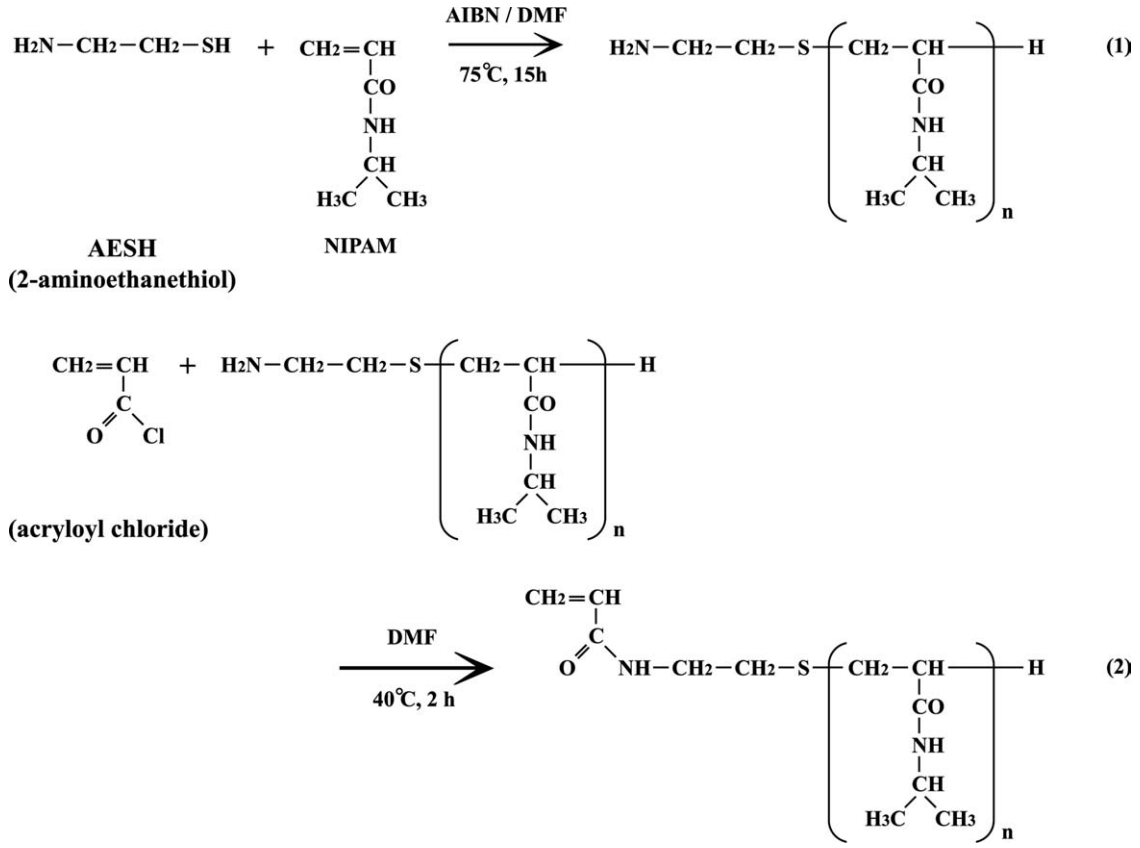


**Figure 2** Preparation scheme of oligo(NIPAM) macromonomer.

temperature, the composition of the solvent, and the pH of solution.[16,17] In this study, the effect of the cross-linker in the preparation of the gel was not investigated; in other words, the effective pore size of the network of the DMAA gel was not considered. We focused on the structural changes in the gel network upon heating.

## EXPERIMENTAL

### Preparation of oligo(NIPAM) macromonomer

The oligo(NIPAM) macromonomer was prepared by the method proposed by Kaneko et al.[18] The preparation scheme is shown in Figure 2. NIPAM was kindly supplied by Kohjin (Japan), and was purified by recrystallization from hexane before use. At the first step, oligo(NIPAM) with a terminal amino group [oligo(NIPAM)-$NH_2$] was prepared by free-radical polymerization using NIPAM as a monomer, 2-aminoethanethiol (AESH) as a chain-transfer agent, and 2,2′-azobisisobutyronitrile (AIBN) as an initiator in *N*,*N*-dimethylformamide (DMF) at 75°C for 15 h. The polymerization conditions are listed in Table I. After polymerization, DMF was removed by vacuum drying, and the concentrated oligo(NIPAM)-$NH_2$ was dissolved in acetone, and precipitated by dropping into a large volume of diethyl ether. The precipitated oligo(NIPAM)-$NH_2$ was recovered by filtration, and dried under vacuum. At the second step, to obtain the oligo(NIPAM) macromonomer, the amino group of oligo(NIPAM)-$NH_2$ was converted to the vinyl-group by reacting with acryloyl chloride in DMF at 40°C for 2 h. After the reaction, the oligo(NIPAM) macromonomer was recovered by a similar procedure as mentioned above using

**TABLE I**
**Polymerization Condition and Materials for Oligo(NIPAM) Macromonomer**

| | | Macro 1 | Macro 2 | Macro 3 |
|---|---|---|---|---|
| | | | g/L | |
| Monomer | *N*-isopropylacylamide (NIPAM) | 257.6 | 128.8 | 32.2 |
| Chain transfer agent | 2-Aminoethanethiol (AESH) | 4.83 | 4.83 | 4.83 |
| Initiator | α,α′-Azobisisobutyronitrile | 3.94 | 3.94 | 3.94 |

diethyl ether. In this study, the molecular weight of oligo(NIPAM) macromonomer was controlled by changing the NIPAM concentration in the first step, and three oligo(NIPAM) macromonomers with different molecular weights were obtained.

