Adsorption of Ammonium and Nitrate Ions by Poly(*N*-isopropylacrylamide) Gel and Poly(*N*-isopropylacrylamide-*co*-chlorophyllin) Gel in Different States

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ABSTRACT: The adsorption of ammonium and nitrate by temperature-stimulus-responsive poly(*N*-isopropylacrylamide) (NIPA) gel and poly(*N*-isopropylacrylamide-*co*-chlorophyllin) (NIPA-CH) gel in different states was investigated. Both the NIPA gel and NIPA-CH gel could adsorb ammonium and nitrate in a swollen state (swollen gel) and a swelling state (swelling gel), and they adsorbed ammonium more than nitrate. When the gels were shrinking (shrinking gel), they could adsorb a little ammonium from solution, but when the gels were in a shrunken state (shrunken gel), they hardly adsorbed ammonium. The adsorption of both ammonium and nitrate increased for the swelling NIPA gel in comparison with the swollen gel. The NIPA-CH gel was the opposite in this respect. The difference in the amounts of adsorption of ammonium and nitrate by the swollen and swelling NIPA-CH gels was more significant than that of the NIPA gels. It was suggested that ions such as ammonium and nitrate could not diffuse into the gels freely. The adsorption of ammonium and nitrate was affected not only by the phase transitions of the gels but also by the electrical charges. The experimental results for the adsorption of ammonium and nitrate during the volume changes of the gels imply that if the gels are applied to the immobilization of microorganisms, they may improve mass transfer between the immobilization matrix and bulk liquid under cyclic temperature changes and promote reactions of the immobilized microorganisms, especially the nitrification of nitrifying bacteria immobilized by the NIPA-CH gel. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 2367–2372, 2005

Key words: adsorption; diffusion; gels; stimuli-sensitive polymers

INTRODUCTION

Immobilized microorganisms can resist being washed out of a reactor and some adverse impacts on reactions. Immobilized enzymes can be used repeatedly; the immobilization of microorganisms or enzymes can also keep them at a high concentration and make it easy to separate them from a solution after a reaction. Because of these advantages, many bioreactors apply immobilized microorganisms or enzymes to reactions. A wide variety of polymers have been used to immobilize biocatalysts. The efficiency of reactions with immobilized biocatalyst is strongly influenced by the mass-transfer and adsorption characteristics of the polymers.

Poly(*N*-isopropylacrylamide) (NIPA) gel is a temperature-stimulus-responsive polymer with a critical temperature (CT) of approximately 34°C. It exhibits a discontinuous volume change, that is, a volume phase transition when the temperature changes across CT.

When the temperature is lower than CT, the shrunken network of the gel swells, and vice versa. Like the NIPA gel, poly(*N*-isopropylacrylamide-*co*-chlorophyllin) (NIPA-CH) gel, first prepared as a light-stimulus-responsive gel, can also respond to a temperature stimulus.[1] Because of the addition of chromophore chlorophyllin trisodium salt to the NIPA gel, the NIPA-CH gel shows a better response to a temperature stimulus than the NIPA gel. Moreover, the NIPA-CH gel has almost the same CT as the NIPA gel. Unlike the NIPA gel, the NIPA-CH gel has some ionizable radicals.

Both the NIPA gel and NIPA-CH gel have crosslinked polymer networks by which they can soak up much water. Water in the gels exits in different states (i.e., nonfreezing water, bound water, and free water).[2] These hydrogels have many pores within their networks. When they undergo phase transitions, the gel states, the pore sizes, and the quantity of water in the gels change, and this causes a complicated effect on the substance diffusion and adsorption. In other words, substance adsorption by the gels not only is governed by interactive hydrophobic forces, electrostatic forces, and hydrogen bonding but also is affected by volume changes and dehydration of the gels

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TABLE I
Compositions of the Gels

Component	NIPA gel	NIPA-CH gel
Monomer N-isopropylacrylamide	7.8 g	7.8 g
Crosslinker N,N'-methylenebisacrylamide	0.133 g	0.133 g
Chlorophyllin coppered trisodium salt	0	0.361 g
Accelerator N,N,N',N'-tetramethylenediamine	0.24 mL	0.24 mL
Initiator ammonium persulfate	40 mg	40 mg
Deionized water	100 mL	100 mL

during the phase transition. At this point, the effects of the phase-transition behavior of gels on substance diffusion and adsorption have not been clearly elucidated.

