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Drug permeation through a temperature-sensitive poly(*N*-isopropylacrylamide) grafted poly(vinylidene fluoride) membrane

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Abstract

Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) is a temperature sensitive polymer, which has been used in the development of thermally controlled devices. In the present study, poly(NIPAA)m grafted poly(vinylidene fluoride) (PVDF) membranes were prepared by an irradiation method and fluxes of model compounds across the various grafted membranes were measured. The effectiveness of various grafted membranes to control drug fluxes by temperature was studied using FITC-dextrans (molecular weights 4400–50 600) and mannitol as model compounds. Also, the effect of environmental conditions on the LCST of the membrane was evaluated. The fluxes of bigger molecules across a temperature sensitive, porous poly(NIPAA)m–PVDF membranes were effectively controlled by temperature, environmental ionic strength and degree of grafting of the membrane, while flux of the smaller molecules was not controlled thermally even at high degree of membrane grafting. These result indicates that the studied membranes are useful in controlling the permeation of high molecular weight compounds such as polynucleotides, peptides and proteins.

Keywords: *N*-Isopropylacrylamide; Porous polymer membrane; Permeability; Temperature sensitivity; Ionic strength

1. Introduction

Various types of on-off switching drug delivery systems have been studied in recent years. These

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systems release the drug in respond to various physiological stimuli according to the needs of the body. These triggering mechanisms include pH (Xie and Liu, 1991), ionic strength (Bodmeier et al., 1996), temperature (Chun and Kim, 1996) and enzymes (Brondsted and Kopecek, 1992). In order to obtain environmentally sensitive delivery systems, polymers with different compositions have been developed and their properties in drug delivery have been studied. Among these polymer-based delivery systems are different membrane-controlled devices for insulin administration (Heller, 1993).

Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) is a temperature sensitive polymer, which shows a lower critical solution temperature (LCST) at about 32°C (Heskins et al., 1968). Poly(NIPAAm) hydrogel applications have been studied in order to develop the temperature sensitive drug delivery systems (Hoffman et al., 1986; Bae et al., 1991a,b). However, poly(NIPAAm) hydrogel releases the drug at temperatures below their LCST temperature, which limits their use in pharmaceutical applications. In order to prepare drug delivery systems which release the drug in physiological conditions, the swelling behaviour of poly(NIPAAm) hydrogel has to be modified by copolymerization with polymers with different hydrophilicity (Okano et al., 1990; Iwata et al., 1991; Yoshida et al., 1994; Chen and Hoffman, 1995) or by modifications in gel composition (Bae et al., 1991a,b; Park and Hoffman, 1994). Also added alcohols (Hirotsu, 1987), salts, and different lengths of the polymer chain (Schild and Tirrel, 1990) affect the swelling behaviour and LCST of poly(NIPAAm) in aqueous solutions.

Some applications for thermally controlled drug deliveries have been constructed by grafting poly(NIPAAm) on porous matrices (Wu et al., 1992; Chun and Kim, 1996). These membranes were more permeable to model drugs above the LCST of poly(NIPAAm) than below that temperature. In these systems the polymer is shrinking to the walls of the pores at physiological temperatures above the LCST of poly(NIPAAm), which allows drug permeation through the membrane. Dinarvand and D'Emanuele (1995) described a thermoresponsive valve-based devices, where drug release is controlled by temperature and polymer volume. Chun and Kim (1996) constructed a composite membrane which allows permeation control also to the small molecules by linking poly(NIPAAm) to a gelatin matrix. Iwata et al. (1991) prepared the temperature sensitive membrane by grafting poly(NIPAAm) onto a porous polyvinylidene fluoride (PVDF) membrane and studied the filtration rate of water through the membrane. The filtration rate of water increased with increasing temperature above LCST of poly(NIPAAm).

In the present study, temperature sensitive membranes were prepared by graft polymerizing *N*-isopropylacrylamide onto inert porous membranes utilizing the electron beam technique. The aim of the study was to investigate the effects of temperature, degree of grafting and ionic strength on the permeation of different size of molecules.

