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Original article

Preparation and Investigation of Poly (N-isopropylacrylamide-acrylamide) Membranes in Temperature Responsive Drug Delivery

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Abstract

Objective(s)
Physiological changes in the body may be utilized as potential triggers for controlled drug delivery. Based on these mechanisms, stimulus–responsive drug delivery has been developed.
Materials and Methods
In this study, a kind of poly (N-isopropylacrylamide-acrylamide) membrane was prepared by radical copolymerization. Changes in swelling ratios and diameters of the membrane were investigated in terms of temperature. On-off regulation of drug permeation through the membrane was then studied at temperatures below and above the phase transition temperature of the membrane. Two drugs, vitamin B_{12} and acetaminophen were chosen as models of high and low molecular weights here, respectively.
Results
It was indicated that at temperatures below the phase transition temperature of the membrane, copolymer was in a swollen state. Above the phase transition temperature, water was partially expelled from the functional groups of the copolymer. Permeation of high molecular weight drug models such as vitamin B_{12} was shown to be much more distinct at temperatures below the phase transition temperature when the copolymer was in a swollen state. At higher temperatures when the copolymer was shrunken, drug permeation through the membrane was substantially decreased. However for acetaminophen, such a big change in drug permeation around the phase transition temperature of the membrane was not observed.
Conclusion
According to the pore mechanism of drug transport through hydrogels, permeability of solutes decreased with increasing molecular size. As a result, the relative permeability, around the phase transition temperature of the copolymer, was higher for solutes of high molecular weight.

Keywords: Acetaminophen, Drug delivery, Hydrogel, Temperature responsiveness, Vitamin B_{12}

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Introduction

Hydrogels are water-swollen networks (crosslinked structures) composed of hydrophilic homopolymers or copolymers. They are rendered insoluble due to the presence of chemical (covalent or ionic) or physical crosslinks (1-2). Stimuli-sensitive hydrogels, which can change their swelling behaviors and other properties in response to environmental stimuli such as temperature, pH, electric field, ultrasound, and special chemical compound, have attracted great interest for their potential applications in technological and biomedical fields (3-6). The researches have been particularly focused on the use of thermo and pH sensitive hydrogels. This partly stems from the observation that several disease states manifest themselves by a change in the physiological parameters of the body, including temperature and pH (7-9). Among temperature sensitive hydrogels, one of the most widely studied systems has been based on poly (N-isopropylacrylamide) (PNIPAAm) gels, which exhibits a temperature-induced volume phase transition in water (10-11). PNIPAAm hydrogels are attracting more and more interest in biomedical applications as they undergo a sharp volume transition in water around 32–34 °C, which is close to the body temperature and the ease with which the transition temperature can be adjusted by copolymerization with other molecules (12). Many scientists believe that the hydrogen bonding effect and hydrophobic forces are the most important reasons in PNIPAAm phase transition process. Therefore, with a change in the ratio of hydrophilic and hydrophobic groups in the polymer, one can adjust the low critical solution temperature (LCST) of the system to match the desired critical region. Furthermore, different media affected the swelling behavior and the LCST of the hydrogel (13). It was indicated that hydrophobic monomers shifted the LCST to lower temperatures and hydrophilic ones in the structure shifted it to higher temperatures (14). An ideal on-off temperature sensitive drug delivery system should have a LCST close to the body temperature. By copolymerization of NIPAAm and hydrophilic monomers such as acrylamide (AAm), the LCST could shift to higher temperatures near 37 °C (15). The goal of this study was doing a relatively complete investigation on swelling of 10:1 P (NIPAAm/AAm) membrane and monitoring of drug permeation through it in response to temperature. Studies on permeation of two drug models with different sizes let us to better understand the mechanism of drug transport through this intelligent hydrogel. Before, some studies were done on drug permeation through this kind of membrane, but they did not focus on permeation of two different drug models and the mechanisms involved on their transport. As NIPAAm shows a negative response to temperature, meaning to release drugs at lower temperatures, applying such membranes as drug delivery systems, need some modifications which is going to be done in the next step. Approaches to develop systems, using hydrogels with positive temperature sensitivity have involved the grafting of poly-NIPAAm onto porous membranes, using interpenetrating porous gels or developing some kinds of composite membranes. A DSC study was also done to investigate the phase transition temperature of the membrane. Two substances, vitamin B_{12} (vit B_{12}) with molecular weight of 1355 g/mol and acetaminophen with molecular weight of 151.17 g/mol were used here as model drugs. Stimuli sensitive drug delivery systems have been used more for peptide and protein delivery. Because of vit B_{12}'s hydrophilic nature and its high molecular weight, this drug has been considered as a good model of proteins in the case of temperature sensitive drug delivery studies (16). The use of acetaminophen as a low molecular weight model in addition to vit B_{12} could give valuable information about the mechanisms involved in temperature sensitivity of the membrane. Various models have ever since been proposed to describe solute diffusion within hydrogel networks in relation to polymer hydration, but still more studies are necessary to better understand the exact mechanism of solute permeation through the hydrogels.