### Analysis of oligo(NIPAM) macromonomer

The vinyl-group of oligo(NIPAM) macromonomer was confirmed in $H^1$ NMR (JEOL, JNM-LA400, 400 MHz) spectra using heavy water as the solvent.

The molecular weight of oligo(NIPAM) macromonomer was estimated by gel permeation chromatography (GPC) (Shimadzu, Column: Shodex KF-804L). Tetrahydrofuran (THF) and polystyrene were used as the solvent and reference, respectively.

### Measurement of transition temperature of oligo(NIPAM)

The transition behavior of oligo(NIPAM) was investigated by measuring the change in transmittance as a function of temperature in water. In the hydrophilic state of the thermosensitive polymer, the solution is transparent because the polymer is soluble in the solution. However, when the change to the hydrophobic state is induced by heating, the solution becomes milky white, because the hydrophobic polymer is insoluble in the aqueous solution. Therefore, the transition temperature can be estimated by measuring the change in the transmittance through the solution as a function of temperature. The transmittance was measured at 600 nm using a spectrophotometer equipped with a temperature-control system (V-530, Japan Spectroscopy). The concentration of oligo(NIPAM) in the aqueous solution was fixed at 0.5 wt %.

### Preparation of DMAA-*graft*-oligo(NIPAM) gel

The DMAA-*graft*-oligo(NIPAM) gel was prepared by radical polymerization using *N*,*N*′-methylenebisacrylamide (MBAA) as the cross-linker. *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS) were used as the polymerization accelerator and initiator, respectively. The polymerization was carried out at 20°C for 6 h using distilled water as a solvent. The preparation conditions of the gels are listed in Table II. Cylindrical and plate-type gels were prepared for the measurements of the swelling properties and the mechanical properties, respectively.

The preparation of the cylindrical gel was carried out in a glass tube with an inner diameter of 3.4 mm. The gels were cut into pieces having lengths equal to the diameter, washed with deionized water, and then dried at room temperature. During the drying process, the gels were placed on a Teflon sheet that was spread on a Petri dish. The gels break if they are dried quickly; therefore, the dish was covered with a thin plastic film with small holes to slow down the drying rate.

**TABLE II**
**Preparation Condition of DMAA-*graft*-oligo(NIPAM) Gel**

| DMAA g/l | MBAA g/l | Oligo (NIPAM) g/l | Oligo (NIPAM) $M_n$ | APS g/l | TEMED g/l |
|---|---|---|---|---|---|
| 32 | 0.6 | 8.0 | 13,000 | 1.16 | 0.114 |
| 32 | 0.6 | 8.0 | 7000 | 1.16 | 0.114 |
| 32 | 0.6 | 8.0 | 2300 | 1.16 | 0.114 |
| 50 | 0.6 | 5.3 | 2300 | 1.16 | 0.114 |
| 50 | 0.6 | 8.0 | 2300 | 1.16 | 0.114 |
| 50 | 0.6 | 9.3 | 2300 | 1.16 | 0.114 |
| 50 | 0.6 | 13.3 | 2300 | 1.16 | 0.114 |

The preparation of the plate-type gel was carried out between two glass plates separated by a 1.5-mm-thick spacer. The gel was removed from the glass plate and washed in water. After washing, the gel was cut into a disc with a diameter of 20 mm.

### Measurement of swelling and mechanical properties of DMAA-*graft*-oligo(NIPAM) gels

The effect of the temperature on the swelling degree of the gels was examined by using the cylindrical gels. The dried gels were immersed in water at 25°C until the swelling of the gels reached equilibrium, and then, the swelling diameter of the gel was measured with a cathetometer. The temperature was then increased to the desired temperature, and the swelling diameter at equilibrium was measured again. This procedure was repeated until the temperature reached 70°C.

The storage modulus of the gel was measured by using the plate-type gel at 30°C. The measurements were performed using a rheology meter (HAAKE MARS rheometer system) at 0.1 Hz.

