

Synthesis of Thermosensitive Copolymer Beads Containing Pyridinium Groups and Their Antibacterial Activity

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ABSTRACT: Thermosensitive 4VP-NIPAAm-4G copolymer beads containing pyridyl groups were first prepared by suspension copolymerization of 4-vinylpyridine (4VP), *N*-isopropylacrylamide (NIPAAm), and tetraethylene glycol dimethacrylate (4G; crosslinking reagent) in a saturated Na₂SO₄ aqueous solution in the presence of surfactant and MgCO₃ as dispersants. Then the copolymer beads containing pyridinium groups were obtained by the quaternization of the copolymer beads with various alkyl iodides (CH₃I, C₄H₉I, C₈H₁₇I) in *N,N*-dimethylformamide. The 4VP-NIPAAm-4G (15 : 97 : 3) copolymer bead and the 4VP-NIPAAm-4G copolymer beads quaternized with butyl iodide exhibited high thermosensitivity in water, although the 4VP-NIPAAm-4G copolymer beads quaternized with methyl iodide or octyl iodide hardly exhibited thermosen-

sitivity. All the quaternized copolymer beads exhibited antibacterial activity against *Escherichia coli* (*E. coli*), although the 4VP-NIPAAm-4G copolymer bead did not. In particular, the copolymer bead quaternized with butyl iodide exhibited the highest antibacterial activity against *E. coli* at 30°C. It was also found that the antibacterial activity of the quaternized 4VP-NIPAAm-4G copolymer beads was greatly affected by not only chain length of alkyl groups in alkyl iodides, with which the 4VP-NIPAAm-4G copolymer beads were quaternized, but also by temperature of the solutions. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 116: 2580–2589, 2010

Key words: adsorption; hydrogels; stimuli-sensitive polymers

INTRODUCTION

It is known that polyNIPAAm is a thermosensitive polymer having lower critical solution temperature (LCST) in an aqueous solution at around 32°C.^{1–5} That is, polyNIPAAm dissolves in water below the LCST and does not dissolve above the LCST. Insoluble crosslinked polyNIPAAm hydrogels swell and shrink reversibly at the temperature below and above the LCST. On account of this unique thermosensitive behavior, various copolymers containing polyNIPAAm have been widely studied from fundamental and practical points of views.^{6–19}

On the other hand, it has been reported that water-soluble pyridinium-type polymers,²⁰ insoluble alkylpyridinium iodides,²¹ or alkylpyridinium iodides immobilized on silica gel^{22–24} had antibacterial activity against bacteria such as *E. coli*.

We have reported that (1) water-soluble copolymers of NIPAAm and vinyl monomer with phosphonic acid groups could be used for the separation

of metal ions from their mixed solution by changing the temperature near the LCST,²⁵ (2) water-soluble 4-vinylpyridine(4VP)-NIPAAm copolymer quaternized with benzyl chloride could adsorb food dyes having negative charges in water and it exhibited high antibacterial activity against bacteria such as *E. coli*,²⁶ (3) the permeability of solutes through the poly(vinyl alcohol)-*graft*-NIPAAm copolymer membranes was controlled by changing temperature,²⁷ and (4) the crosslinked copolymer hydrogels consisting of NIPAAm and vinyl monomer with phosphonium groups had high water-absorption ability and antibacterial activity depending on temperature.²⁸

In addition to these studies, we have reported that insoluble resins with quaternary ammonium groups,²⁹ phosphonium groups,³⁰ or immobilized phenolic derivatives³¹ exhibited high antibacterial activity against bacteria and the antibacterial activity of them was greatly affected by the chemical and physical structure of the resins. We have reported that such water-insoluble resins having antibacterial activity could prevent residual toxicity of bactericide moieties in water, because they could be easily separated from water after use.

So far only a few reports^{32,33} on the study of physicochemical properties of 4- (or 2-) vinylpyridine-NIPAAm copolymers can be found. But in the

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reports, the synthesis of the quaternized copolymers and the antibacterial activity of them have not been investigated.

In the previous report,³⁴ we have reported the synthesis of 4VP-NIPAAm-4G copolymer beads and the copolymer beads quaternized with alkyl iodides and that they had high adsorption ability for sodium dodecylbenzene sulfonate or bisphenol-A by hydrophobic interaction between the copolymer beads and such solutes.

In this study, we prepared thermosensitive copolymer beads containing pyridinium groups by the quaternization of 4VP-NIPAAm-4G copolymer beads with various alkyl iodides having different length of alkyl chains and investigated the thermosensitivity of the copolymer beads. We studied the antibacterial activity of the quaternized copolymer beads against *E. coli* under various conditions and the temperature dependence of the antibacterial activity; also discussed the relation between the antibacterial activity and the chemical structure of the copolymer beads.

EXPERIMENTAL

Materials

NIPAAm was kindly supplied by Kohjin Co. (Tokyo, Japan), and purified by recrystallization from tetrahydrofuran/*n*-hexane (15 : 85 vol. ratio) mixed solution. 4-Vinylpyridine (4VP) (Nacalai Tesque Co. Tokyo, Japan) was purified by distillation under reduced pressure (65°C/15 mmHg) under nitrogen atmosphere. Tetraethylene glycol dimethacrylate (4G; crosslinking reagent) was purchased from Aldrich Japan (Tokyo, Japan) and used without further purification. 2,2'-Azobisisobutyronitrile (AIBN) (Nacalai Tesque Co. Tokyo, Japan) was purified by recrystallization from ethanol (Wako Pure Chemical Industries, Osaka, Japan). *N,N*-dimethylformamide (DMF) was purchased from Wako Pure Chemical Industries, (Osaka, Japan) and dried with a molecular sieve overnight before use. Alkyl iodides (methyl iodide, butyl iodide, and octyl iodide) were purchased from Tokyo Chemical Industry (Tokyo, Japan) and used without further purification. Other chemical compounds were of reagent grade and were used without further purification.