2. Materials and methods

2.1. Materials

Caffeine and FITC-dextrans (MW 4400, 9300, 19 600 and 50 600) were obtained from Sigma Chemical Co. (St Louis, MO). Mannitol was from Merck (Darmstadt, Germany) and [^{3}H]mannitol (22.4 Ci/mmol) from Dupont (NEN Products, Boston MA). Disodium hydrogen phosphate dihydrate and sodium dihydrogen phosphate dihydrate for buffer were from Merck (Darmstadt, Germany).

2.2. Preparation of the PVDF–NIPAAm membranes

Hydrophobic PVDF membranes (Millipore) with pore sizes 0.45 μm were used as received. Ion-exchanged water was used throughout the study. NIPAAm was prepared from isopropyl amine and vinyl chloride according to a procedure described by Svarfvar et al. (1996).

Preirradiation grafting was accomplished by first irradiating the PVDF membranes under nitrogen atmosphere (< 220 ppm O_2) using an Electrocurtain electron accelerator (Energy Sciences

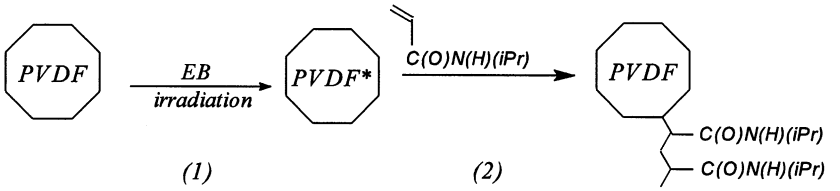

Fig. 1. The reaction mechanism when grafting *N*-isopropylacrylamide onto poly(vinylidene fluoride) membranes. In stage (1), the PVDF membrane is irradiated with electrons, and in stage (2) the irradiated membrane is reacted with *N*-isopropylacrylamide.

Inc.) operating at an acceleration voltage of 175 kV. The membranes were irradiated with 100 kGy. Immediately after irradiation the membranes were immersed at ambient temperature in a graft solution containing 20% *N*-isopropylacrylamide (NIPAAm). This solution was continuously purged with nitrogen in order to remove oxygen. The reaction mechanism is depicted in Fig. 1. After grafting the membranes were Soxhlet extracted with water to remove a remaining monomer and dried in vacuo at 40°C overnight. The degree of grafting was determined gravimetrically according to:

$$G = \frac{m_1 - m_0}{m_0} 100 \text{ wt\%}$$

where m_0 represents the mass of the original PVDF membrane and m_1 represents the mass of the grafted, extracted and dried PVDF membrane. The thickness of the grafted membrane varied between 121 and 137 μm depending on the degree of grafting.

2.3. Convective permeability of the grafted membranes

Convective permeability of the grafted membranes was studied by introducing a volume of flow through the membrane and measuring the corresponding pressure difference across the membrane. A more detailed description of the method is given in our earlier paper (Hautojärvi et al., 1996). The flow was produced by an Ismatech peristaltic pump and the pressure difference was measured with a SenSym SCX05DNC pressure sensor. Measurements were carried out using two different permeating solutions: distilled water and 0.5 M KCl. During the measurement the cell and the permeating solution were kept in a termosted water bath allowing the exact control of the temperature. Measurements were carried out at the temperature range 20–40°C. For each value of permeability, at least four different values of the flow were used in order to check the linearity between flow and the pressure difference. The convective permeability of the membrane (P) was obtained from the slope of pressure difference versus volume flow

$$\Delta p = \frac{1}{P}\frac{l}{A} V_a$$

where Δp is the pressure different across the membrane, l is the geometric thickness of the membrane, A is the area of the flow and V_a is the volume flow across the membrane.