It has been reported that the alternating swelling and shrinking of gels can control substance permeation from gels to bulk solutions in a drug delivery system.[3] Adsorbing water into and squeezing water out of gels acts like a hydraulic pump that can facilitate reactions with immobilized microorganisms. These behaviors currently are attracting a lot of attention.

To apply these gels to the immobilization of microorganisms in the future, we need to investigate the effects of polymer network changes due to temperature stimuli on substrate diffusion and adsorption. Furthermore, the adsorption characteristics of gels with or without a temperature stimulus will be helpful for understanding the behaviors and volume transitions of gels. Ammonium and nitrate are the main nitrogen sources for microorganisms, they always dissolve in solution, and they exist as ions with a pH near neutrality. They are also important substances for nitrogen removal in nitrification during biological wastewater treatment. In this study, the adsorption characteristics of the NIPA gel and NIPA-CH gel for ammonium and nitrate in different states were investigated.

EXPERIMENTAL

Preparation of the NIPA gel and NIPA-CH gel

Both the NIPA gel and NIPA-CH gel were synthesized by free-radical polymerization in water. The compositions of the gels are shown in Table I. All the reagents used in these experiments were from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan); they were extra-pure-grade and were not purified furthermore.

N-Isopropylacrylamide, N,N'-methylenebisacrylamide, and chlorophyllin coppered trisodium salt (used only in the NIPA-CH gel) were dissolved in 100 mL of deionized water. The solution was

purged with nitrogen gas for 20 min and cooled at 7°C for 30 min. N,N,N',N'-Tetramethylenediamine and ammonium persulfate were added to the mixture to initiate the polymerization. The polymerization was carried out in a mold at 7°C for at least 24 h until the gelation was complete. The gel was dipped in a large amount of deionized water for the removal of unreacted monomers and was allowed to swell completely. Then, the gel was cut into approximately 4 mm × 4 mm × 4 mm cubes. The gel cubes were dipped into 20 ± 0.5°C or 40 ± 0.5°C pure water before use for at least 24 h so that they could reach equilibrium.

Adsorption experiments

The adsorption experiments were performed in NH_4NO_3 or NH_4Cl solutions at 20 ± 0.5°C or 40 ± 0.5°C. The gels were separated from deionized water, wiped for the removal of excessive water on the surface, and weighed. The gels were placed into different solutions with a volume ratio to the gel below 50 at 20 ± 0.5°C or 40 ± 0.5°C. The concentration, pH, density, and weight of the solution and the weight of gel were measured at both the beginning and end of each experiment. The volume was calculated from the weight and density of the solution. The ammonium and nitrate concentrations were determined spectrophotometrically with automated analytical equipment (Autoanalyzer 3, Bran+Luebbe Co.).

RESULTS

Effects of the gel volume change on the ammonium adsorption

The NIPA gel and NIPA-CH gel, which had almost the same dry weights, were completely swollen at 20°C or shrunken at 40°C in pure water. Then, the gels were placed into 20 or 40°C NH_4Cl solutions so that they would undergo phase transitions or remained unchanged. The NIPA gel and NIPA-CH gel were placed at 20°C (remaining swollen), 40°C (remaining shrunken), from 20 to 40°C (shrinking), and from 40 to 20°C (swelling) in the solutions. The kinetic concentration changes of ammonium in the solutions are shown in Figure 1.

