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Molecular separation by thermosensitive hydrogel membranes

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Abstract

A new method for separation of molecules of different size is presented. The method is a useful addition to conventional separation methods which depend mainly on gel permeation chromatography using size exclusion. In the new method, hydrogel membranes are used which swelling level can be thermally controlled. In this study, a crosslinked poly(N-isopropylacrylamide-co-butylmethacrylate 95:5mol%) membrane is used and three solutes of distinct molecular size: two dextrans with molecular weights of 150,000 and 4,400 g/mol respectively and uranine with a molecular weight of 376 g/mol. The swelling of the membranes as function of temperature was measured as well as the influence of the swelling level on the permeability of the three solutes. The influence of the swelling level and the solute size on the permeability was as expected from the free-volume theory. Based on these permeability phenomena, separation was performed in a continuous way by varying the membrane swelling at the appropriate time. A linear relationship between inverse membrane hydration and solute diffusion was found for uranine and dextran (MW=4,400), indicating the validity of the free-volume theory.

Keywords: hydrogels; diffusion; solute separation; thermosensitive membrane; poly(N-isopropylacrylamide-co-butylmethacrylate 95:5mol%) membrane

Introduction

Hydrogels are insoluble hydrophilic polymer networks which show significant swelling in water [1]. The swelling forces in the network, caused by the interaction between water and the hydrophilic polymer chains, are counterbalanced by the elastic forces of the chains. The degree of swelling can be influenced by changing the crosslink density, the chemical nature of the monomers, or by the incorporation of comonomers. Recently, hydrogels have been developed, the swelling of which can be affected by specific external stimuli such as pH [2,3], ionic strength [4], certain chemicals [5], photo-irradiation [6], electric field [7] and temperature [8–11].

Hydrogels with a strong temperature dependent swelling are obtained by crosslinking of polymers which have a lower critical solution temperature ($LCST$) in aqueous solutions, such as poly-N-isopropylacrylamide [p(NIPAAm)] [10,12]. The polymer–polymer and polymer–water interactions in these gels are signifi-

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cantly influenced by temperature. One way to influence the swelling characteristics of these hydrogels is by using an appropriate hydrophilic/hydrophobic balance in the hydrogel. By polymerizing NIPAAm and butylmethacrylate (BMA), a hydrophobic monomer, in a ratio of 95:5 mol%, combined with a crosslinking agent, a hydrogel can be obtained which shows a linear swelling change with temperature [11]. This feature allows an accurate control of swelling of the gel by temperature.

Thermosensitive hydrogels based on p(NIPAAm) have been used for the temperature controlled delivery of bioactive compounds [13], the regulation of the activity of embedded enzymes [10,13,14], and as extraction solvents for proteins [9,15].

While macromolecules can be extracted from solutions using these gels, conventional methods, mainly gel permeation chromatography, are needed for the separation of molecules of different size from a mixture.

In this study, we present a new way to perform separation of solutes of different size, making use of the possibility of accurate control of the swelling of thermosensitive gels. With this technique, separation is performed in a two compartment diffusion cell using a hydrogel membrane made of crosslinked p(NIPAAm–co-BMA), with temperature as the only variable required to perform separation.

From the free-volume theory of diffusion in swollen hydrogels [16–20] it can be expected that the permeation of solutes through the hydrogel membrane is dependent on the swelling level of the membrane and on the solute's molecular size. According to this theory

$$\ln\left(\frac{D_m}{D_0}\right)=\phi_2\left(\frac{-Bq_2}{V_f}\right)\left(\frac{1}{H}-1\right) \qquad (1)$$

where D_m and D_0 are the diffusion coefficients of the solute in the membrane and in water, respectively, q_2 is the cross-sectional area of the solute, V_f is the free volume in the hydrogel, H is the volume fraction of water in the gel, B is a constant, and ϕ_2 is the screening effect of the polymer network which equals unity for a mesh size significantly larger than the solute's diameter [1,21–23]. This equation implies that the separation of the mixture of molecules having a distinct difference in molecular size may be achieved by varying the degree of swelling of the membrane.

In this study, two fluorescein isothiocyanate(FITC)-labelled dextrans with molecular weights of 150,000 and 4,400 g/mol respectively (dextran 150K and dextran 4,4K) and uranine with a molecular weight of 376 g/mol were chosen to perform separation experiments because they can easily be quantified in a mixture. Separation of these solutes is performed based on predictions of the free-volume theory concerning the influence of the swelling level and the solute size on the permeability.