Synthesis of thermosensitive copolymer beads

NIPAAm is an amphiphatic monomer, but 4VP is a hydrophobic monomer. Therefore, 4VP-NIPAAm-4G copolymer beads were prepared by suspension polymerization in an aqueous dispersant as follows³⁵: 50% chloroform solutions of monomers (4VP, NIPAAm, and 4G) mixed at prescribed ratios were first prepared. The monomer solutions were bubbled

with nitrogen for 5 min and 0.1 g of AIBN was added to the solutions as an initiator. *n*-hexadecyltrimethylammonium bromide was added to the monomer solutions as a surfactant. Dispersant media (400 cm³) was prepared by dispersing 2 g of magnesium carbonate in a saturated sodium sulfate aqueous solution. The dispersant medium was treated with ultrasonic and placed in three-necked flask equipped with a condenser, a nitrogen inlet, and a stirrer stick. The dispersant medium was bubbled with nitrogen for 60 min and the monomer solution was added while stirring. Polymerization was carried out under stirring at 400 rpm at 50°C for 4 h under nitrogen atmosphere. In this experiment, a saturated sodium sulfate aqueous solution was used to prevent NIPAAm from dissolving into an aqueous phase from a chloroform phase. Surfactant and magnesium carbonate were used to prevent copolymer beads from sticking together during polymerization. After polymerization, the products were filtered off and washed with 0.1 mol dm⁻³ HCl solution to remove magnesium carbonate. After that, the copolymer beads were washed with propanone using soxhlet extractor to remove residual monomers, initiator, etc., from copolymer beads for over 24 h and they were dried in air. Additionally, as the 4VP-NIPAAm-4G copolymer beads swell and shrink below or above the LCST as mentioned later, the copolymer beads were alternately soaked in water at 10 and 60°C to eliminate residual monomers from copolymer beads. After drying, 4VP-NIPAAm-4G (abbreviated as VPNG) copolymer beads were obtained. The scheme of the synthesis of VPNG and the quaternized copolymer beads (abbreviated as QVPNG-R) is shown in Figure 1. The 4VP content in VPNG copolymer beads was calculated from anion exchange capacity because of 4VP units in the copolymer beads. The anion exchange capacity of the copolymers was measured as follows: The dried copolymer bead (0.25 g) was placed in a 100 cm³ glass-stoppered Erlenmeyer flask. 50 cm³ of 0.1 mol dm⁻³ HCl solution were poured in to the flask. The mixture was shaken for 15 h at 30°C. The anion exchange capacity was determined by titrating the concentration of HCl in the supernatant with a 0.1 mol dm⁻³ AgNO₃ solution after adding 10 cm³ of 0.2 mol dm⁻³ NaHCO₃ solution and 5 cm³ of 2% (w/v) starch solution. A 0.2% (w/v) fluorescein aqueous solution was used as an indicator in this titration. The chemical structure of VPNG copolymer bead was confirmed by infrared spectra.

Quaternization of VPNG copolymer beads

The quaternization of VPNG copolymer beads was carried out at 70°C for a prescribed time under nitrogen atmosphere in a sealed glass autoclave as

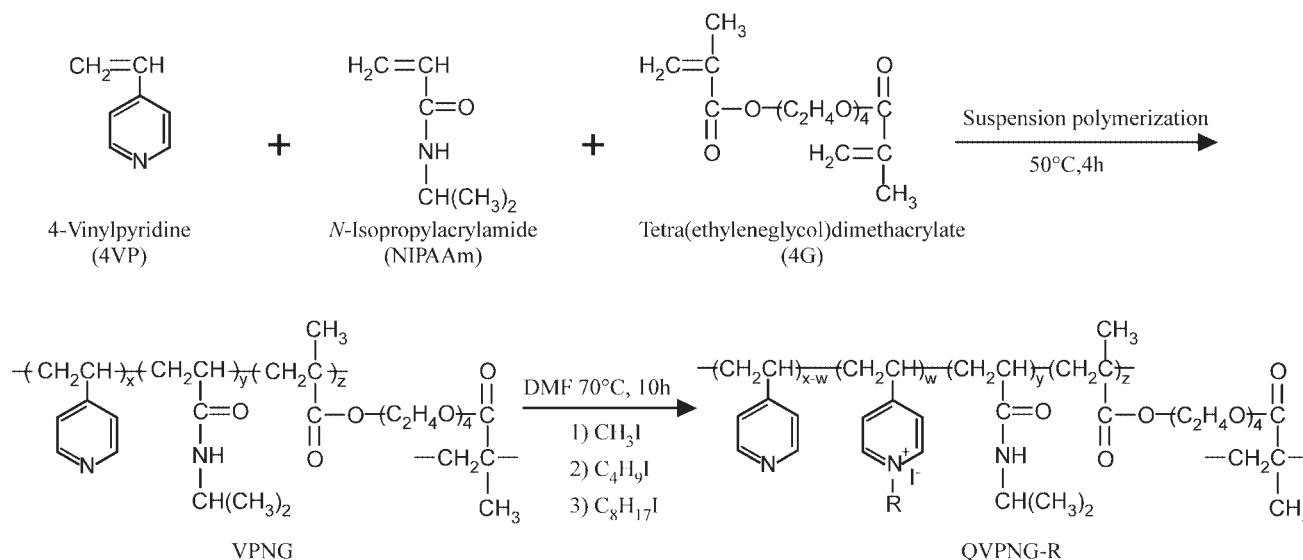


Figure 1 Synthesis of 4VP-NIPAAm-4G (VPNG) copolymer beads and quaternization of the copolymer beads.

follows: 0.5 g of dried VPNG copolymer bead was put into 20 cm³ of DMF, followed by addition of alkyl iodides (10 times excess of 4VP moiety in the copolymers). The quaternized copolymer beads (QVPNG-R, R=M, B, O) were purified by Soxhlet extraction with propanone for over 24 h, and then the copolymer beads were obtained by vacuum drying. M, B, and O in QVPNG-R (R=M, B, O) copolymer beads stand for methyl, butyl, octyl groups in alkyl iodides used for the quaternization, respectively. Pyridyl groups in VPNG copolymers ought to be converted to pyridinium groups by the quaternization. Therefore, the content of pyridinium groups in the QVPNG-R was determined by measurement of I⁻ in the quaternized copolymer beads. The content of I⁻ in the QVPNG-R copolymer beads was determined by thiocyanic acid titration (Volhard method).