2.4. Determination of drug flux through the membranes

Studies were performed in glass side-by-side diffusion cells (3.25 ml/compartment) (Crown Glass Co. Inc., Somerville, NJ). The area of the membrane exposed was 0.64 cm^2 and 50 μM FITC-dextran solutions were typically used as donor solutions, but the effects of molecular weight on drug permeation through the membrane were studied with 5 μM FITC-dextran solutions. Concentrations of caffeine and mannitol solutions were 0.5 mM. The 0.5 mM mannitol solution contained radio labelled mannitol 160 μCi/mmol. Drugs were dissolved in 6 mM phosphate buffer solution at pH 7.0 with the ionic strength of 0.2 excluding the studies dealing with effects of ionic strength. Ionic strength was adjusted with 1 M NaCl. A blank buffer was placed in receiver compartments of the diffusion cells.

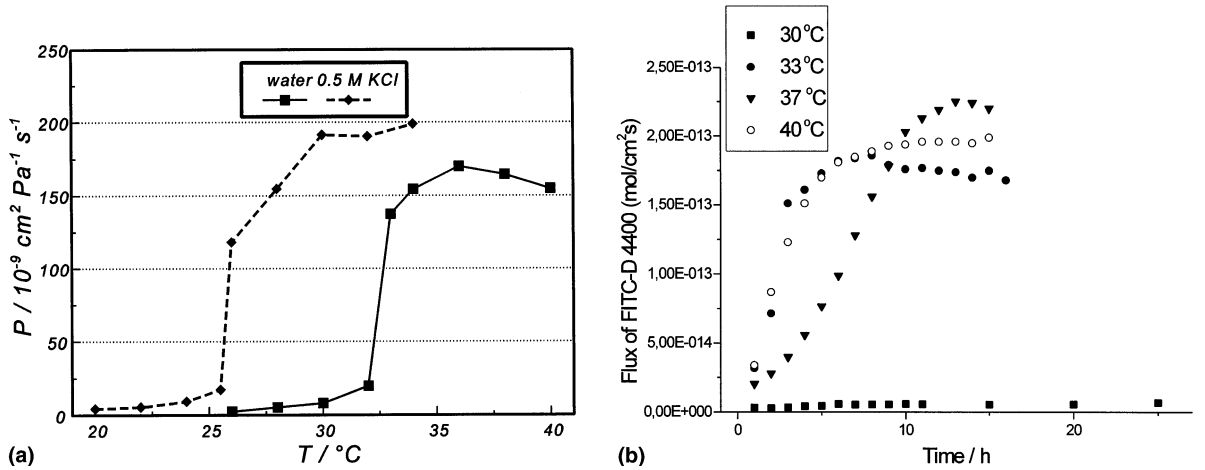

Fig. 2. (A) The convective permeability of the 7 wt% grafted poly(NIPAAm)–PVDF membrane. (B) Effect of temperature on the flux of the FITC-dextran (MW 4400) across the 28 wt% grafted poly(NIPAAm)–PVDF membrane.

Both donor and receiver solution were constantly circulating at a flow rate of about 1 ml/h (Ismatech, MCP V5.10, Switzerland).

Fractions (1 ml) were collected from the receiver compartment. Caffeine concentrations were measured spectrophotometrically (Hitachi 220, Tokyo, Japan) at wavelength 272 nm. FITC-dextran concentrations were determined fluorometrically (Luminescence Spectrophotometer LS50B, Perkin Elmer Ltd., Buckinghamshire), excitation at 495 nm and emission at 515 nm. Mannitol concentrations were determined using liquid scintillation counting (Rackbeta 1218 liquid scintillation counter, LKB Wallac, Turku). Then, 4.5 ml of aqueous counting scintillant (Ultima Gold) was added to the samples of 500 μl. Counting was continued for 360 s.

When the stationary state has been reached the diffusional flux (J) of the permeating drug can be obtained using the equation:

$$J = \frac{V_a c_a}{A}$$

where V_a is the flow rate of the buffer in a receiver compartment, c_a is the steady state concentration of the permeant in a receiver compartment and A is the area of the membrane exposed.