The screening effect of the polymer network was evaluated as well as the relationship between $1/H$ and $\ln(D_m/D_0)$ to evaluate if the diffusion of the solutes in the membrane followed free-volume theory. The screening effect was evaluated by comparing the diameters of the solutes with the mesh size of the network. The mesh size at different temperatures was calculated from swelling data and from crosslink density data as obtained from compression experiments. To evaluate the relationship between $1/H$ and $\ln(D_m/D_0)$, solute partitioning coefficients were measured as a function of H to obtain D_m as a function of H ($D_m=P/K_d$, where P is the permeability of the membrane and K_d is the solute's partitioning coefficient). Since the swelling level of the membranes used is inversely proportional to temperature between 15°C and 25°C, $1/H$ could be modified by temperature.

Experimental

Membrane synthesis

The copolymerization of NIPAAm (4g, Kodak, recrystallized in hexane) and BMA (0.266 g, Polysciences Inc., distilled at 57°C/17 mmHg) was carried out in dioxane (4.27 ml) at 80°C for 12 hr, with EGDMA (ethyleneglycoldimethacrylate, 0.074 ml (1 mol%), Polysciences Inc., deinhibited with inhibitor-remover, Aldrich) as a crosslinker and BPO (tert-butylperoxyoctanoate, 0.012 ml) as an initiator. Dried nitrogen was bubbled through the solution for 10 min prior to polymerization. The solution was poured between two glass plates which were separated by a rubber gasket (diameter 2 mm). After polymerization for 16 hr, the glass plates were immersed in water for one day and separated in an ultrasound bath. The membranes were soaked in 100/0 v/v%, 75/25 v/v%, 50/50 v/v%, and 25/75 v/v% methanol/water mixtures for one day each and in deionized water for 4 days to remove unreacted compounds. The resulting membranes had a thickness of 1–2.5 mm, depending on the temperature.

Swelling

Three samples of the hydrogel were dried for 3 days at room temperature and then dried under vacuum at 60°C for 2 days. The volume of the dried samples (V_p) was determined by measuring the decrease in weight after immersion in water. The equilibrium swelling of the samples in water, defined as the ratio of the gel volume to the polymer volume fraction in the swollen gel [$S=(V_p+V_w)/V_p$], was determined by the weight of the swollen samples, assuming volume additivity of water and polymer in the swollen hydrogel. The weight of the swollen samples was determined after less than 0.3% change in weight over time was detected. Before measuring the weight, excess surface water was blotted with lint-free tissue paper.

Permeability

The permeability of the membrane for each of the three solutes (uranine $MW=376$, Aldrich, FITC-dextran $MW=4400$ and FITC-dextran $MW=150{,}000$, both from Sigma) was measured as function of temperature using a 2×10 ml two chamber diffusion cell. The initial donor concentration of the solutes was 1 mg/ml and the effective surface area for diffusion was 4.9 cm^2. Donor and receiver chambers were stirred (150 rpm, diameter propellor 11 mm). The permeability (P) was calculated from the rate of diffusion at steady-state [24]: $\ln (\Delta c)_{t_2}/(\Delta c)_{t_1}=(2AP\Delta t)/(Vh)$, where A is the membrane surface area, V is the chamber volume, h is the membrane thickness, and $(\Delta c)_{t_x}$ is the concentration difference between donor and receiver at time t_x. The concentration of the solutes in the receiver and donor cells was measured with a UV/Vis-spectrophotometer (Perkin–Elmer, Lambda 7, λ_{max}: 490nm).

Separation

The concentration of the three solutes in the donor cell at the beginning of separation experiments was 1 mg/ml for each solute. The receiver cell fluid was replaced by fresh water twice a day to maintain sink conditions. The concentrations of the three solutes were measured in all the replaced receiver solutions and each day in the donor cell as function of time using HPLC (Waters 250Å ultrahydrogel column, Shimadru RF-535 fluorescent detector, Em=515 nm, Ex=490 nm, mobile phase: water) and a UV/Vis-spectrophotoscopy. With the mobile phase and wavelengths employed, the HPLC showed two separate peaks for the dextrans and no peak for uranine. After calibration curves were made, the dextrans could be quantified in a mixture of solutes. At 490 nm, the absorption of uranine, measured with the UV/Vis-spectrophotometer, was more than hundred times stronger than of the dextrans. By combining absorption data at 490 nm with

the HPLC results for the dextrans, uranine could thus be quantified.