Measurement of swelling volume of the copolymer beads

To evaluate the swelling behavior of VPNG and QVPNG-R copolymer beads, the swelling volumes were measured as follows: 0.2 g of dried copolymer beads was first soaked in deionized water in 5 cm³ measuring cylinder for 24 h at room temperature (24–25°C), and then swelling volume of the copolymer beads was measured after the copolymer beads were allowed to stand at each temperature for 1 h. The swelling volume of the copolymer beads at each temperature was calculated using eq. (1).

$$\text{Swelling volume (cm}^3\text{ g}^{-1}) = \frac{\text{Apparent volume (cm}^3\text{)}}{\text{Weight of dried copolymer beads (g)}} \quad (1)$$

Measurement of surface charge of copolymer beads in water

The surface charge of the quaternized copolymer beads in water was determined by colloidal titration method as follows.^{28,36} About 5 cm³ of deionized water were added to the weighed dry copolymer beads in a flask, and then the flask was allowed to stand for 48 h. To this flask, 25 cm³ of 1/400 mol dm⁻³ potassium poly vinyl sulfate (PVSK) (degree of polymerization: 2,000) solution (Wako Pure Chemical Co. Osaka, Japan) were added and the flask was shaken at various temperatures for 24 h. After that 5 cm³ of supernatant was pipetted and 5 cm³ of a 1/200 mol dm⁻³ methyl glycol chitosan (degree of polymerization: 600) solution (Wako Pure Chemical Co. Osaka, Japan) were added to the solution. The solution was titrated with a 1/400 mol·dm⁻³ PVSK solution using toluidine blue as an indicator. The same procedure was performed in the absence of copolymer beads. The surface charges of the copolymer beads in water were calculated using Eq. (2)

$$X = \frac{1}{400}(V_1 - V_2) \times f \times \frac{V_3}{5} \times \frac{1}{W} \quad (2)$$

Where X is surface charge of the copolymer beads (mmol g⁻¹), f is the factor of 1/400 mol dm⁻³ PVSK solution, V₁ is the titration volume of the 1/400 mol dm⁻³ PVSK solution (cm³), V₂ is the titration volume of 1/400 mol dm⁻³ PVSK solution in the blank test (cm³), V₃ is the total volume of finally surrounding water of the copolymer beads (cm³), and W is the weight of dried copolymer beads (g).

Measurement of adsorption capacity of the VPNG and QVPNG-R copolymer beads for sodium dodecyl benzenesulfonate or sodium benzenesulfonate

We have previously reported that thermosensitive hydrogels from trialkyl-4-vinylbenzyl phosphonium chloride-NIPAAm-*N,N'*-methylenebisacrylamide copolymers could adsorb more sodium dodecyl benzenesulfonate (SDBS) than sodium benzenesulfonate (SBS), because SDBS was mainly adsorbed by hydrophobic interaction on the copolymers and the adsorption of SDBS at the temperature below the LCST increased with increasing amounts of trialkylphosphonium chloride introduced into the copolymers. In addition, we have also reported that the antibacterial activity of the copolymer hydrogels was greatly affected by not only the content of phosphonium groups but also by the chain length of alkyl groups in trialkylphosphonium chloride of the copolymers.²⁸ This suggests that the antibacterial activity of the copolymer hydrogels was affected by not only positive charges but also by the hydrophobicity of the copolymers. Therefore, to evaluate the effect of hydrophobicity of the VPNG and QVPNG-R copolymer beads on the antibacterial activity, the adsorption capacity of the copolymer beads for SDBS or SBS was measured as follows: 0.05 g of dried copolymer bead was put into 25 cm³ of deionized water in a 50 cm³ Erlenmeyer flask and the flask was shaken at 20°C for 24 h to complete the swelling of the copolymer beads. And then 25 cm³ of SDBS (or SBS) solution (five times excess of 4VP moiety in the copolymers) was poured into the flask. Then the flask was shaken for further 24 h at various temperatures. After that the copolymer beads were filtered off and then the residual concentration of SDBS (or SBS) in the solution was measured with spectrophotometer (SHIMADZU UV-1600PC) after dilution of the solution until to a desired concentration. The amount of SDBS (or SBS) adsorbed was estimated by measuring absorbance at 261 nm for SDBS and 263 nm for SBS, respectively, by eq. (3).

Adsorption (mmol g⁻¹)

$$= \left\{ \frac{(B - S)}{M} \times A \times V \times \frac{1}{1000} \right\} \times \frac{1}{W} \quad (3)$$

Where *B* is the concentration of SDBS (or SBS) in the blank test (ppm), *S* is the concentration of SDBS (or SBS) after adsorption (ppm), *M* is the molecular weight of SDBS (or SBS), *A* is the degree of dilution of the solutions measured (g mol⁻¹), *V* is the initial volume of solutions (dm³), *W* is the weight of copolymer beads added (g).