### Measurement of the temperature dependence of diffusivity of rhodamine B through the DMAA-*graft*-oligo(NIPAM) gel membrane

The diffused amount of rhodamine B through the gel membrane for time $t$ is expressed by the following equation.

$$M = \frac{V \cdot C(t)}{A} \tag{1}$$

Here, $M$ is the diffused amount of rhodamine B, $V$ is the solution volume in the downstream cell, $A$ is the cross-sectional area of the gel membrane, and $C(t)$ is

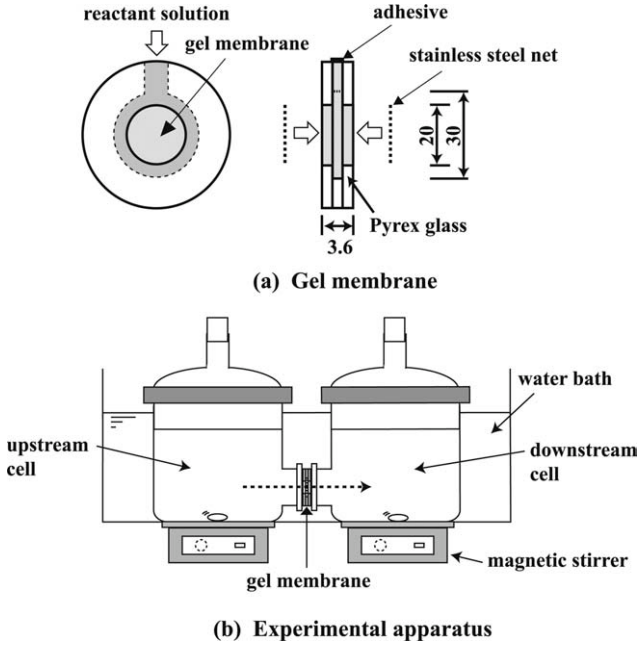


**Figure 3** Diagram of (a) the gel holder for the gel membrane and (b) the experimental apparatus.

the concentration of rhodamine B downstream at time $t$. $C(t)$ is assumed to be sufficiently smaller than the initial rhodamine B concentration upstream, $C_0$. This implies that the change in the concentration of rhodamine B in the upstream cell is negligible and that $C_0$ is the same as the initial concentration of rhodamine B in the upstream cell.

Using Fick's law under the assumption mentioned above, $M$ is expressed by the following equation.

$$M = D\frac{C_0}{d} \cdot t \tag{2}$$

Here, $D$ is the diffusion coefficient of rhodamine B, and $d$ is the thickness of the gel membrane.

From these equations, the diffusion coefficient is obtained by the following equation.

$$D = \frac{V \cdot d}{A \cdot C_0} \cdot \frac{C(t)}{t} \tag{3}$$

Therefore, the diffusion coefficient was estimated by measuring the increasing rate of the concentration of rhodamine B in the downstream cell.

The gel membrane was prepared in the center hole of a gel holder composed of three glass plates, as shown in Figure 3(a). The walls of the glass plates with holes were treated with chlorodimethylvinylsilane to introduce double bonds on the wall surface.[19] The gel was prepared by radical polymerization. The preparation procedure was as follows. The hole was covered by two slide glasses, and the reactant solution, which was previously degassed, was poured into the hole through a cut in the center plate. The reactant solution was composed of two aqueous solutions. One was the monomer solution containing DMAA, oligo(NIPAM), MBAA, and TEMED, and the other was an APS solution. After the polymerization of the gel was complete, the slide glasses covering the hole were removed, and the surface of the gel membrane was reinforced by a stainless steel net, which was attached to the glass plate using quick-drying glue, as shown in Figure 3(a). Then, the membrane was washed by immersion in distilled water. The DMAA gel membrane was also prepared without oligo(NIPAM) and used as a reference.

The apparatus for the measurement of the diffusivity of rhodamine B through the gel membrane is shown in Figure 3(b). The measurement procedure was as follows. The gel membrane was put between two permeation cells of capacity 500 mL. Water was poured into these cells, and the desired amount of rhodamine B was added into the upstream cell. These cells were immersed in a water bath. After the temperature reached the desired value, the solution sample was taken from the downstream cell at various time intervals, and the concentrations of rhodamine B in these samples were measured using a UV–Vis spectrophotometer at 553 nm. After the time lag, the concentration of rhodamine B in the downstream cell increased linearly with time. The diffusivity was estimated from the slope by using eq. (3).

## RESULTS AND DISCUSSION

### $H^1$ NMR spectra of oligo(NIPAM) macromonomers and their molecular weights

Figure 4 shows representative $H^1$ NMR spectra of the prepared oligo(NIPAM) macromonomer. The three peaks from 5.6 to 6.3 ppm denoted by *h*, *i*, and *j* correspond to the hydrogen in the vinyl-group of the oligo(NIPAM) macromonomer, and the peak denoted by *a* is the hydrogen in the isopropyl group of NIPAM. From these results, it is confirmed that the oligo(NIPAM) macromonomer was successfully prepared.

The molecular weights of the prepared oligo(NIPAM) and their polydispersity values are listed in Table III. The molecular weights are about 2300, 7000, and 13,000, respectively. As indicated by their polydispersity values, the distributions of the molecular weights of these oligo(NIPAM) macromonomers are relatively wide.