Because the solutions were very low in concentration, the volume changes of the gels were negligibly small after they were placed in the solutions without any temperature change. In this case, the concentration changes of the solutions were attributed to solute adsorption. The concentration of ammonium in a solution after the shrunken NIPA gel or NIPA-CH gel was placed it at 40°C showed almost no change, and the volume of the solution did not change, either. This implies that ammonium was not adsorbed. The con-
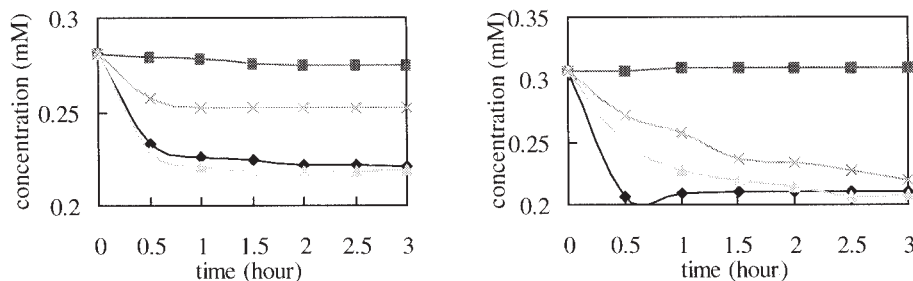
Figure 1 Kinetic changes in the ammonium concentrations of the solutions after the NIPA gel (left) or NIPA-CH gel (right) was added: (■) the gel shrank in 40°C pure water in advance and was then put into a 40°C solution (the volume of the gel remained unchanged in the solution), (×) the gel shrank in 40°C pure water in advance and was then put into a 20°C solution (the gel swelled in the solution), (◆) the gel swelled in 20°C pure water in advance and was then put into a 40°C solution (the gel shrank in the solution), and (▲) the gel swelled in 20°C pure water in advance and was then put into a 20°C solution (the volume of the gel remained unchanged in the solution).

centration of the solution after the swollen NIPA gel or NIPA-CH gel was placed in it at 20°C declined and reached equilibrium within 3 h. When gels shrink or swell because of a phase transition, water in the gels is squeezed out or adsorbed from the ambient solution, and this results in a volume change of the solvent. The concentration of a solution depends on both the solvent volume and the quantity of the solute. The concentration of the solution, in which the NIPA-CH gel shrank, decreased and reached equilibrium rapidly, and with the shrinking NIPA gel in it, the concentration decreased slowly. We concluded that the decrease in the concentration of the solution could be attributed to the increase in the volume of the solvent due to a discharge of water by the gel other than ammonium adsorption because the quantity of ammonium adsorbed by the shrinking gel was reduced in comparison with the quantity under the swollen condition (Fig. 2). This implies that a concentration change in a bulk solution cannot directly reflect the adsorption of gels with a volume change.
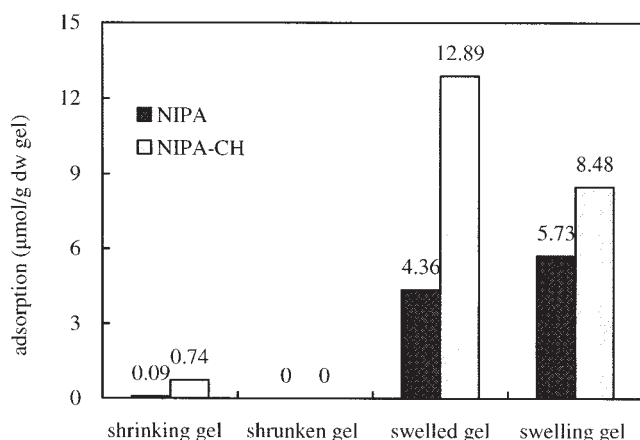
Figure 2 shows the quantities of ammonium adsorbed by the NIPA gel and NIPA-CH gel in different states. The shrinking gels adsorbed little ammonium, and the completely shrunken gels did not adsorb ammonium at all. The swollen and swelling NIPA gels and NIPA-CH gels adsorbed more ammonium, and the adsorption behaviors of the swollen and swelling gels were studied.

Selective adsorption on ammonium and nitrate by the NIPA gel and NIPA-CH gel

The adsorption behaviors of the swelling and swollen NIPA gels and NIPA-CH gels in 0.3 mM NH$_4$NO$_3$ solutions were investigated. The adsorption of ammonium and nitrate is presented in Figure 3.

Ammonium was adsorbed more than nitrate by the swelling and swollen NIPA gels and NIPA-CH gels. This suggests that both the NIPA gels and NIPA-CH gels were apt to adsorb ammonium. The difference in the amounts of adsorption of ammonium and nitrate by the NIPA gel was less than that by the NIPA-CH gel; that is, the selective adsorption of ammonium by the NIPA-CH gel was greater than that by the NIPA gel. The contributions of the volume change to the adsorption of ammonium and nitrate during swelling by the NIPA gel and NIPA-CH gel were different. The swelling NIPA gel adsorbed ammonium slightly better than the swollen one, but there was almost no difference in nitrate absorption; on the other hand, the NIPA-CH gel showed little difference in ammonium absorption between its swelling and swollen forms, but the difference in nitrate absorption was obvious.