The results of the permeability experiments were used for the separation experiments. First a temperature of 25°C was selected (low swelling) to make permeation of uranine possible with only trace permeation of the dextrans. After uranine depletion a temperature of 20°C was selected to make permeation of dextran 4,4K possible with maintaining only trace permeation of dextran 150K.

Compression modules

Discs (2.7 mm^2 in area and 1.81 mm in thickness) were cut from the membranes and equilibrated in water at 24°C. A depth gauge (B.C. Ames Co. Nathan, MA, bench comparator), as described by Cluff et al. [25], was used to measure the unidirectional compression modules. Teflon sheets were placed between the plates and the samples to minimize friction and thus minimize nonuniform deformation. The uniform deformation under different weights was determined by measuring the deformation under a certain weight after no change of the deformation over time was detected. The samples were immersed in water (24°C) for 10 min between measuring deformation under different weights to recover its undeformed dimensions. The deformation was kept below 10% of the initial dimensions.

Partition coefficient (K_d)

The partition coefficient, defined as the ratio of the solute concentration in the membrane to that in the outer solution, was determined for both uranine and dextran 4,4K at different temperatures at a solute concentration of 1 mg/ml. For each solute and temperature, three discs (volume between 0.1 and 0.3 cm^3, volume determined by weight at 23°C), previously equilibrated in water at the desired temperature, were immersed in 20 ml solute solution of the same temperature. The solute solution was kept at this temperature in a waterbath. After 3, 5 and 7 days one disc was taken out of the solution, blotted with tissue paper, and placed in 10 ml fresh water. Each following day the disc was removed from the water, blotted with tissue paper, and placed in 10 ml fresh water, until no measurable amount of solute was released from the discs. The same was done for all of the three discs. The concentration of solute released in water was determined photospectroscopically.

The concentration of the solute in the discs after immersion in the solute solution (for respectively 3, 5 and 7 days) was calculated by measuring the total released amount of solute from the discs and the volume of the discs (as determined by weight) at the experimental temperature.

Results

Swelling

The swelling of crosslinked p(NIPAAm-co-BMA) as a function of temperature is shown in Fig. 1. The gel shows a negative thermosensitivity; this is mainly due to hydrophobic interactions between hydrophobic groups in the polymer which induce entropy triggered dehydration as temperature increases [8]. Compared to the swelling of crosslinked p(NIPAAm), a lower temperature for deswelling and gel collapse is observed, and there ex-

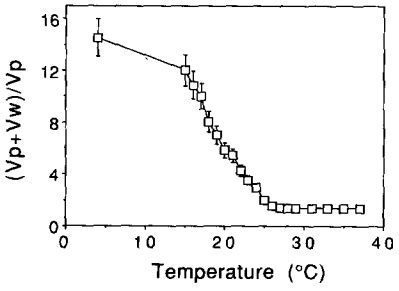

Fig. 1. Swelling of poly(NIPAAm-co-BMA, 95:5 mol%), crosslinked with 1 mol% EGDMA, as function of temperature. Errorbars represent standard deviation ($n=3$).

ists no sharp transition in the swelling as function of temperature [11]. Between 16°C and 25°C, the swelling of the p(NIPAAm–co-BMA) network decreases from $S=12$ to $S=2$ proportionally with increasing temperature. This property makes it possible to accurately control swelling with temperature in this region.

Permeability

The permeability of the membrane for the three solutes as function of temperature is shown in Fig. 2. As expected, the permeability of uranine is the highest at all temperatures, followed by the permeability of dextran 4,4K. The permeability of the solutes decreases with increasing temperature (decreased swelling). The highest temperature at which significant permeation is possible is 27°C for uranine and decreases for solutes of increasing size (23°C for dextran 4,4K and less than 20°C for dextran 150K). No permeation of solutes is observed above 28°C. Considering the swelling characteristics, permeation of small molecules is possible at low swelling, while an increased swelling level is required for the permeation of solutes of increasing molecular size, in accordance with free-volume theory. Based on these swelling and solute size dependent diffusion properties, separation was performed.

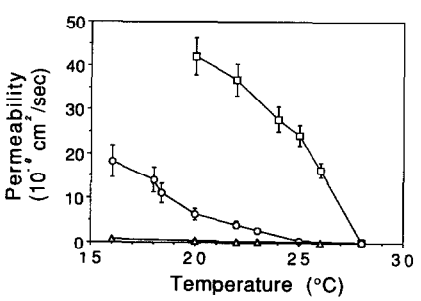

Fig. 2. Permeability of uranine (□), dextran 4.4K (○), and dextran 150K (△) through crosslinked poly(NIPAAm-co-BMA, 95:5 mol%) membrane. Errorbars represent standard deviation in slope of curve of receiver concentration of solute as function of time at steady-state.