3. Results and discussion

3.1. Thermally controlled behaviour of membranes

The convective permeability of the 7 wt% grafted poly(NIPAAm)–PVDF membrane is presented in Fig. 2A. In the case of the distilled water, the permeability increases strongly at the temperature 32–34°C and stays then almost constantly at the range 35–40°C. When salt is added into the permeate, the increase of the permeability happens at lower temperature—around 25–26°C, otherwise the curves have similar shapes. The same effect was observed in drug fluxes, when the

Table 1
The effect of ionic strength on the fluxes of the FITC-dextran (MW 9300) at various temperatures

Temperature (°C)	Flux (J) ($\times 10^{-13}$ mol/cm^2 s) Ionic strength	
	0.1	1.0
25	7.8 ± 1.7	2.8 ± 3.5
30	5.0 ± 1.8	127.1 ± 11.2
37	160.0 ± 59.4	178.1 ± 16.9

Mean (± S.D.) values are shown ($n = 3$).

Table 2
Fluxes of the molecules with different molecular weights through the 28 wt% grafted poly(NIPAm)–PVDF membrane below and above the LCST of the poly(NIPAAm)

Molecular weight of the permeant	Flux (J) ($\times 10^{-14}$ mol/cm^2 s)	
	25°C	37°C
Mannitol (MW 182)	102.0 ± 6.7	93.0 ± 7.0
FITC-D (MW 4400)	ND	1.49 ± 0.24
FITC-D (MW 9300)	ND	0.64 ± 0.04
FITC-D (MW 19 600)	NT	0.62 ± 0.28
FITC-D (MW 50 600)	NT	0.01 ± 0.00

Mean ($\pm$ S.D.) values are shown ($n = 3$).
ND, not detectable (limit of detection for flux is 10^{-18} mol/cm^2 s)
NT, not determined.

ionic strength of the buffer was increased (Table 1). When the ionic strength increased from 0.1 to 1.0 increased the flux of the FITC-dextran (MW 9300) about 25 times at 30°C.

The thermosensitivity of the PVDF–NIPAAm membranes and the importance of solute molecular size were also confirmed by measuring the diffusional flux of several compounds through the membrane at temperatures on both sides of the LCST of poly(NIPAAm). Changing the temperature from 25 to 37°C did not affect the flux of mannitol (MW 182) across the 28 wt% grafted membrane (Table 2), but the fluxes of FITC-dextrans (MW 4400 and 9300) across the 28 wt% and 36 wt% grafted membranes increased with increasing temperature (Fig. 2B, Table 2). The flux of FITC-dextran (MW 4400) was about 30 times greater at temperatures above the LCST of poly(NIPAAm) than below the LCST (30°C). At 30°C, no significant increase in flux was observed during 50 h, which indicates hindered flux of FITC-dextran through the membrane or considerably longer lag-time than above the LCST of poly(NIPAAm). At 25°C, the fluxes of FITC-dextrans were not detectable. The flux of FITC-dextran (MW 4400) was not responsive to a temperature change when temperature was increased from 33 to 40°C. At temperatures above the LCST of the poly(NIPAAm), no significant differences in the time for achieving the steady state as a function of temperature were observed (Fig. 2B).

The fluxes of FITC-dextrans are decreased with increasing molecular weight of permeant at 37°C. Flux of the FITC-dextran (MW 4400) through membrane at 37°C was 2.4-folds and 150-folds greater than that of FITC-dextrans (MW 19 600 and MW 50 600), respectively. This result is in good agreement with the earlier study which indicates that apparent diffusivities of FITC-dextrans through poly(NIPAAm) hydrogels decrease with the increase in solute size (Dong et al., 1994).

The reversibility of the thermal response of NIPAAm–PVDF membranes is illustrated in Fig. 3. The flux of FITC-dextran (MW 4400) across 28 wt% membranes was studied as a function of time, while the temperature was changed from 37 to 25°C and back. When the temperature is decreased from 37 to 25°C, the change in the flux of FITC-dextran was slower than the change in the flux, when the temperature was increased from 25 to 37°C. At constant temperatures the fluxes of permeant increased while steady state was achieved.