Measurement of antibacterial activity

Measurement of the antibacterial activity of the copolymer beads was carried out as follows; The bacterium used in this study was *E. coli* (IFO3301), which was obtained commercially from the National Institute of Technology and Evaluation, Chiba, Japan. About 0.4 g of the copolymer bead and 20 cm³ of sterile deionized water were placed into a 50 cm³ Erlenmeyer flask, and the copolymer beads were completely swollen at various temperatures (20, 30, and 40°C) for 24 h. After that 20 cm³ of cultured cell suspensions containing *E. coli* of about 10⁷–10⁸ cells cm⁻³ were added into the flask, and the flask was shaken at various temperatures for a prescribed time (1–6 h). After contacting the copolymer beads with a bacterial suspension for a prescribed time, the suspension was diluted several times with sterile water, and 0.1 cm³ of the diluted suspension was spread on an agar plate made of nutrient agar. The plate was kept at 30°C for 15–24 h, and the number of viable cells was calculated from the numbers of the colonies formed on the plate.

RESULTS AND DISCUSSION

Synthesis of the 4VP-NIPAAm-4G copolymer beads

We have investigated the conditions for suspension copolymerization of amphiphatic NIPAAm and hydrophobic 4VP to get spherical copolymer beads in a good yield (more than 80%). In this study, we prepared spherical 4VP-NIPAAm-4G (VPNG) copolymer beads by suspension copolymerization under the best conditions as described in the previous report.²⁷ Table I shows the results of VPNG copolymer beads obtained at various monomer ratios in feed and QVPNG-R copolymer beads obtained by the quaternization of the VPNG copolymer bead with alkyl iodides (CH₃I, C₄H₉I, and C₈H₁₇I) with different chain length of alkyl groups. The contents of 4VP in the copolymer beads, which were estimated from anion exchange capacity, were over about 83% of 4VP molar ratios calculated from molar ratios in feed. Figure 2 shows IR spectra of Poly4VP, polyNIPAAm, and VPNG(15 : 97 : 3) copolymer. In the IR spectrum of poly4VP, the characteristic absorption band at 1600 cm⁻¹ due to NH in pyridyl group was observed. In the IR spectrum of polyNIPAAm copolymer, the characteristic absorption bands at 1660 cm⁻¹ because of amide I and 1550 cm⁻¹ because of amide II were observed. These characteristic adsorption bands could be found in the IR spectrum of VPNG copolymer. In the IR spectrum of VPNG copolymer, characteristic absorption band at 1720 cm⁻¹ because of carbonyl group in 4G was also observed. These results

TABLE I
Characterization of VPNG and QVPNG-R Copolymer Beads

4VP : NIPAAm : 4G (Molar ratio in feed)	4VP content in copolymer beads ^a $\times 10^{-1}$ (mmol g ⁻¹)		Quaternization agent	Pyridinium (N ⁺) content in quaternized copolymer beads $\times 10^{-1}$ (mmol g ⁻¹)	Quaternization (%)
	Found	Calcd.			
10 : 97 : 3	6.5	7.7	CH ₃ I	5.1	78.5
			C ₄ H ₉ I	5.1	77.7
			C ₈ H ₁₇ I	5.4	82.5
15 : 97 : 3	9.2	11.1	CH ₃ I	7.3	79.0
			C ₄ H ₉ I	6.2	67.7
			C ₈ H ₁₇ I	6.4	70.0
20 : 97 : 3	12.5	14.2	CH ₃ I	9.5	76.3
			C ₄ H ₉ I	10.0	80.0
			C ₈ H ₁₇ I	9.9	79.1

^a 4VP content was determined from anion exchange capacity of the VPNG copolymer beads.

indicate that VPNG copolymer consists of 4VP, NIPAAm, and 4G. Figure 3 shows the scanning electron micrograph of the VPNG(15 : 97 : 3) copolymer beads obtained at 4VP : NIPAAm : 4G = 15 : 97 : 3 molar ratio in feed. Although each copolymer bead had almost spherical shape, it was found that some beads joined each other and formed clusters even after drying. In suspension copolymerization of 4VP, NIPAAm, and 4G at 10 : 97 : 3 and 20 : 97 : 3 molar ratios in feed, spherical copolymer beads were not obtained in a good yield. Therefore, the VPNG (15 : 97 : 3) copolymer bead was mainly used for following experiments. The numbers in parentheses after VPNG stand for 4VP : NIPAAm : 4G molar ratios in feed, respectively.

Quaternization of VPNG copolymer beads

VPNG copolymer beads were quaternized with alkyl iodides (CH₃I, C₄H₉I, and C₈H₁₇I) with different length of alkyl chains at 70°C for 10 h in DMF. Table I also shows the pyridinium contents in QVPNG-R copolymer beads obtained and the quaternization

(%) of QVPNG-R copolymer beads. The quaternization (%) of VPNG-R copolymers was calculated using eq. (4)

$$\text{Quaternization (\%)} = 100 \times \frac{\text{(the content of pyridinium groups in the VPNG-R copolymer beads (mmol g}^{-1}\text{))}}{\text{(the content of pyridyl groups in VPNG copolymer beads (mmol} \cdot \text{g}^{-1}\text{))}} \quad (4)$$

The pyridinium contents in the QVPNG-R copolymer beads increased with increasing 4VP contents in VPNG copolymer beads, whenever different alkyl iodides were used for the quaternization. The quaternization (%) of all QVPNG-R copolymer beads were in the range from about 68 to 83%. The large difference of the quaternization (%) could not be observed between the VPNG-R copolymer beads, which were

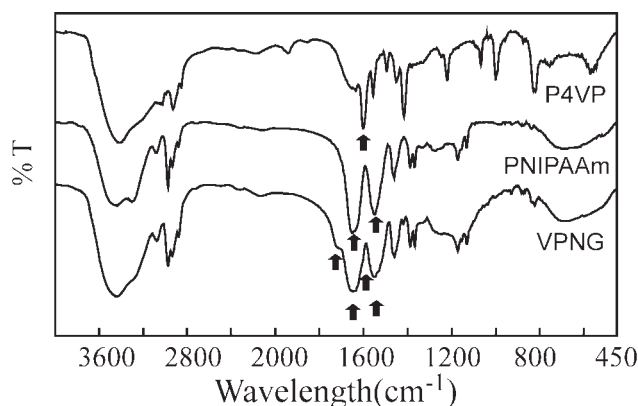


Figure 2 IR spectra of PolyNIPAAm, Poly4VP, and VPNG copolymer.