### Transition behaviors of oligo(NIPAM)

Figure 5 shows the change in the transmittance through the oligo(NIPAM) solution. For the solutions containing oligo(NIPAM) whose molecular weights, $M_n$, are 7000 and 13,000, the transmittance sharply

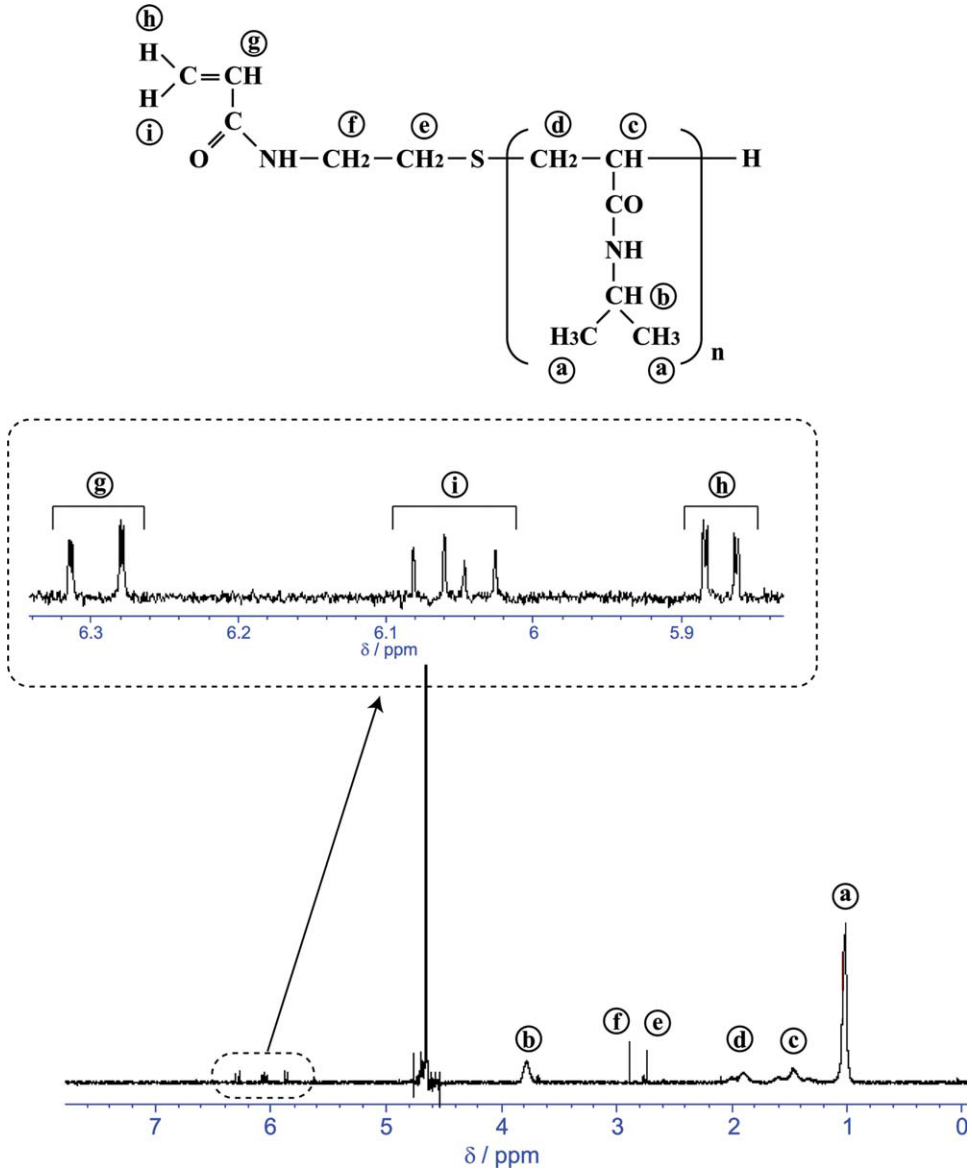


**Figure 4** $H^1$ NMR spectrum of the oligo(NIPAM) macromonomer with corresponding H-labeled assignments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

decreased at around 32°C, which coincides with the LCST of poly(NIPAM). For the solution containing oligo(NIPAM) of $M_n$ 2300, the decrease in the transmittance started from about 32°C and continued to about 45°C. Several articles have reported that the LCST increases as the molecular weight of oligo(NIPAM) decreases,[20–22] and our result agrees with this trend.