Our results from convective permeability of the membranes and drug permeation studies confirmed the temperature-sensitive behaviour of the PVDF–(NIPAAm) membrane as earlier reported by Iwata et al. (1991). The permeability depends on the conformation of the grafted NIPAAm chains: above the LCST, poly(NIPAAm) grafted onto porous PVDF membrane shrinks and thus, the pores in the membrane are left open allowing free diffusion of drugs through the pores. Below the LCST, poly(NIPAAm) is in the swollen state and hydrogel fills the pores of the membrane.

Studied PVDF–(NIPAAm) membranes were capable of temperature sensitive release of FITC-dextrans (MW 4400 and 9300), while the flux of much smaller mannitol molecule was equal on collapsed and swollen polymer states. Earlier Brazel and Peppas (1996) have reported that temperature and pH sensitive hydrogels of poly(*N*-isopropylacrylamide-co-methacrylic acid) are able to control the release of streptokinase, while release of a smaller molecule as heparin cannot be controlled. Poly(NIPAAm) containing membranes may, however, control also the release of small molecules, since the flux of 4-acetaminophen (MW 151) through composite membrane of poly(NIPAAm) in the gelatin was increased substantially by increasing temperature from 25 to 40°C (Chun and Kim, 1996).

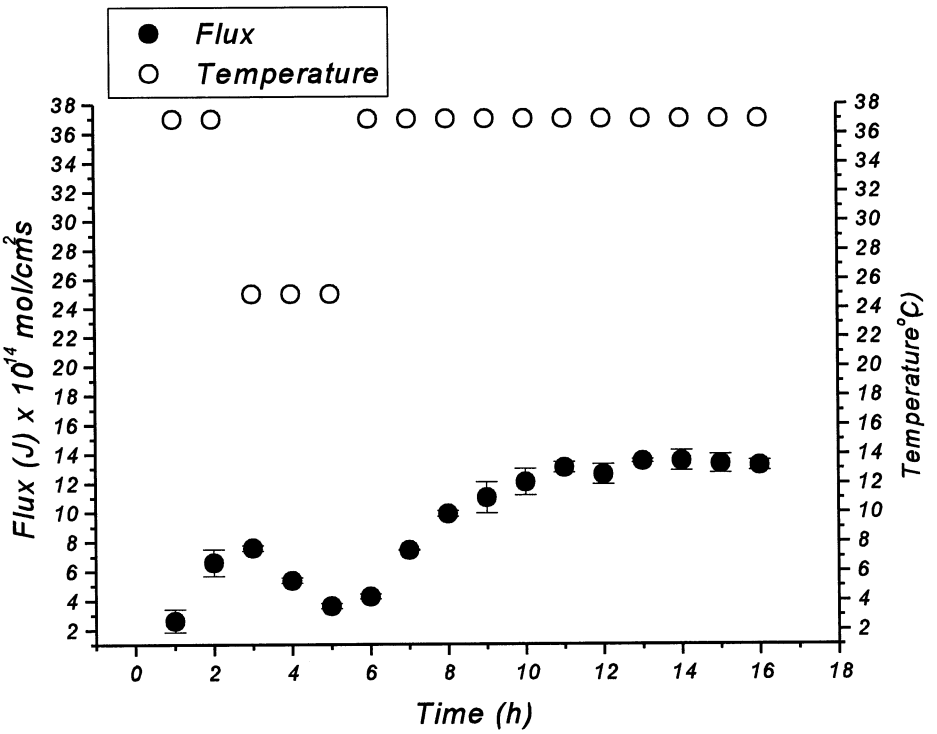

Fig. 3. The flux of the FITC-dextran (MW 4400) across the 28 wt% grafted membrane upon temperature change from 37 to 25°C and back to 37°C in PBS pH 7.0 with ionic strength 0.2. The bars represent the standard deviation ($n = 3$).