Materials and Methods

Materials

N-isopropylacrylamide (NIPAAm), acrylamide (AAm), N,N-methylen-bis-acrylamide (MBAAm), N,N,N,N-tetra-methyl-ethylenediamine (TEMED) and ammonium persulfate (AP) were obtained from Aldrich, vit B_{12} and acetaminophen were kindly donated by Iran Hormone Pharmaceutical Co., Iran. Potassium mono-hydrogen phosphate and potassium dihydrogen phosphate were obtained from Merck, Germany. All other chemicals used were of analytical reagent purity and water was double-distilled and freshly prepared.

Preparation of temperature sensitive membranes

P (NIPAAm-co-AAm) was prepared by free radical copolymerization in water at room temperature. AP and TEMED were used as an initiator and accelerator, respectively. NIPAAm (1.3 mol%), AAm (0.21 mol%) and MBAAm (0.019 mol%) were dissolved in 60 ml distilled water and the solution was bubbled with nitrogen for 20 min prior to copolymerization in order to remove the remaining oxygen. After that, 0.017 mol% AP and 40 µl TEMED were added to the solution and stirring under nitrogen gas was continued for one more min. The solution immediately was injected between two glass plates of a gel caster system with a 0.75 mm silicon rubber as a spacer and copolymerization was allowed to proceed for 4 hr at room temperature. The membranes were then cut into disks of 4 cm diameter by the use of a steel punch and were immersed in a large bowel containing distilled water for 1 day to remove the unreacted species (17).

Thermal analysis

Sections of temperature sensitive membranes were pre-equilibrated in distilled water for 24 hr at 15 °C. A differential scanning calorimeter (Mettler-Toledo, USA) was used in this study to determine the LCST of the membranes. The samples were taken out from water and any excess water on the surfaces was removed by blotting with filter paper. Finally, the discs were coarsely crushed, using a mortar and pestle and approximately 5 mg samples of hydrogels were weighed into aluminum pans, which were then sealed. An empty closed aluminum pan was used as the reference cell. Then the temperature was increased from -20 °C to 100 °C at the rate of 2 °C /min in an N_2 gas flow.

Swelling measurements of the membrane at different temperatures

Three pre-weighed dry hydrogel discs with diameter of 4 cm and thickness of 0.75 mm were immersed in two beakers, one containing double distilled water and another containing phosphate buffer solution (pH= 7.4). The temperature of these solutions was maintained by immersing the beakers in a water bath (Memmert, Germany). The samples were allowed to equilibrate at the given temperature, then removed from the media and tapped with filter paper to dry the gel surface and then reweighed. This procedure was repeated until no change in weight could be measured. Swelling Ratio (SR) of discs was determined as the ratio of the weight of hydrated discs equilibrated at a certain temperature (W_s) over the weight of dry discs (W_d) according to the equation below:

$$\%SR = \frac{Ws}{Wd} \times 100$$

These measurements were carried out at different temperatures (27, 31, 32, 41, 44 °C) below and above the LCST of the membrane. To investigate the effect of media on the swelling ratio of the membranes, phosphate buffer solution and distilled water were chosen here as different media. These studies were generally performed in triplicate.