Separation

The results of the separation experiments are shown in Fig. 3. During the first 100 hr the temperature was maintained at 25°C, following with 20°C for the remaining period. At 25°C, only permeation of uranine was significant. After 100 hr, most of the uranine (94.3%) had permeated, while 3.6% of dextran 4,4K and no measurable amount of dextran 150K had passed the membrane. After 100 hr, with the temperature lowered to 20°C, permeation of dextran

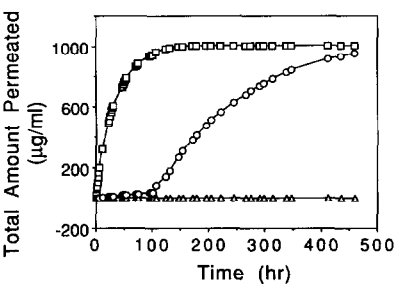

Fig. 3. Total amount of uranine (□), dextran 4.4K (○), and dextran 150K (△) permeated through crosslinked poly(NIPAAm-co-BMA, 95:5 mol%) membrane. Initial donor concentration: 1 mg/ml for each solute. Temperature: 0–100 hr: 25°C, 100–460 hr: 20°C.

TABLE 1

Composition of mixtures of solutes permeated through crosslinked p(NIPAAm-co-BMA) membrane and total amounts of each solute accumulated in receiver cell during different time intervals

Time interval (hr)	Composition of mixture accumulated in receiver cell (wt.%)	Percentage of initial donor amount permeated
0–100	96.3% uranine 3.7% dextran 4,4K	uranine: 94.3% dextran 4,4K: 3.6% dextran 150K: 0%
100–460	6.4% uranine 93.6% dextran 4,4K	uranine: 5.7% dextran 4,4K: 91.4% dextran 150K: 0%

Composition donor after 460 hr: 95% dextran 150K and 5% dextran 4,4K

4,4K became apparent, while dextran 150K still showed only trace permeation. In Table 1 the compositions of the mixtures of solutes permeated through the membrane during the different time intervals is given, together with the total amounts of each solute accumulated in the receiver cell during the same time periods. It was found that the diffusional behavior of the solutes during the separation experiments was as could be expected from the permeability experiments.

Discussion

Free-volume theory for solute diffusion

The free-volume theory predicts, based on eqn. (1), a linear relationship between $1/H$ and $\ln(D_m/D_0)$ in absence of a significant effect ($\phi_2=1$), and a rapid decrease of $\ln(D_m/D_0)$ with increasing $1/H$ as the mesh size approaches the diameter of the solute ($\phi_2<1$). To evaluate if the diffusion of the solutes in the membrane followed free-volume theory, the determination of both the screening effect and the relationship between $1/H$ and $\ln(D_m/D_0)$ is required.

Screening effect of network

Data from several authors [22,26–28] show that the extent of the screening effect is related to the ratio of the diameter of the solute and the mesh size. The mesh size (ξ) is a structural parameter of the network and is usually characterized by the average distance between crosslinks. The data from the authors showed that for several solutes and hydrogel networks, good agreement is found between experimental data and the free-volume theory if $\phi_2=1-r/\xi$ is used in eqn. (1) [1]. The parameter r characterizes the solute's size as the diameter of an equivalent sphere for the solute [1]. This equals the diameter for dextran since dextran is a random coil molecule [29,30] which is characterized by a spherical domain [29,31]. By comparing the diameter of the solutes with the mesh size of the hydrogel network, the screening effect can thus be evaluated.

In this analysis, the diameters of the solutes are calculated from their diffusion coefficients using the Einstein–Stokes equation in which the diffusion coefficients are obtained from data of the sedimentation coefficients and molar volumes. The mesh size is calculated from the molecular weight between crosslinks which is obtained from the compressibility modules.