**TABLE III**
**Molecular Weight and Polydispersity of Oligo(NIPAM)**

| | Macro 1 | Macro 2 | Macro 3 |
|---|---|---|---|
| | | g/L | |
| Number average molecular weight ($M_n$) | 13,000 | 7000 | 2300 |
| Polydispersity | 1.65 | 1.92 | 1.34 |

### Swelling properties of DMAA-*graft*-oligo(NIPAM) gel

Figure 6 shows the effect of the molecular weight of oligo(NIPAM) on the temperature dependence of the swelling diameter of the cylindrical gels in water. The concentrations of DMAA and oligo(NIPAM) in the preparation of the gels were fixed at 50 and 8.0 g/L, respectively. The result for the DMAA gel is also shown for comparison. The swelling diameter of the DMAA gel decreased gradually as the temperature increased, but this decrease is negligible for its use in biochips. The swelling diameter of the DMAA-*graft*-oligo(NIPAM) gel prepared with oligo(NIPAM) of $M_n$ 2300 decreased slightly with temperature in the same manner as DMAA gel. On the other hand, the gels prepared with oligo(NIPAM)s of $M_n$ 7000 and 13,000 shrank

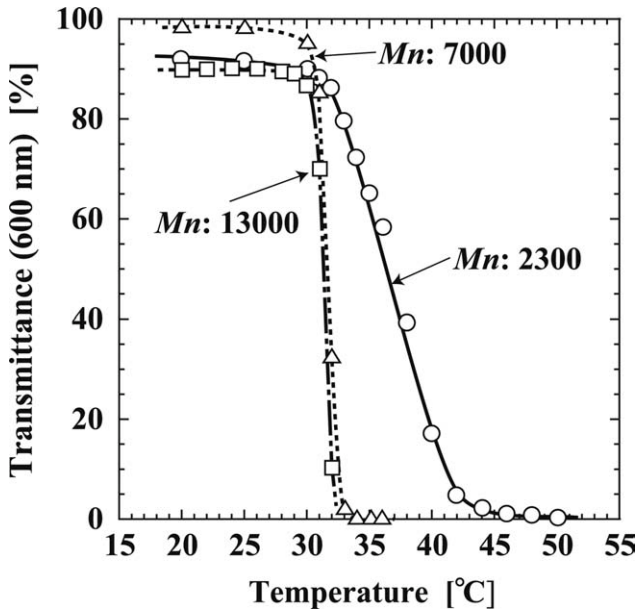


**Figure 5** Transition behaviors of oligo(NIPAM) in water. The concentration of oligo(NIPAM) is fixed at 0.5 wt %.

largely from about 32°C onwards; in other words, thermosensitivity was observed clearly. This result implies that for high molecular weights such as $M_n$ 7000 and 13,000, the oligo(NIPAM) chains grafted on the gel network collapse on heating above the LCST, and the inter-molecular interaction between the oligo(NIPAM) molecules shrinks the whole gel network. On the other hand, for $M_n$ 2300, the shrinkage of the gel was not

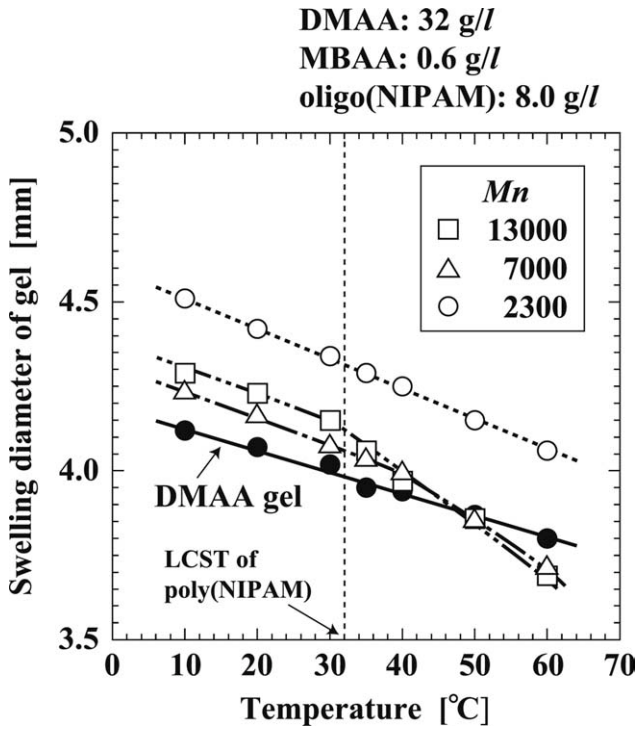


**Figure 6** Temperature dependence of the swelling diameter of DMAA and DMAA-*graft*-oligo(NIPAM) gels.

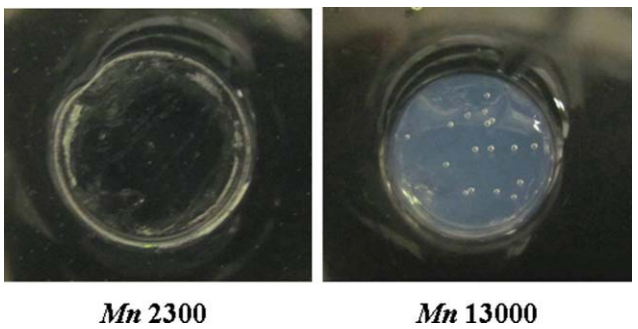


**Figure 7** Photographs of DMAA-*graft*-oligo(NIPAM) gels at 60°C, with different $M_n$. The concentrations of DMAA and oligo(NIPAM) were fixed at 32 and 8.0 g/L, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

large, because the oligo(NIPAM)s collapsed mainly by intra-molecular interactions mainly occurs.