Swelling measurements of the membrane by thermal cycling

The effect of thermal cycling on swelling ratios and diameters of the membranes was also investigated in order to show reversibility and reproducibility of thermosensitivity. The experimental method was similar to that described above, except that the temperature was fluctuated between 30 °C (below the LCST) and 46 °C (above the LCST) in two hr

cycles. The chosen medium was phosphate buffer solution here (pH= 7.4).

Drug permeation studies

The permeation of vit B_{12} and acetaminophen through the membranes at temperatures above and below the LCST of P (NIPAAm-co-AAm) was investigated, using a purpose-built horizontal side by side diffusion cell. First of all, the membrane was clamped between two cells of apparatus with a permeation area of 1.98 cm^2. Before each test, system was soaked in the medium for at least two hours at each temperature. The diffusion cell immersed in a 27, 32, 41, 44 ºC thermostated water bath with temperature fluctuations of ±0.1 ºC (Memmert, Germany). Donor part of the diffusion cell was filled with 1% solution of either vit B_{12} or acetaminophen and the receptor chamber was filled with drug free phosphate buffer solution (pH= 7.4). At different time intervals, an aliquot of 4 ml was taken out and replaced with the same volume of phosphate buffer. The solution of each compartment was stirred at 100 rpm to eliminate boundary layer effect. In order to meet the requirement of sink condition, the solute concentration in the donor cell was kept at least 100-fold greater than that in the receptor cell during the entire experiment. The appearance of solute in the receptor chamber was monitored over time by ultraviolet absorbance at 361 nm and 243 nm for vit B_{12} and acetaminophen, respectively. The amount permeated was then plotted against the time. Solute permeation, Flux (J) =M/t.S; was calculated according to the following equation based on Fick's first law of diffusion with assumptions including: (1) steady state was reached in the membrane after a short lag time, t_L; (2) the area for permeation, S, and solute concentration in the donor cell, Cd; were constant; (3) sink condition was maintained at the receptor side.

$$M= PSC_dt$$

where M was the amount of drug model permeated, P was permeation coefficient of the membrane at different temperatures, S was available area for drug permeation, and C_d was drug concentration in donor cell and t was the time. All experiments were performed in triplicate.

Statistical analysis

Data were analyzed by 1-way analysis of variance (ANOVA), using SPSS statistical package. Statistical differences yielding $P\leq .05$ were considered significant. Duncan or Tukey's multiple-comparison *post hoc* tests were applied when necessary.

Results and Discussion

Thermal analysis results

Figure 1 revealed the DSC thermogram of the 24 hr water equilibrated P (NIPAAm-co-AAm) membrane. An endothermic peak was observed at 36.6 °C. Moreover, the onset point of the peak, determined by the intersection point of the two tangents for the baselines and the downward slope of the peak, was found at 36.3 °C. The LCST of the PNIPAAm homopolymer was about 32 °C according to the studies (14). Incorporating hydrophilic monomers such as acrylamide shifted the LCST of the membrane to a higher temperature (36.6 °C) near the body temperature because it favored hydrophilic interactions inside the copolymer matrix and as a result, shrinkage happened at higher temperatures. This again confirmed that the phase transition in P (NIPAAm-co-AAm) membranes happened as a result of a change in hydrophobic/hydrophilic balances inside the hydrogel. At temperatures below the LCST, hydrophilic forces were dominant and hydrogel was in a swollen state. At temperatures above the LCST, hydrophobic interactions were more distinct than hydrophilic forces and as a result, the membrane became dehydrated. Our results were in a close agreement with the result of previous studies done on the LCST of a kind of P (NIPAAm-co-AAm) membrane (15).