3.2. Effect of degree of grafting

As seen in Table 3, it is possible to affect the flux of FITC-dextran (MW 4400) across the membrane by changing the degree of membrane grafting. The flux of the FITC-dextran (MW 4400) decreased ca. two orders of magnitude when degree of grafting increased from 6 to 36 wt%. When degree of grafting was increased to 88 wt%, the penetration of FITC-dextran (MW 4400) was not detectable at 25 or 37°C. Okahata et al. (1986) reported that even low molecular weight molecules cannot pass through the PVDF membrane, if the degree of grafting is high. In present study, however, flux of the mannitol did not decrease with increasing degree of grafting below the LCST of poly(NIPAAm) and flux of mannitol

Table 3
Effect of the degree of grafting on the flux of the FITC-dextran (MW 4400) in PBS pH 7.0 with ionic strength 0.2 at 37°C

Degree of grafting (wt%)	Flux (J) ($\times 10^{-14}$ mol/cm^2 s)
6	1073 ± 481
15	114.9 ± 13.9
28	17.7 ± 0.7
36	15.2 ± 11.3
88	Not detectable[a]

Mean ($\pm$ S.D.) is shown ($n = 3$).
[a] Detection limit for flux is 10^{-18} mol/cm^2 s.

Table 4
Effect of the degree of grafting on the flux of the mannitol at 25 and 37°C in PBS pH 7.0 with ionic strength 0.2

Degree of grafting (wt%)	Flux (J) ($\times 10^{-14}$ mol/cm^2 s)	
	25°C	37°C
28	102.0 ± 6.7	93.0 ± 7.0
36	86.9 ± 30.3	95.4 ± 21.4
88	136.1 ± 22.6	54.4 ± 9.8

Mean ($\pm$ S.D.) values are shown ($n = 3$).

across the 88 wt% grafted membrane was surprisingly faster at 25 C° than at 37 C° (Table 4). Also the flux of another small molecule, caffeine, across the 88 wt% grafted membrane was faster at 25°C than at 37°C (data not shown).

The fluxes of mannitol and caffeine across the 88 wt% grafted membrane were faster in the swollen than in collapsed polymer state. This result suggests, that at high degrees of grafting poly(NIPAAm) is in the swollen state and chains do not shrink on the pore walls, but form the hydrogel on the PVDF. The density of the hydrogel is lower at temperatures below the LCST of the poly(NIPAA)m than at temperatures above the LCST of poly(NIPAA)m. That allows smaller molecules to diffuse through the water-filled pores of a swollen polymer. For bigger molecules as FITC-dextrans the permeation through the pore of the membrane requires an open pore and they are not able to pass through the membrane, when swollen hydrogel formed at higher grafting rates blocks the pores.

4. Conclusions

Temperature sensitive (NIPAAm) grafted PVDF membrane was prepared by graft polymerization. Degree of grafting and molecular weight of the permeant have an important role in controlling the flux through the membrane. Our results suggest that such membrane is useful in controlling the release of large molecules such as oligonucleotides, peptides and proteins.

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References

Bae, Y.H., Okano, T., Kim, S.W., 1991a. 'On-Off' thermocontrol of solute transport. II. Solute release from thermosensitive hydrogels. Pharm. Res. 8 (5), 624–628.

Bae, Y.H., Okano, T., Kim, S.W., 1991b. Thermocontrol of solute transport. I. Temperature dependence of swelling of *N*-isopropylacrylamide networks modified with hydrophobic components in water. Pharm. Res. 8 (5), 531–537.

Bodmeier, R., Guo, X., Sarabia, R.E., Skultety, P.F., 1996. The influence of buffer species and strength on diltiazem HCl release from beads coated with the aqueous cationic polymer dispersions, Eugradit RS, RL 30D. Pharm. Res. 13, 52–56.