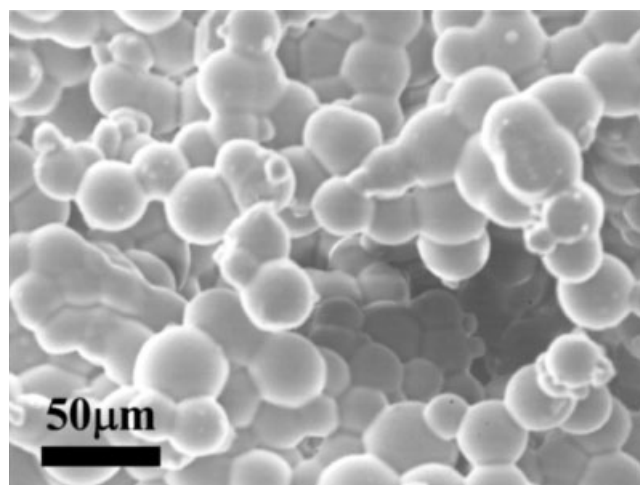


Figure 3 Scanning electron micrograph of the VPNG (15 : 97 : 3) copolymer beads obtained by suspension polymerization.

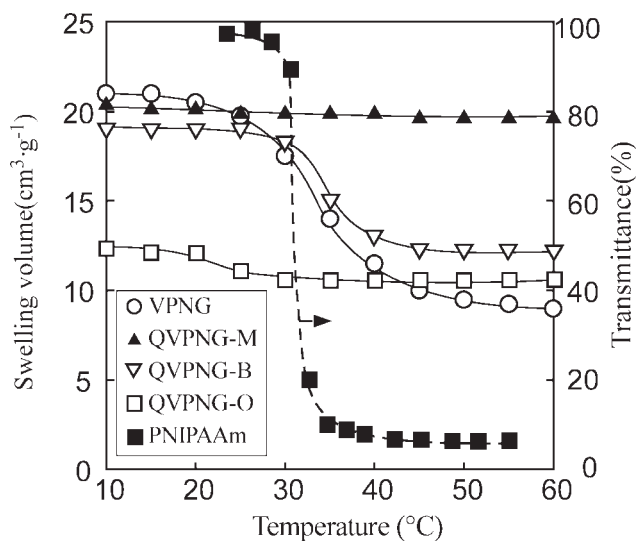


Figure 4 Temperature dependence of the swelling volume of the VPNG(15 : 97 : 3) copolymer beads and the quaternized (QVPNG-R) copolymer beads in deionized water.

obtained by the quaternization of the same VPNG copolymer bead with different alkyl iodides

Swelling behavior of VPNG and QVPNG copolymer beads

As aforementioned, polyNIPAAm is a thermosensitive polymer with lower critical solution temperature (LCST) in an aqueous solution at around 32°C.¹⁻⁵ That is, polyNIPAAm dissolves in water below the LCST and does not dissolve above the LCST. As crosslinked polyNIPAAm hydrogels are insoluble in water at any temperature, they swell and shrink reversibly at the temperature below and above the LCST in water. Figure 4 shows the swelling volume of the VPNG(15 : 97 : 3) copolymer bead and the quaternized QVPNG-R copolymer beads in deionized water at various temperatures. In Figure 4, the temperature dependence of the change in the transmittance (%) of light at 660 nm of polyNIPAAm aqueous solution is also shown. Figure 4 shows that the transmittance (%) of light at 660 nm of a polyNIPAAm aqueous solution decreased rapidly at around 32°C and became almost constant values less than 10% above 35°C. This means that polyNIPAAm dissolves in water below the LCST (32°C) of polyNIPAAm and does not dissolve above the LCST. It has been reported that the rapid decrease in the transmittance(%) of light at 660 nm above the LCST is because of the flocculation of polyNIPAAm by hydrophobic interaction between isopropyl groups in the polymers above the LCST. The swelling volume of the VPNG copolymer bead was sharply decreased above around 30°C because of the thermosensitivity of polyNIPAAm. The QVPNG-M copolymer bead obtained by quaternization with methyl

iodide showed the highest swelling volume above 30°C, but the change of the swelling volume with temperature was little due to its high hydrophilicity of this copolymer bead in the temperature range measured. In contrast, the QVPNG-O copolymer bead obtained by the quaternization with octyl iodide showed low swelling volume due to high hydrophobicity of octyl groups in the copolymer bead and only a slight decrease of the swelling volume was observed above 20°C. On the other hand, only the QVPNG-B copolymer beads obtained by the quaternization with butyl iodide showed a sharp decrease in the swelling volume above 30°C similar to the VPNG copolymer bead. These results indicate that the thermosensitivity of QVPNG-R copolymer beads were greatly affected by the length of alkyl groups in alkyl iodides used for the quaternization of pyridyl group in VPNG copolymer beads.