Swelling results of the membrane at different temperatures

As it is indicated in Figure 2, swelling of the membranes in phosphate buffer solution

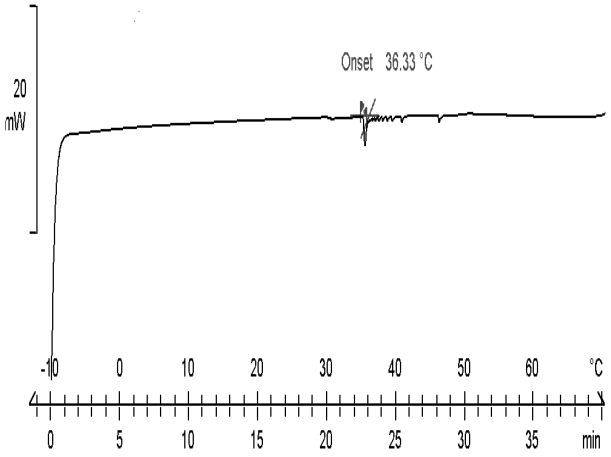

Figure 1. DSC thermogram of the P (NIPAAm-co-AAm) membrane.

(pH= 7.4) and distilled water, were significantly decreased at temperatures above the LCST of the membranes compared to those of below it. This can be described by the fact that AAm was hydrophilic, whereas the isopropyl group of NIPAAm was hydrophobic. Hydrogen bonding between water molecules and hydrophilic groups of the hydrogel was strong at temperature below the LCST. As a result of that, copolymer was fully swollen in these temperatures. At higher temperatures, water was partially expelled from the hydrogel and dominance of hydrophobic forces caused the membrane to become dehydrated. Because of low ionic strength in distilled water and consequently the presence of a low osmotic pressure medium, swelling ratios were higher at distilled water compared to phosphate buffer solution at all temperatures.

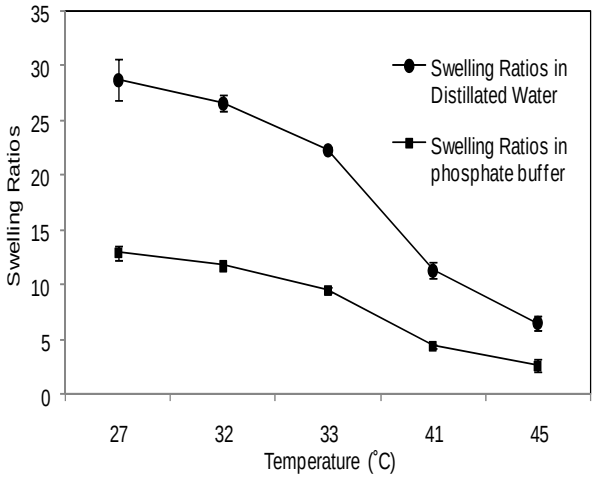

Figure 2. Swelling ratios of the P (NIPAAm-co-AAm) membrane at different temperatures (n=3).

Swelling results of the membrane by thermal cycling

Figure 3 and Figure 4 showed the swelling ratios and diameters of the membrane, respectively, with two thermal cycling between 30 °C (below the LCST of the membrane) and 46 °C (above the LCST of the membrane). It was indicated that the swelling ratios and diameters of the membrane were 5 times more at 30 °C (below the LCST) compared to 46 °C (above the LCST). These figures showed not only temperature sensitivity in swelling of the membrane, but also a good reversibility and reproducibility of the temperature response. These results confirmed that a change in hydrophobic-hydrophilic balances inside the membrane in response to a temperature variation was reversible and reproducible. Reversibility in thermoresponsivity was a main factor of an efficient on–off drug-delivery system. A temperature sensitive drug delivery system was likely to undergo numerous repeated volume phase transitions in its manufacture and in the process of modulating drug release. In a study done by Wang *et al*, some kinds of thermoresponsive P (NIPAAm-co-AAm) nanogels with different monomer ratios were prepared and their characteristics were investigated in terms of temperature. It was indicated that these nanogels underwent a sol-gel transition at about body temperature and the response was reversible but with a clear hysteresis. They ascribed this to the fact that the nanogel particles were shrunken compact particles at elevated temperatures, and the surrounding water entered into particles with difficulty to swell the nanogel (18). In another work done by Li *et al*, some P (NIPAAm) membranes were subjected to 15 thermal cycles and the effect of repeated thermal cycling on the thermoresponsive swelling behavior of crosslinked P (NIPAAm) membranes were investigated. Their results showed that repeated thermal cycling led to the formation of the cracks on the surface of the hydrogels and resulted a decreased degree of swelling in the crosslinked Poly (NIPAAm) membranes at temperatures below the LCST due to the presence of created cracks (19).