The concentration changes of ammonium and nitrate in solutions of NH$_4$NO$_3$ due to swollen and shrunken NIPA gels and NIPA-CH gels at 20°C are shown in Figure 4. The concentrations of ammonium and nitrate in the solutions decreased after the swollen NIPA-CH gel and NIPA gel were added because of ion diffusion from the solutions to the gels and ad-
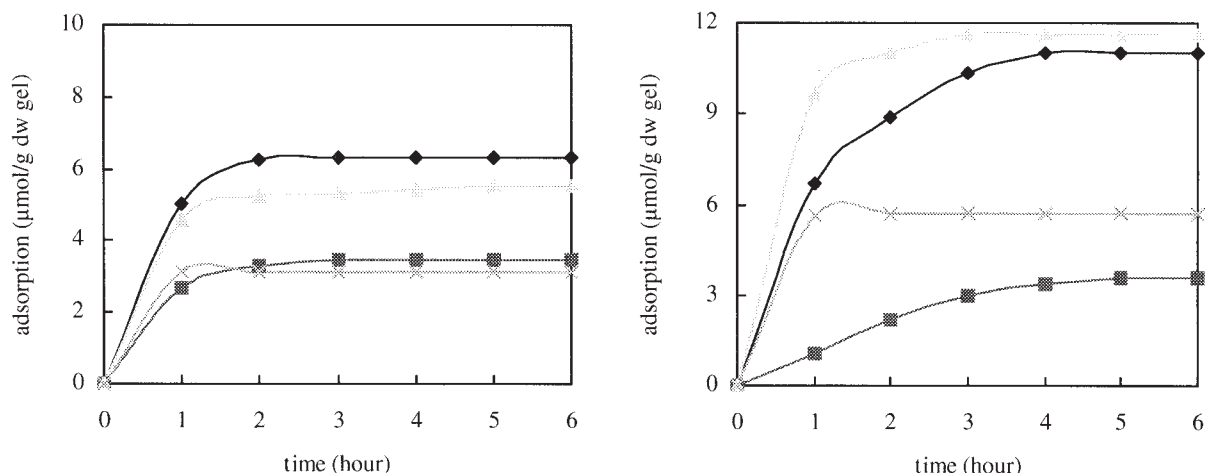

Figure 2 Adsorption of ammonium by the gels in different states. The figures above the bars indicate adsorption quantities.

Figure 3 Adsorption of ammonium and nitrate by the NIPA gel (left) and NIPA-CH gel (right): (■) the adsorption of nitrate by the swelling gel, (×) the adsorption of nitrate by the gel in the swollen state, (♦) the adsorption of ammonium by the swelling gel, and (▲) the adsorption of ammonium by the gel in the swollen state.

sorption by the gels. Ammonium decreased in concentration and nitrate was almost unchanged after the shrunken NIPA gel was added to the solutions. In contrast, nitrate increased after the shrunken NIPA-CH gel was added. This implied that the rate of water absorption by the swelling NIPA-CH gel was faster than that of nitrate, and this resulted in the nitrate concentration rising; however, the rate of ammonium adsorption was faster than that of water, and this resulted in a concentration decline. The adsorption on nitrate by the NIPA-CH gel and the adsorption on nitrate by the NIPA gel differed.