It has been shown by several authors [8,25,32,33] that accurate data of the crosslinking density can be obtained by uniaxial compression experiments if friction between the sample and the plates of the compression device can be avoided. Compression experiments have the advantage that a simple apparatus can be used. The stress–strain relationship for the crosslinked p(NIPAAm-co-BMA) hydrogel obtained from the compression experiments is shown in Fig. 4. The effective crosslinking density in the dried state (ν_e^*) can be obtained from the stress–strain relationship using eqn. (2) [1,34]:

$$\tau=RT\left(\frac{\phi_{P,0}}{\phi_P}\right)^{2/3}\phi_P\nu_e^*\left((\alpha-\frac{1}{\alpha^2}\right) \qquad (2)$$

where τ is the applied stress, α is the linear deformation factor, and $\phi_{P,0}$ and ϕ_P are the polymer volume fraction in the relaxed state (as

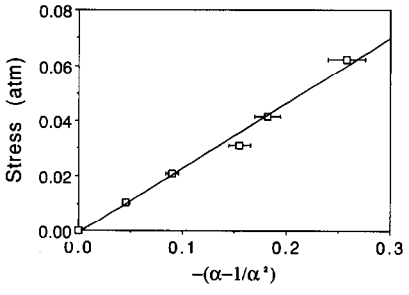

Fig. 4. Stress–strain relationship in the compression of swollen poly(NIPAAm-co-BMA, 95:5 mol%) crosslinked with 1 mol% EGDMA.

crosslinked polymer after polymerization) and in the swollen gel state respectively. The crosslink density thus obtained was 2.86×10^{-5} mol/cm^3, which is comparable to values found by other authors for similar networks [8,35]. The crosslink density can be used to calculate the average molecular weight between crosslinks ($\bar{M}_c$) [1,36]:

$$\bar{M}_c=\frac{1}{\nu_e^*\bar{v}} \tag{3}$$

where $\bar{v}$ is the specific volume of dried polymer. The mesh size can be calculated from the average distance between crosslinks [1,37,38]:

$$\xi=\gamma(c_n\cdot nl^2)^{1/2} \tag{4}$$

where n is the average number of C–C bonds between crosslinks ($=2\bar{M}_c/M_r$, M_r is the molecular weight of the repeat unit), l is the C–C bond length, c_n is Flory's rigidity factor of the polymer chain ($c_n=\bar{r}_0/nl^2$ where $\bar{r}_0$ is the average end-to-end distance of the polymer chain under unperturbed conditions), and γ is the linear expansion factor of the network due to swelling which equals $S^{1/3}$. The rigidity factor of the p(NIPAAm–co-BMA) chain is not known, but can be estimated from rigidity factors of similar chains and from end-to-end distance data of linear p(NIPAAm). Heskins and Guillet [12] measured an average radius of gyration ($\bar{r}_g$) of 400Å for linear p(NIPAAM) ($\bar{M}_n=1.3\times10^6$) in water at 33°C (theta temperature). Since $\bar{r}_0=\bar{r}_g\sqrt{6}$ and $nl^2=2(\bar{M}_c/M_r)l^2$, c_n of p(NIPAAm) at 33°C is calculated to be 18.5. The rigidity factors in water of similar polymer chains are: c_n of p(acrylamide) = 14.8, c_n of p(dimethylacrylamide) = 9.6 [39]. Using a rigidity factor of 18.5 for the p(NIPAAm–co-BMA) chain, the mesh size of the p(NIPAAm–co-BMA) network can be calculated as function of temperature using eqn. (4). In Table 2 the values of the mesh size of the p(NIPAAm–co-BMA) network is given at 20°, 25°, and 28°C.

TABLE 2

Estimated mesh size of p(NIPAAm–co-BMA 95:5 mol%)), crosslinked with 1 mol% EGDMA, at 20°, 25°, and 28°C

Temperature (°C)	Mesh size (Å)
28	168
25	195
20	279

The hydrodynamic diameters of the solutes can be calculated from the Einstein–Stokes equation:

$$D_0=\frac{kT}{3\pi\eta d} \tag{5}$$

where k is the Boltzmann constant, η is the viscosity of the solvent, and d and D_0 are the hydrodynamic diameter and the diffusion coefficient of the solute respectively.

D_0 of the dextrans can be obtained from their sedimentation coefficient [31]:

$$M=\frac{N_A kT}{1-\rho/\rho_2}\times\frac{s}{D_0} \tag{6}$$

where M is the molecular weight, s is the sedimentation coefficient (s from Ref. [40]), and D is the diffusion coefficient of the solute, and ρ and ρ_2 are the densities of the solvent and the solute respectively (ρ_2 from Ref. [29]).

The diffusion coefficient of the solutes can also be estimated from Wilke and Chang's [41] semi-empirical equation:

$$\frac{D_0\eta}{T}=7.4\times10^{-8}\frac{\sqrt{XM}}{V_b^{0.6}} \tag{7}$$

where X is an association parameter ($=2.6$ for water), η is the viscosity of the solvent, M is the molecular weight of the solvent, and V_b is the molar volume of the solute (V_b of solutes from Refs. [29,42]).