Brazel, C.S., Peppas, N.A., 1996. Pulsatile local delivery of thrombolytic and antithrombotic agents using poly(*N*-isopropylacrylamide-co-methacrylic acid) hydrogels. J. Control. Release 39, 57–64.

Brondsted, H., Kopecek, J., 1992. Hydrogels for site-specific drug delivery to the colon: in vitro and in vivo degradation. Pharm. Res. 9, 1540–1545.

Chen, G., Hoffman, A.S., 1995. Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH. Nature 373, 49–52.

Chun, S.-W., Kim, J.-D., 1996. A novel hydrogel-dispersed composite membrane of poly(*N*-isopropylacrylamide) in a gelatin matrix and its thermally actuated permeation of 4-acetaminophen. J. Control. Release 38, 39–47.

Dinarvand, R., D'Emanuele, A., 1995. The use of thermoresponsive hydrogels for on-off release of molecules. J. Control. Release 36, 221–227.

Dong, L.C., Hoffman, A.S., Yan, Q., 1994. Dextran permeation through poly(*N*-isopropylacrylamide) hydrogels. J. Biomater. Sci. Polymer Ed. 5, 473–484.

Hautojärvi, J., Kontturi, K., Näsman, J., Svarfvar, B., Viinikka, P., Vuoristo, M., 1996. Characterization of graft modified porous polymer membranes. Ind. Eng. Chem. Res. 35, 450–457.

Heller, J., 1993. Modulated release from drug delivery devices. Crit. Rev. Ther. Drug. Carrier Syst. 10 (3), 253–305.

Heskins, M., Guillet, J.E., 1968. Solution properties of poly(NIPAAm). J. Macromol. Sci.-Chem. A2 (8), 1441–1455.

Hirotsu, S., 1987. Phase transition of a polymer gel in pure and mixed solvent media. J. Phys. Soc. Jpn. 1, 233–242.

Hoffman, A.S., Afrassiabi, A., Dong, L.C., 1986. Thermally reversible hydrogels. II. Delivery and selective removal of substances from aqueous solutions. J. Control. Release 4, 213–222.

Iwata, H., Oodate, M., Uyama, Y., Amemiya, H., Ikada, Y., 1991. Preparation of temperature-sensitive membranes by graft polymerzation onto a porous membrane. J. Membr. Sci. 55, 119–130.

Okahata, Y., Noguchi, H., Seki, T., 1986. Thermoselective permeation from a polymer-grafted capsule membrane. Macromolecules 19, 493–494.

Okano, T., Bae, Y.H., Jacobs, H., Kim, S.W., 1990. Thermally on-off switching polymers for drug permeation and release. J. Control. Release 11, 255–265.

Park, T.G., Hoffman, A.S., 1994. Estimation of temperature-dependent pore size in poly(*N*-isopropylacrylamide) hydrogel beads. Biotechnol. Prog. 10 (1), 82–86.

Schild, H.G., Tirrel, D.A., 1990. Microcalorimetric detection of lower critical solution temperatures in aqueous polymer solutions. J. Phys. Chem. 94, 4352–4356.

Svarfvar, B., Ekman, K., Sundell, M., Näsman, J.H., 1996. Electron-Beam graft modified membranes with externally controlled flux. Polym. Adv. Technol. 7, 839–846.

Wu, X.S., Hoffman, A.S., Yager, P., 1992. Synthesis and characterization of thermally reversible macroporous poly(*N*-isopropylacrylamide) hydrogels. J. Polym. Sci. 30, 2121–2129.

Xie, H.-Q., Liu, Y., 1991. Acrylic acid polymers with uniform polystyrene side-chains and their intermolecular complexes used as a chemical valve. Eur. Polym. J. 27 (12), 1339–1343.

Yoshida, R., Sakai, K., Okano, T., Sakurai, Y., 1994. Modulating the phase transition temperature and thermosensitivity in *N*-isopropylacrylamide copolymer gels. J. Biomater. Sci. Polym. Ed. 6 (6), 585–598.

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