DISCUSSION

A gel is considered a state between solid and liquid, and so its adsorption of a substance is like substance diffusion into the gel to a great extent. A substance diffuses through pores into the gel via water in pores, and the diffusion rate depends on the concentration difference of the solutions inside and outside the gel, the size of the molecules in the bulk solution, the sizes of the pores, and the temperature. Even through ammonium and nitrate have low molecular weights and have small molecules, they could not seem to diffuse freely into the swollen NIPA gel and NIPA-CH gel. There were significant differences between the ammonium and nitrate diffusion in the NIPA gel and NIPA-CH gel. The NIPA-CH gel had carboxyl radicals and ionized at a neutral pH, so the NIPA-CH gel was an ionized gel with a negative charge. Adsorption concerned the charge of the adsorbate. Ammonium was apt to be adsorbed into the NIPA-CH gel because of its positive charge. The NIPA gel was a nonionic gel, but the swollen NIPA gel adsorbed ammonium more than nitrate, too. This implied that the polar groups of
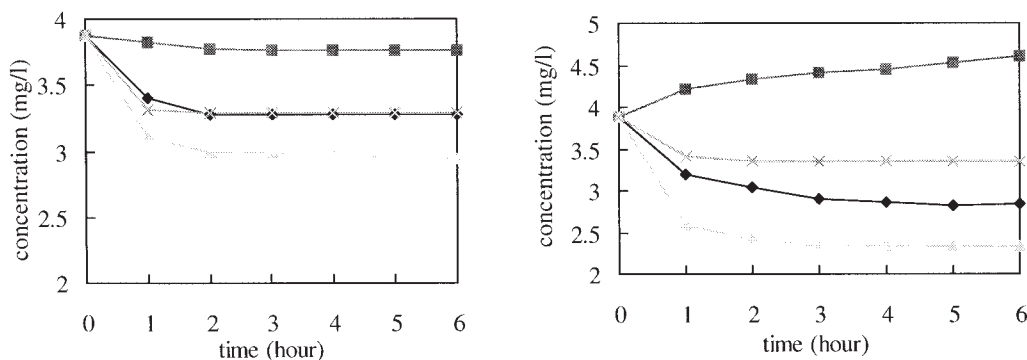

Figure 4 Concentration changes of ammonium and nitrate in solutions of NH_4NO_3 after the NIPA gel (left) or NIPA-CH gel (right) was added at 20°C: (■) the concentration change of nitrate after the shrunken gel was added and the gel underwent swelling, (×) the concentration change of nitrate after the swollen gel was added and the gel did not change in volume, (♦) the concentration change of ammonium after the shrunken gel was added and the gel underwent swelling, and (▲) the concentration change of ammonium after the swollen gel was added and the gel did not change in volume.

amide in the side chain of NIPA could not dissociate but had higher affinity for ammonium than nitrate.

Raising the temperature usually promoted diffusion, but it also affected the structure of the NIPA gel and NIPA-CH gel because of gel shrinkage. For a bigger molecule such as insulin, its diffusion inside the NIPA gel was restricted by gel shrinkage due to a decrease in the pore size, but for a small molecule such as glucose, it was mainly controlled by a temperature below 30°C, not by a gel network.[4] Many studies have shown that electrostatic interactions play an important role in adsorption on charged species.[5–8] The shrinkage of the NIPA gel had no effect on the diffusion of the inorganic electrolytes (e.g., zinc, nickel, and chromium) between the gel and the external solution because the complex formation among the radicals and metals in the NIPA gel was dominant in both the swollen gel and the shrunken gel.[9] Protein adsorption by a polystyrene-core/NIPA-shell particle strongly depended on the temperature, and the adsorption increased as the incubation temperature increased even after the gel shrank.[6] This study shows that even small ions such as ammonium cannot be adsorbed by the shrunken NIPA gel and NIPA-CH gel at 40°C. When the temperature rose above 34°C, the NIPA gel and NIPA-CH gel began to shrink. During their phase transition, the adsorption of cationic ammonium was very low. This suggested that the diffusion of ammonium was seriously hindered and that the adsorption conditions were different for the swollen gel and shrunken gel. The adsorption of metal by the NIPA gel was due to complex formation, and the adsorption of protein by the shrunken hydrogel was due to hydrophobic action. The greater adsorption of ammonium, in comparison with that of nitrate, and the difference in the adsorption rate between the NIPA gel and NIPA-CH implied that the adsorption involved the charge or polarity of the gels. That the two shrunken gels could not adsorb ammonium suggested that the hydrophilicity of the gels played a critical role in adsorption. In the shrinking stage, the NIPA gel and NIPA-CH gel were reduced in volume, and their hydrophobicity increased. This resulted in water and other substances being squeezed out.