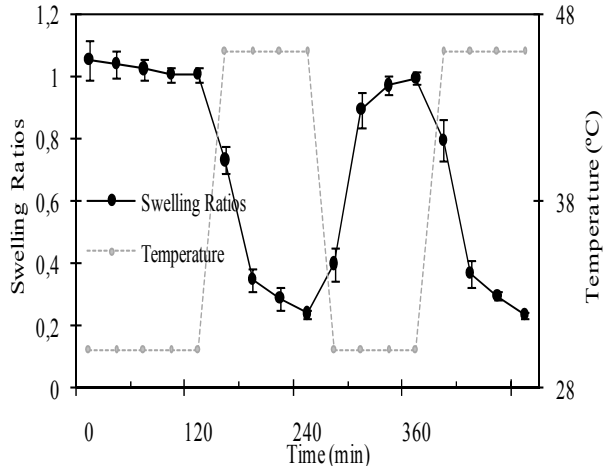

Figure 3. Swelling Ratios of the P (NIPAAm-co-AAm) membrane by thermal cycling between 30 ºC and 46 ºC (n=3).

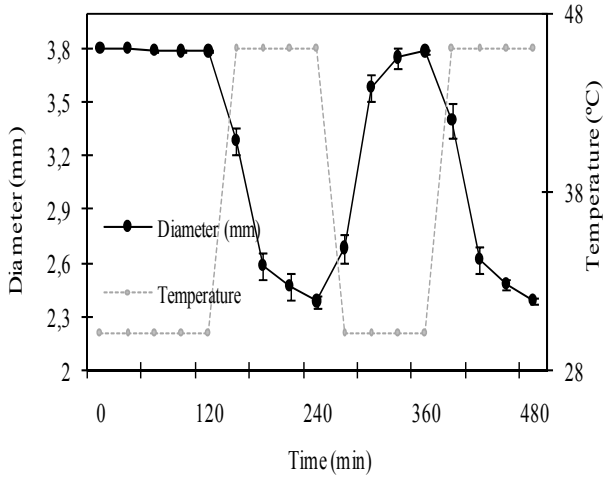

Figure 4. Diameters of the P (NIPAAm-co-AAm) membrane by thermal cycling between 30 ºC and 46 ºC (n=3).

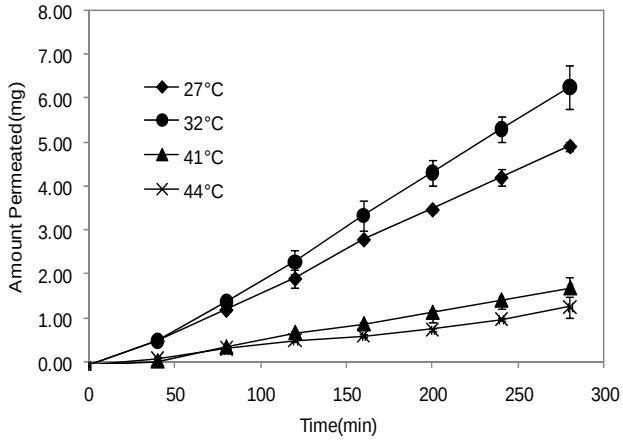

Figure 5. Vit B_{12} permeation through P (NIPAAm-co-AAm) membranes at different temperatures, ($p < 0.0001$) (n=3).