Furthermore, the swelling diameter of the DMAA-*graft*-oligo(NIPAM) gels was larger than that of the DMAA gel at low temperatures. These phenomena are due to the fact that the cross-linking is hindered by the copolymerization of the oligo(NIPAM)s. The swelling diameter of the gel grafted with oligo(NIPAM) of $M_n$ 2300 was larger than those of other gels. The reason might be that the number of molecules of oligo(NIPAM) of $M_n$ 2300 to be copolymerized is larger than those of other oligo(NIPAM)s, because the amount of oligo(NIPAM) was fixed at 8.0 g/L.

Figure 7 shows the photographs of the gels prepared with oligo(NIPAM)s of $M_n$ 2300 and 13,000. The DMAA concentration in the preparation of the gel was 32.0 g/L, and that of oligo(NIPAM)s was 8.0 g/L. Cylindrical gels with diameter and length of 10 mm were used, and heated to 60°C, which is higher than the LCST of these oligo(NIPAM)s. The gel prepared with oligo(NIPAM) of $M_n$ 2300 is almost transparent, while that of $M_n$ 13,000 is opaque. It is well known that the network distribution of the transparent gel is homogeneous macroscopically, whereas that of the opaque gel consists of coarse and dense networks, that is, an inhomogeneous structure. The transparency of the gel prepared with oligo(NIPAM) of $M_n$ 2300 suggests that the collapse of the oligo(NIPAM) chains grafted on the gel network is mainly caused by intra-interactions of the oligo(NIPAM) molecules and that the structural change shown in Figure 1 occurs. On the other hand, in the case of the gel prepared with oligo(NIPAM) of $M_n$ 13,000, it can be considered that the collapse of the oligo(NIPAM) chains is caused by inter-molecular interactions and forms inhomogeneous structures.

Figure 8 shows the effect of the copolymerization ratio of oligo(NIPAM) on the swelling diameter of the gel. Oligo(NIPAM) of $M_n$ 2300 was used. The DMAA concentration in the preparation of the gel was fixed at 50 g/L, and the concentration of

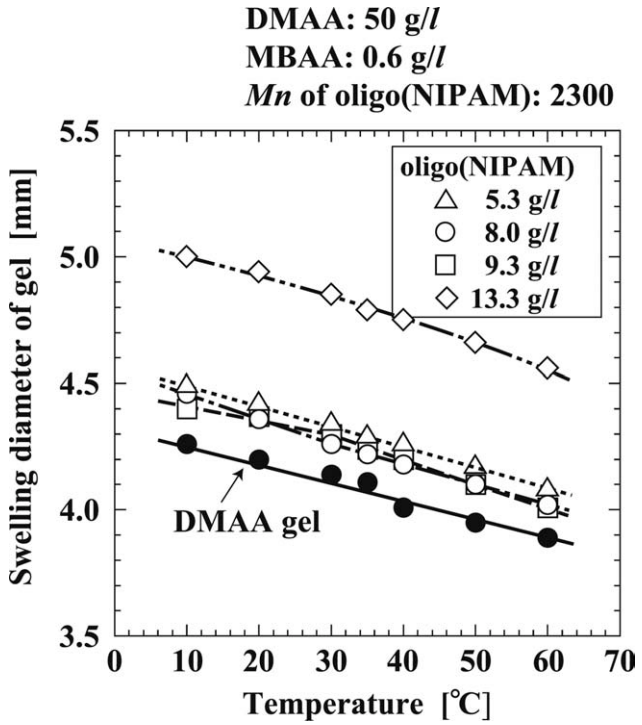


**Figure 8** Effect of the oligo(NIPAM) copolymerization ratios on the swelling diameter of the gel.

oligo(NIPAM) was varied from 5.3 to 13.3 g/L. The swelling diameters of the gels prepared with the oligo (NIPAM) concentrations of 5.3, 8.0, and 9.3 g/L were very close to each other, unlike the swelling diameter of the 13.3 g/L concentration oligo(NIPAM), which was larger. These results suggest that the cross-linking is hindered by copolymerization of oligo(NIPAM) with higher copolymerization ratios; in other words, the cross-linking density decreases. It was also found that gelation was particularly difficult when the concentration of oligo(NIPAM) was higher than 13.3 g/L.

### Mechanical properties of DMAA-*graft*-oligo(NIPAM) gels

Figure 9 shows the effect of the copolymerization ratio of the oligo(NIPAM)s on the storage modulus of the gel at 30°C. The compositions of gels were the same as those shown in Figure 8. The storage modulus decreased on copolymerizing the oligo(NIPAM)s; however, these values scarcely changed when the oligo(NIPAM) concentrations were 5.3–9.1 g/L and decreased largely at 13.3 g/L. These results suggest that the cross-linking density decreases by copolymerizing the oligo(NIPAM) of higher molecular weights, as mentioned above.