The volume phase transition of gels is explained by the Donnan theory.[10] The swelling pressure comes from the difference between the osmotic pressure of a shrunken gel and the outer solution, and as a result of gel swelling, the ions between the gel and the outer solution are redistributed. In deionized water, because of osmotic pressure, only water molecules were adsorbed by the shrunken NIPA-CH gel and NIPA gel during the swelling of the gels. In contrast to water molecules, counterions such as ammonium in a solution were preferentially adsorbed into the ionized swelling gels to balance the osmotic pressure between the gels and the outer solution. Unlike ammonium, nitrate could not be drawn into the swelling NIPA-CH gel because they had the same negative charge, and so the nitrate concentration rose, whereas the ammonium concentration decreased, with a decreasing volume of the solution during the NIPA-CH gel swelling. The volume of the swelling gels at equilibrium in the solution was smaller than that in pure water because of adsorbed ions. The Donnan theory is also applicable to the nonionic NIPA gel.[11] The swelling polar network in the NIPA gel adsorbed ions (ammonium and nitrate) or water, but the adsorption of ammonium and nitrate by the NIPA gel was not as large as that by the NIPA-CH gel. This was because of the strong electrostatic interaction between the shrunken NIPA-CH gel and the adsorbate; during its swelling, the difference in the adsorption between ammonium and nitrate became bigger than that of the swollen NIPA-CH gel. It was concluded that the difference for ammonium and nitrate between the swelling and swollen NIPA gels was smaller than that of the NIPA-CH gel, and this was attributed to the weak electrostatic force of the NIPA gel.

Mass-transfer resistance between a bulk liquid and an immobilized microorganism is a limiting factor for the reaction of an immobilized microorganism in conventional gels.[12,13] The results of the adsorption of ammonium and nitrate by the NIPA and NIPA-CH gels show that the two gels under a temperature stimulus could improve substrate diffusion between an immobilized microorganism and a bulk liquid. Because the volume change (from shrunken to swollen) allows the NIPA gel to adsorb more ammonium and nitrate, if it is applied to an immobilized microorganism, the diffusion between the gel and bulk liquid will be promoted under a temperature stimulus. The NIPA gel as an immobilization matrix is very promising for improving mass transfer to immobilized microorganisms that need ammonium.

Nitrifying bacteria are playing a key role in nitrogen removal in wastewater treatment, but they are often washed out from wastewater-treatment reactors, and so the immobilization of nitrifying bacteria is applied in practice to overcome this problem. Nitrifying bacteria immobilized in the NIPA-CH gel under a temperature stimulus will work better than in the NIPA gel or other matrices with no volume change, such as poly(vinyl alcohol), because the volume change of the NIPA-CH gel can put more ammonium and less nitrate into the gel and facilitate the reaction of the immobilized nitrifying bacteria.

When the gels are shrinking or are completely shrunk, their adsorption of ammonium and nitrate decreases or even ceases, and this will negatively affect the reaction of an immobilized microorganism. On the other hand, the gels discharge almost all nitrate and some ammonium included in them during their shrinking (the data are not shown here). For alleviat-

ing the inhibition of production, the shrinkage of gels can facilitate a reaction, but it also results in substrate reduction in the gels, which will lower the reaction rate. For the application of these gels to immobilizing microorganisms with a temperature stimulus, it is important to determine the optimal frequency of shrinking and swelling in a cycle of volume change.

CONCLUSIONS

Both swollen and swelling NIPA gels and NIPA-CH gels adsorbed ammonium and nitrate, and ammonium was adsorbed more than nitrate in both gels. When the gels were shrinking, their adsorption decreased dramatically. Completely shrunken gels even at high temperatures did not adsorb ammonium at all. The adsorption of both ammonium and nitrate increased for the swelling NIPA gel in comparison with the swollen NIPA gel. The adsorption of the NIPA-CH gel was the opposite. The difference between ammonium and nitrate adsorption was more significant between the swollen NIPA-CH gel and swelling NIPA-CH gel than between the swollen NIPA gel and the swelling NIPA gel. This suggested that even small ions such as ammonium and nitrate could not diffuse into the gels freely.

Volume changes improved ammonium and nitrate transfer to the gels, and this could promote the reaction of immobilized microorganisms, especially for the nitrification of nitrifying bacteria immobilized by the NIPA-CH gel.

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