Antibacterial activity of quaternized copolymer beads

The antibacterial activity of QVPNG-R copolymer beads containing pyridinium groups against *E. coli* was investigated. First, the effect of the amount of copolymer beads added on the antibacterial activity was investigated at 30°C using the QVPNG(15 : 97 : 3)-B copolymer bead. Figure 5 shows the changes in viable cell numbers with time by an addition of various amounts (0.04–0.4 g) of the QVPNG-B copolymer bead. The number of viable cell numbers decreased sharply with time by an addition of 400 mg of copolymer beads. This rapid decrease in the number of viable cell numbers with time

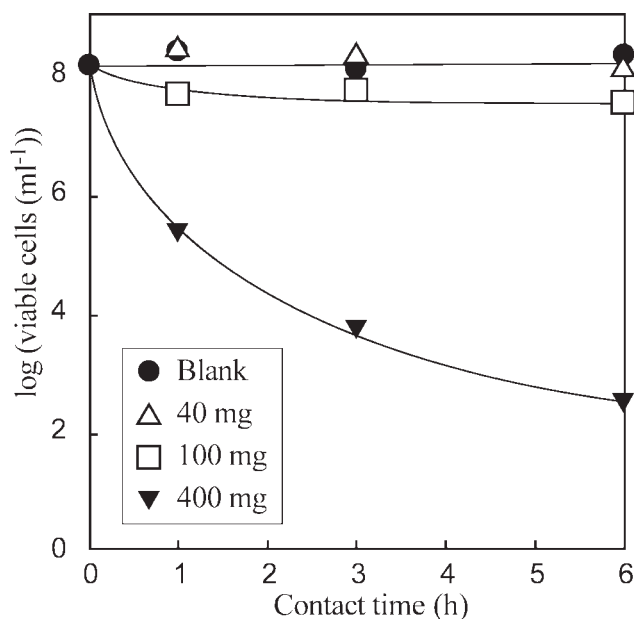


Figure 5 Effect of the amount of the QVPNG(15 : 97 : 3)-B copolymer bead added on its antibacterial activity.

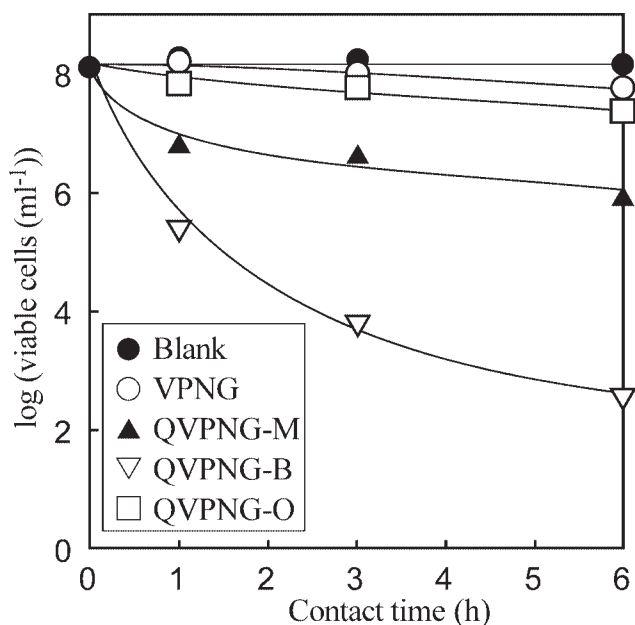


Figure 6 Antibacterial activity of the VPNG(15 : 97 : 3)-R copolymer beads quaternized with various alkyl iodides at 30°C.

indicates high antibacterial activity of the copolymer beads. Therefore, 400 mg of copolymer beads were used for the following measurements of the antibacterial activity of the copolymer beads.

Figure 6 shows the effect of alkyl chain length in pyridinium groups of the QVPNG(15 : 97 : 3)-R copolymer beads on the antibacterial activity at 30°C. In Figure 6 the antibacterial activity of the VPNG(15 : 97 : 3) copolymer containing no pyridinium groups bead is also shown. The VPNG(15 : 97 : 3) copolymer bead exhibited no antibacterial activity against *E. coli*. From Figure 6 the order of the antibacterial activity of the QVPNG(15 : 97 : 3)-R copolymer beads is as follows:

$$\text{QVPNG-B} > \text{QVPNG-M} > \text{QVPNG-O}$$

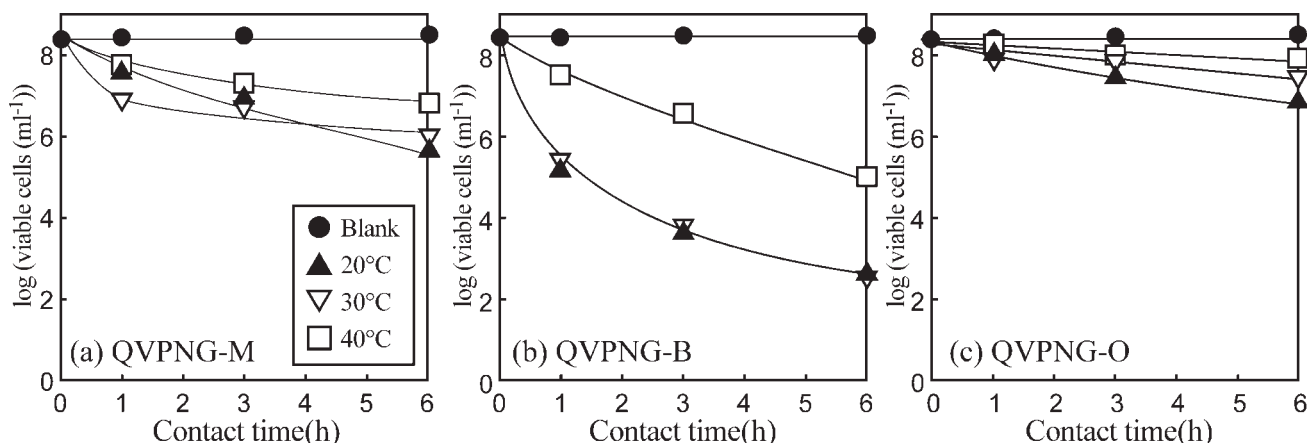


Figure 7 Antibacterial activity of the QVPNG-R copolymer beads against *E. coli* at different temperature.

Thus, it was found that the antibacterial activity of the QVPNG(15 : 97 : 3)-R copolymer beads was also greatly affected by alkyl chain length in pyridinium groups of the copolymer beads as well as the thermosensitivity of the copolymer beads as shown in Figure 4.