### Temperature dependence of diffusivity of rhodamine B through DMAA-*graft*-oligo(NIPAM) gel membrane

To confirm the structural changes, as shown in Figure 1, the temperature dependence of the diffusivity

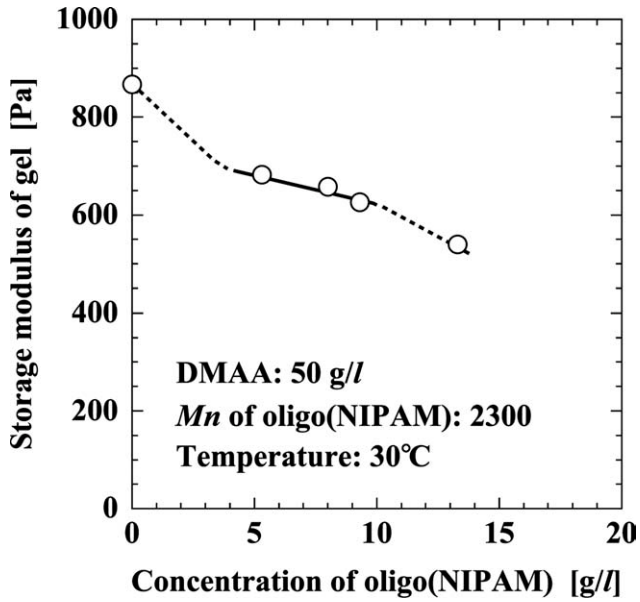


**Figure 9** Effect of the oligo(NIPAM) copolymerization ratios on the storage modulus of the gel at 30°C.

of rhodamine B through the DMAA-*graft*-oligo(NIPAM) gel membrane was investigated. Figure 10 shows the effect of the oligo(NIPAM) concentrations in the preparation of the gel on the diffusivity of rhodamine B. The DMAA concentration was fixed at 50 g/L, and oligo(NIPAM) of $M_n$ 2300 was used. Because it is considered that the gel prepared with oligo(NIPAM) of $M_n$ 2300 shows the structural change shown in Figure 1, as mentioned above. The diffusivity through the DMAA gel was also investigated for comparison. The diffusivity through the

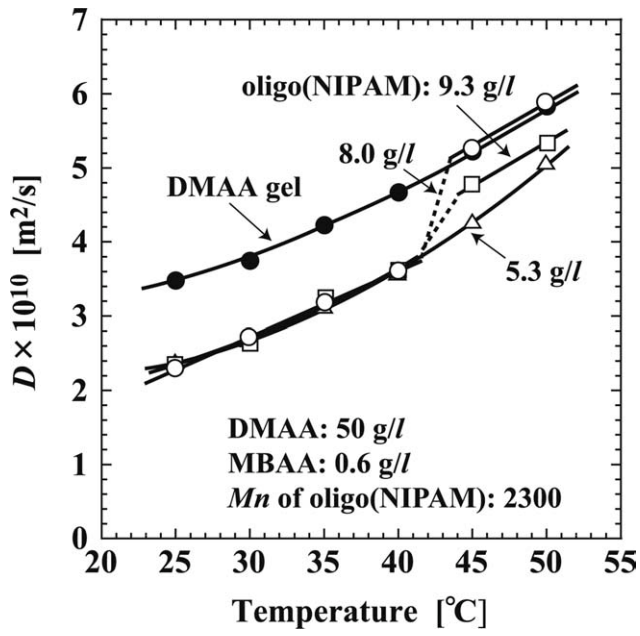


**Figure 10** Effect of the oligo(NIPAM) concentration in the preparation of the gel on the diffusivity of rhodamine B.

DMAA gel membrane increased monotonously with temperature. In general, the diffusivity is proportional to temperature and is inversely proportional to the viscosity of the solution, according to Einstein-Stokes's law.[23] On the other hand, diffusivity through the DMAA-*graft*-oligo(NIPAM) gel membrane increased stepwise between 40 and 45°C, when the concentration of oligo(NIPAM) was 8.0 g/L. The temperature was closely related to the LCST of oligo(NIPAM) shown in Figure 5. This implies that at a temperature lower than 40°C, the oligo (NIPAM) chains expand in the gel network and hinder the diffusion of rhodamine B. On heating the gel above 45°C, which is above the LCST of oligo (NIPAM) with $M_n$ 2300, the oligo(NIPAM) chains collapsed, and the diffusion resistance to rhodamine B decreased. As a result, the diffusivity of rhodamine B increased stepwise. Furthermore, at 9.3 g/L, a similar increase in the diffusivity was observed, but the increase in the diffusivity was smaller than that at 8.0 g/L. At 5.0 g/L, the stepwise increase was not observed. These measurements were carried out twice, and the difference in the data was very small—<5%. From these results, it can be inferred that the structural change in the gel network shown in Figure 1 occurs by selecting suitable molecular weights and copolymerization ratios of the oligo(NIPAM).