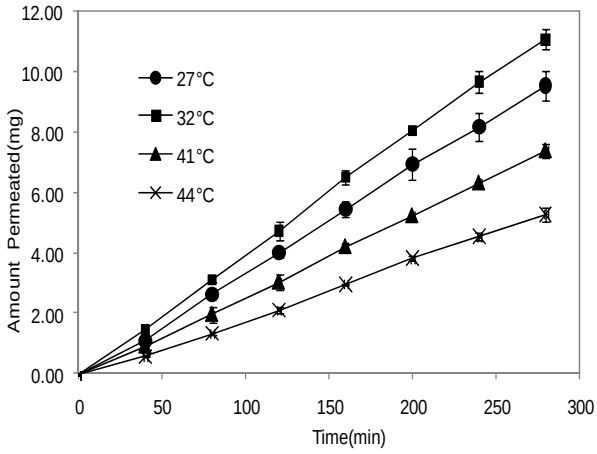

Figure 6. Acetaminophen permeation through P (NIPAAm-co-AAm) membranes at different temperatures, ($p < 0.009$) (n=3).

Drug permeation results

Figure 5 and Fig. 6 showed the permeation of vit B_{12} and acetaminophen through P(NIPAAm-co-AAm) membranes at different temperatures, respectively. As it can be seen, vit B_{12} permeation through the membranes at temperatures below the LCST of the hydrogel increased substantially compared to that at temperatures above it. However, for acetaminophen, as indicated in Figure 4, the variation of temperature around the LCST could not produce much greater changes in drug permeation through the membrane.

This could be attributed to a relationship between molecular size and the mechanism of drug transport. Generally, There were two mechanisms proposed to explain diffusion through the hydrogels: the partition and pore mechanisms. The partition mechanism was assumed to involve the dissolution of the solute into the polymer structure. The pore mechanism assumed that the solute moves through the bulk water phase of the swollen polymer. Strong hydrogen bonding between water molecules and carbonyl and amido groups of the P (NIPAAm-co-AAm) membrane made the copolymer swell in water at low temperatures. A hypothetical network of pores within the swollen hydrogel had been suggested since the diffusion was seen to be affected by the size of the solute involved. Solute transport was most of the time based on diffusion through these water filled pores. However, the water molecules were partially expelled from hydrogel as the temperature was increased above the LCST. This caused dramatic

decrease in water content of the pores and consequently a significant reduction in drug diffusion due to shrinkage of the copolymeric matrix (18, 20). A main reason for better on-off permeation of vit B_{12} through the membrane might be due to its larger molecular weight (1355 g/mol) compared with acetaminophen (151.17 g/mol). According to the pore mechanism of drug transport, diffusion of larger molecular weight drug models through the membrane was much more dependent on the pore size and water content of the membrane than the smaller ones. As a result, the difference in drug transport around the LCST was much more distinct for vit B_{12} than acetaminophen. Zhang *et al* indicated that molecular size of solutes played an important role in determining solute permeability across a kind of composite poly (N-isopropylacrylamide-co-methacrylic acid) membrane. They indicated that the permeability of the solutes decreased with increasing molecular size. As a result, the relative permeability, around the LCST, was higher for solutes with a higher molecular weight in their study. They also proposed that the alteration of porosity of the membrane due to copolymer swelling/shrinkage in response to temperature was the major factor responsible for the changes in solute permeability across the composite membrane (16).

As it was indicated in Figure 5 and Figure 6, drug permeation through the membrane at 32 °C was a little higher than that at 27 °C. Drug permeation through a normal polymer was expected to increase when temperature rose due to an increase in diffusion coefficients of drug models. Inversely, for a temperature sensitive hydrogel, an increase in temperature above the LCST caused dehydration of the polymer and made a decrease in permeation of drug models through the membrane. Here when temperature increased from 27 °C to 32 °C, the copolymer was still fully hydrated. Therefore, drug permeation was more affected by an increase in drug diffusion coefficients in terms of temperature. As a result, a small increase in permeability of drug models at 32°C compared to 27 °C was observed (21).