Figure 7 shows the effect of temperature on the antibacterial activity of the QVPNG(15 : 97 : 3)-R copolymer beads against *E. coli*. All QVPNG-R copolymer beads exhibited high antibacterial activity at 20°C or 30°C, and the lowest antibacterial activity at 40°C, although the antibacterial activities of three QVPNG-R copolymer beads were different as shown in Figure 7. The QVPNG-B copolymer bead exhibited higher antibacterial activity than the QVPNG-M or QVPNG-O copolymer bead at every temperature and the antibacterial activity of the QVPNG-B copolymer bead decreased remarkably at 40°C. This remarkable decrease in the antibacterial activity is because of high shrinkage (low swelling volume) of the copolymer bead in water at 40°C as shown in Figure 4. Such remarkable decreases in the antibacterial activity at 40°C were not observed in the case of the QVPNG-M and QVPNG-O copolymer beads.

As shown before in Figure 4, the QVPNG(15 : 97 : 3)-M copolymer bead had high swelling volume in water because of its high hydrophilicity and the QVPNG(15 : 97 : 3)-O copolymer bead had low swelling volume due to its low hydrophilicity (high hydrophobicity). Figure 4 also showed that only the QVPNG(15 : 97 : 3)-B copolymer bead had high thermosensitivity as well as the VPNG(15 : 97 : 3) copolymer bead or polyNIPAAm, but the QVPNG-M and QVPNG-O copolymer beads had low thermosensitivity in the temperature range studied. These results indicate that only the QVPNG-B copolymer bead having almost the same hydrophilicity–hydrophobicity balance as the VPNG(15 : 97 : 3) copolymer bead

TABLE II
Positive Surface Charges of the VPNG and the QVPNG-R Copolymer Beads in Water at Various Temperatures

Abbreviation of copolymer beads	Positive surface charges $\times 10^{-2}$ (mmol g^{-1})			Pyridinium (N^+) content in QVPNG-R $\times 10^{-1}$ (mmol g^{-1})
	20°C	30°C	40°C	
VPNG	0.0	0.0	0.0	0.0
QVPNG-M	3.5	2.3	1.5	7.3
QVPNG-B	2.5	2.2	0.1	6.2
QVPNG-O	3.0	2.1	0.2	6.4

or polyNIPAAm exhibited high antibacterial activity against *E. coli*.

Surface charge of QVPNG-R copolymer beads in water

It is known that bacteria such as *E. coli* have negative charges on their surface in water. Therefore, such bacteria must be attracted by electrostatic interaction between cationic polymers and bacteria. We have reported that the antibacterial activity of water-insoluble polymers containing cationic groups such as quaternary ammonium groups²⁹ or phosphonium groups³⁰ was affected by both electrostatic interaction and hydrophobic interaction between the surface of water-insoluble polymers and bacteria. So positive charges on the surface of the QVPNG(15 : 97 : 3)-R copolymer beads were first determined at various temperatures by colloidal titration method.^{28,36} Table II shows the positive charges on the surface of various QVPNG-R copolymer beads quaternized with various alkyl iodides measured at 20°C, 30°C, and 40°C. It was found that VPNG(15 : 97 : 3) copolymer bead containing no pyridinium groups had no positive surface charges and the positive surface charges of the QVPNG-R copolymer beads swollen in water are fairly smaller than the pyridinium (N^+) contents in the dried copolymer beads. This indicates that only a small part of positive charges in the copolymer beads appeared on the surface of copolymer beads swollen in water. The positive charges of all the QVPNG-R copolymer beads decreased with increasing temperatures as shown in Table II. This is because the shrinkage of the QVPNG-R copolymer beads in water increased with increasing temperature. Furthermore, it was found that the positive charges on the surface of the QVPNG-B and QVPNG-O copolymer beads at 40°C became fairly smaller than that of the QVPNG-M copolymer bead. This result corresponds to the fact that the QVPNG-B and QVPNG-O copolymer beads were more shrunken than the QVPNG-M copolymer bead at 40°C as shown in Figure 4.

Adsorption of SDBS or SBS with QVPNG-R copolymer beads

We have reported that VPNG(15 : 97 : 3) copolymer beads having no charges could adsorb SDBS by hydrophobic interaction.³⁴ We have also reported that the antibacterial activity of the copolymer hydrogels having cationic groups was affected by not only positive charges but also hydrophobicity of the copolymers.²⁸ Next, to compare the hydrophobicity of the QVPNG(15 : 97 : 3)-R copolymer beads containing pyridinium groups, the adsorption ability of the QVPNG-R copolymer beads for SDBS or SBS was measured at 20°C, 30°C, and 40°C. SDBS and SBS have the same anionic sulfonate groups. SDBS has long dodecyl group in the molecule but SBS does not. The results are shown in Figure 8. The adsorption ability of the VPNG(15 : 97 : 3) copolymer bead for SDBS or SBS is also shown in Figure 8.