## CONCLUSIONS

The amphiphilic DMAA gel grafted with thermosensitive oligo(NIPAM) was prepared by copolymerizing DMAA with oligo(NIPAM). The effect of the molecular weight and the copolymerization ratio of oligo(NIPAM) on the structural changes in the gel network by heating were investigated. Oligo(NIPAM) macromonomers with three different molecular weights, that is, $M_n$ of 2300, 7000, and 13,000, were synthesized. The transition from hydrophilic to hydrophobic of oligo(NIPAM)s of $M_n$ 7000 and 13,000 was observed at about 32°C, while that of $M_n$ 2300 was observed between 32 and 45°C.

The gel grafted with the oligo(NIPAM)s of $M_n$ 7000 and 13,000 shrank above 32°C, in contrast to the swelling behavior of the gel grafted with oligo (NIPAM) of $M_n$ 2300 that resulted in negligible shrinkage, ideal for its use in biochips. In the case of high molecular weight oligo(NIPAM)s, inter-molecular interactions between the oligo(NIPAM) chains caused the shrinkage of the gel, and in the case of low molecular weight oligo(NIPAM)s, the shrinkage of the gel was not large because the collapse of the oligo(NIPAM) was caused by intra-molecular interactions.

To confirm the structural changes, the temperature dependence of the diffusivity of rhodamine B through the gel membrane was investigated. The diffusivity through the gel grafted with oligo(NIPAN) of $M_n$ 2300 increased stepwise between 40 and 45°C.

These results suggest that by selecting the molecular weight and the copolymerization ratio of the oligo(NIPAM), structural changes occur without a large volume change in the gel.

The authors are grateful for the help provided by Ms. Y. Okada and Mr. Y. Nakaya, graduate students of Hiroshima University.

## References

1. Proudnikov, D.; Timofeev, E.; Mirzabekov, A. Anal Biochem 1998, 259, 34.
2. Rubina, A. Y.; Pan'kov, S. V.; Dementieva, E. I.; Pen'kov, D. N.; Butygin, A. V.; Vasiliskov, V. A.; Chudinov, A. V.; Mikheikin, A. L.; Mikhailovich, V. M.; Mirzabekov, A. D. Anal Biochem 2004, 325, 92.
3. Pan, Z.; Li, Y.; Shan, Q.; Hong, X.; Zhou, D.; Zhang, M.; Chen, P.; Xiao, P.; Lu, Z. Electrophoresis 2008, 29, 2424.
4. Ootsuki, C.; Miyauchi, H. Patent 2001, WO01/98781.
5. Arenkov, P.; Kukhtin, A.; Gemmell, A.; Voloshchuk, S.; Chupeeva, V.; Mirzabekov, A. Anal Biochem 2000, 278, 123.
6. Takahashi, A.; Sakohara, S. J Appl Polym Sci 2011, 122, 1530.
7. Yoshinari, E.; Fukukawa, H.; Horie, K. Polymer 2005, 46, 7741.
8. Muniz, E. C.; Geuskens, G. Macromolecules 2001, 34, 4480.
9. Guilherme, M. R.; Silva, R.; Girotto, E. M.; Rubira, A. F.; Muniz, E. C. Polymer 2003, 44, 4213.
10. Guilherme, M. R.; Silva, R.; Rubira, A. F.; Geuskens, G.; Muniz, E. C. React Funct Polym 2004, 61, 233.
11. Takei, Y. G.; Aoki, T.; Sanui, K.; Ogata, N.; Sakurai, Y.; Okano, T. Macromolecules 1994, 27, 6163.
12. Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. Nature 1995, 374, 240.
13. Zhang, J.; Chu, L. Y.; Cheng, C. J.; Mi, D. F.; Zhou, M. Y.; Ju, X. J. Polymer 2008, 49, 2595.
14. Kubota, N.; Matsubara, T.; Eguchi, Y. J Appl Polym Sci 1998, 70, 1027.
15. Kuckling, D.; Wohlrab, S. Polymer 2002, 43, 1533.
16. Sakohara, S.; Takioka, T.; Nikai, T.; Takatani, K.; Kagaku kogaku Ronbunshu 2003, 29, 62.
17. Çaykara, T.; Akçakaya, İ. J Appl Polym Sci 2007, 104, 2140.
18. Kaneko, Y.; Sakai, K.; Kikuchi, A.; Yoshida, R.; Sakurai, Y.; Okano, T. Macromolecules 1995, 28, 7717.
19. Tsuji, T.; Konno, M.; Sato, S. J Chem Eng Jpn 1990, 23, 447.
20. Xia, Y.; Yin, X.; Burke, N. A. D.; Stöver, H. D. H. Macromolecules 2005, 38, 5937.
21. Xia, Y.; Burke, N. A. D.; Stöver, H. D. H. Macromolecules 2006, 39, 2275.
22. Shan, J.; Zhao, Y.; Granqvist, N.; Tenhu, H. Macromolecules 2009, 42, 2696.
23. Tsunomori, F.; Ushiki, H. Phys Lett A 1999, 258, 171.