Permeation (fluxes= M/t.S) of drug models through the membranes at different

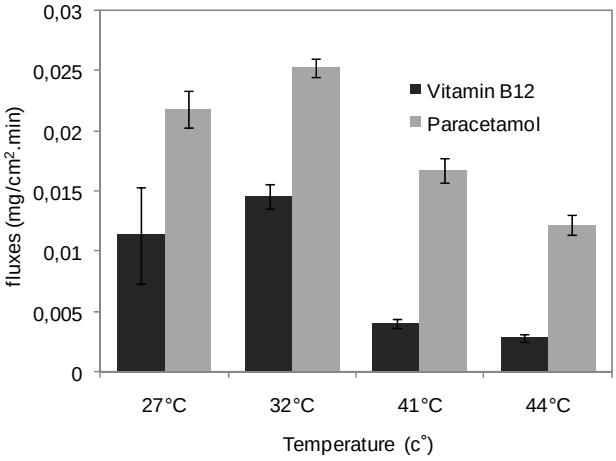

Figure 7. fluxes of vit B_{12} and acetaminophen through the membrane at different temperatures (n= 3).

Permeation (fluxes= M/t.S) of drug models through the membranes at different temperatures were obtained from the slope of permeation curve at each temperature as it was described in this text before according to the equation $M=PSC_dt$, where M was the amount of drug model permeated, P was permeation coefficient of the membrane at different temperatures, S was available area for drug permeation, and C_d was drug concentration in donor cell and t was the time.

In Figure 7, permeation of both vit B_{12} and acetaminophen through the membranes were shown at different temperatures. It was indicated that fluxes of vit B_{12} was lower than that of acetaminophen at all temperatures due to its larger size. It was also observed that vit B_{12} fluxes increased substantially when temperature changed from 32 °C (below the LCST of the hydrogel) to 41 °C (above the LCST), whereas relative fluxes of acetaminophen, around the LCST did not change a lot.

According to the pore mechanism of drug transport, at temperatures above the LCST when the copolymer became shrunken, drug permeation decreased markedly specially for larger molecular weight models such as vit B_{12}. As the membrane did not completely dehydrated at temperatures above the LCST, small amount of drug permeation through the membrane was still detected at these temperatures and complete on-off could not be achieved. Dinarvand *et al* indicated that erythromycin permeation (a high molecular

weight drug model) through a kind of hydrogel membrane was remarkably higher at temperature below the LCST compared to that at temperatures above it, but this difference in permeability around the phase transition temperature of the membrane was not noticeable for hydroxyl urea (a low molecular weight drug model) (17).

Conclusion

This study indicated that a kind of crosslinked P (NIPAAm/AAm) membrane could act successfully as a reversible, reproducible, and thermoresponsive drug delivery barrier for large drug molecules such as vitamin B_{12}. This membrane showed a volume phase transition temperature at about 35.6 °C in water. The LCST of PNIPAAm homopolymers were just about 32 °C. It was shown that incorporating hydrophilic monomers such as acrylamide inside the copolymer matrix shifted the LCST of the membrane to higher temperatures near the body temperature which was ideal for a temperature sensitive drug delivery system. Swelling ratio and diameter of the membrane increased to several orders of magnitude when temperature changed below the LCST of the copolymer. Strong hydrogen bonding between water molecules and carbonyl and amido groups of the P (NIPAAm-co-AAm) membrane made the copolymer swell in water at low temperatures. At higher temperatures, water was partially expelled from the copolymer matrix and dehydration of the membrane was occurred. It was indicated that permeation of two drug models, acetaminophen and vit B_{12} through the membrane could be thermally modulated. According to the pore mechanism of drug transport through the hydrogels, the P (NIPAAm-co-AAm) membrane showed more temperature sensitivity and so better on-off control for large molecular weight models such as vit B_{12}.

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