Figure 8(a) shows that the VPNG and the QVPNG-R copolymer beads had high adsorption ability for SDBS, although the temperature dependences of their adsorption abilities are different. Figure 8(b) shows that the VPNG and the QVPNG-R copolymer beads had only very low adsorption ability for SBS and the adsorption abilities of the QVPNG-R copolymer beads for SBS decreased slightly with increasing temperature. As aforementioned, the surface positive

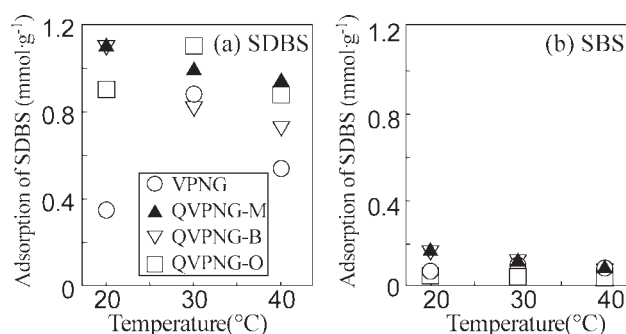


Figure 8 Adsorption ability of the VPNG(15 : 97 : 3) copolymer bead and the QVPNG-R copolymer beads for SDBS (a) and SBS (b) at various temperatures.

charges of the QVPNG-R copolymer hydrogels decreased with increasing temperature. Therefore, the adsorption of SDBS with the QVPNG-R copolymer beads is mainly because of the electrostatic interaction between the QVPNG-R copolymer beads and SDBS. The low adsorption of SDBS with the VPNG copolymer bead is due to only weak hydrophobic interaction between the VPNG copolymer bead and SDBS, because the VPNG copolymer bead contains no pyridinium groups. Figure 8(a) also shows that the adsorption abilities of the QVPNG-M and the QVPNG-B copolymer beads for SDBS decreased with increasing temperature. These results indicate that high adsorption of SDBS with the QVPNG-R copolymer beads was brought about by both high hydrophobic interaction and low electrostatic interaction between the QVPNG-R copolymer beads and SDBS. Furthermore, it was found that the adsorption abilities of the QVPNG-B copolymer bead for SDBS at 30°C and 40°C were lower than those of the QVPNG-M copolymer bead at those temperatures. This is because of higher shrinkage (lower swelling volume) of the QVPNG-B copolymer bead than the QVPNG-M copolymer bead at 30 and 40°C. Because it is considered that SDBS is more difficult to penetrate into the more shrunken copolymer beads. It has been reported that hydrophobic interaction increases with temperature below about 50°C.³⁵ Therefore, it is considered that the adsorption of SDBS with the VPNG copolymer bead must increase with increasing temperature. But the adsorption ability of the VPNG copolymer bead for SDBA was the maximum at 30°C and decreased again at 40°C as shown in Figure 8(a). This decrease of the adsorption ability of the VPNG copolymer bead for SDBS at 40°C is due to higher shrinkage of the copolymer bead in water at 40°C. The adsorption ability of the QVPNG-O copolymer bead for SDBS was also the maximum at 30°C and decreased again at 40°C as well as the VPNG copolymer bead. The decrease of the adsorption ability of the QVPNG-O copolymer bead for SDBS at 40°C is due to both higher shrinkage and lower surface charges of the copolymer bead in water at 40°C. Furthermore, it was found that the QVPNG-O copolymer bead had higher adsorption ability for SDBS at 30°C than the QVPNG-M or the QVPNG-B copolymer beads, although the QVPNG-O copolymer bead showed higher shrinkage (lower swelling volume) in water than the QVPNG-M and the QVPNG-B copolymer beads at 30°C as shown in Figure 4. This higher shrinkage of the QVPNG-O copolymer hydrogels means that the copolymer became more hydrophobic in water. Therefore, the higher adsorption ability of the QVPNG-O for SDBS at 30°C is due to high hydrophobic interaction in spite of high shrinkage of the copolymer bead in water at 30°C.

Form these results it was found that the QVPNG-R copolymer beads having antibacterial activity could highly adsorb SDBS by both hydrophobic interaction and electrostatic interaction. These results suggest that QVPNG-R copolymer beads can adsorb bacteria having negative charges by both hydrophobic interaction and electrostatic interaction. On the other hand, it is considered that the antibacterial activity of these QVPNG-R copolymer beads was brought about by the destruction of the cell wall of bacteria by the interaction between QVPNG-R copolymer beads and bacteria. Therefore, it is considered that the stronger the interaction between the copolymer beads and bacteria is, the higher the antibacterial activity will be. However, the order of the antibacterial activity of QVPNG-R copolymer beads (QVPNG-B > QVPNG-M > QVPNG-O) does not correspond to the order of magnitude of surface positive charges (Table II) or of the hydrophobicity (Fig. 8) of the copolymer beads in water. From these results both positive charges and suitable hydrophilic-hydrophobic balance as shown in the QVPNG-B copolymer bead were found to be necessary for high antibacterial activity of QVPNG-R against *E. coli*. However, the exact mechanism of the destruction of the cell wall of bacteria with the copolymer beads having cationic groups is not obvious at present.

CONCLUSIONS

QVPNG-R copolymer beads containing pyridinium groups were obtained by the quaternization of VPNG copolymer beads with alkyl iodides (CH₃I, C₄H₉I, and C₈H₁₇I) with different length of alkyl chains. The VPNG(15 : 97 : 3) copolymer bead and the QVPNG(15 : 97 : 3)-B copolymer bead exhibited high thermosensitivity, although the QVPNG(15 : 97 : 3)-M and QVPNG(15 : 97 : 3)-O hardly exhibited thermosensitivity. Thus, the thermosensitivity of the QVPNG(15 : 97 : 3)-R copolymer beads was greatly affected by the length of alkyl chain in alkyl iodides used for the quaternization of the VPNG copolymer beads.

The order of the antibacterial activity of the QVPNG-R copolymer beads against *E. coli* was as follows:



Thus, the antibacterial activity of the QVPNG-R copolymer beads was also affected greatly by the length of alkyl chain in pyridinium groups of the copolymer beads. The QVPNG-B copolymer bead, which had the highest thermosensitivity, exhibited the highest antibacterial activity against *E. coli*. And the antibacterial activity of the QVPNG-R copolymer beads was also influenced by temperature. It was

also found that the antibacterial activity of the QVPNG-R copolymer beads was affected by both the electrostatic interaction and hydrophobic interaction between the copolymer beads and bacteria.

From these results, it is concluded that QVPNG-R copolymer beads should have suitable hydrophilicity (positive charges)- hydrophobicity balance for high antibacterial activity against bacteria such as *E. coli* in water.

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