An estimation of the hydrodynamic diameter of the dextrans can also be obtained from

TABLE 3

Diffusion coefficients and molecular dimensions of uranine, dextran 4,4K and dextran 150K

Solute	Diffusion coefficient D_0 from eqn. (6) (cm^2/sec)	Diffusion coefficient D_0 from eqn. (7) (cm^2/sec)	Hydrodynamic diameter (from Einstein-Stokes) (Å)	End-to-end distance from eqn. (8) (Å)
Uranine		5.4×10^{-6}	10.3	
Dextran 4,4K	1.1×10^{-6}	1.3×10^{-6}	49	47
Dextran 150K	0.19×10^{-6}	0.16×10^{-6}	247	275

the relationship between the end-to-end distance and the molecular weight of dextrans. For dextran it is found that

$$\left(\frac{\bar{r}_0^2}{M_n}\right)^{1/2} = 0.71\,\text{Å} \qquad (8)$$

in water at 25°C [29]. The dimensions and diffusion coefficients of the solutes thus obtained are given in Table 3.

A comparison of the data of Table 2 and Table 3 shows that the hydrodynamic diameter of both uranine and dextran 4,4K are significantly lower than the mesh size at all temperatures. From these results it can that be concluded that no major screening effect is expected for uranine and dextran 4,4K and that the minor screening of dextran 4,4K will be approximately constant in the temperature range of the experiments. For dextran 150K, however, a strong screening effect can be expected, which may contribute to the extremely low permeability of this solute.

Relationship between 1/H and ln(D_m/D_0)

To obtain $\ln(D_m/D_0)$ as function of $1/H$, both P and K_d have to be determined as function of H ($D_m = PK_d$). Since H is determined by temperature, as shown in Fig. 1, P and K_d as function of H can be obtained from P as function of temperature (Fig. 2) and K_d as function of temperature.

The partition coefficient of uranine and dextran 4,4K as function of temperature is given in Fig. 5. The partition coefficient of dextran is roughly proportional to membrane hydration. This is in accord with Yasuda's analysis which assumes $K_d = \alpha H$ [17].

The partition coefficient of uranine in the membrane is equal to the membrane hydration at high hydration (low temperature), but increases significantly with decreasing hydration. This is most likely due to hydrophobic interactions between the gel matrix and the solute since the gel becomes a more hydrophobic environment with increasing temperature. This interaction is possible since uranine possesses, in addition to hydrophilic properties due to its charge, also hydrophobic properties [43]. Hydrophobic interactions are generally responsible for the affinity between dye molecules and the hydrophobic moieties in hydrogels [44]. The sudden drop in partitioning at 28°C may be due to the collapsed state of the gel at this

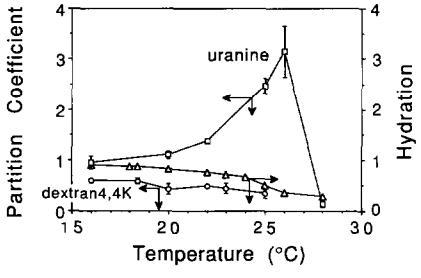

Fig. 5. Equilibrium membrane hydration (△) and partition coefficients of uranine (□) and dextran 4,4K (○) in crosslinked poly(NIPAAm-co-BMA, 95:5 mol%) at a solute concentration of 1 mg/ml as function of temperature.

temperature. In the collapsed state strong polymer–polymer interactions are present which probably lead to the breaking of the dye–polymer interactions. This causes uranine to be only present in the (small) water fraction of the gel.

The relationship between $\ln(D_m/D_0)$ and $1/H$-1 for uranine and dextran 4,4K, as obtained from the permeability and partitioning experiments, is shown in Fig. 6. Since the permeability of dextran 150K was too low to give meaningful data for $\ln(D_m/D_0)$, only the two smaller solutes are shown. This plot shows a linear relationship between $\ln(D_m/D_0)$ and $1/H$-1 for both solutes, indicating that diffusion of these solutes in the membrane follows free-volume theory. The lower negative slope for uranine compared to dextran is caused by uranine's smaller size as predicted by the free-volume theory [16]. In the free-volume theory analysis, the value of $\ln(D_m/D_0)$ at the intercept should equal zero ($H=1$; $D_m=D_0$). Slightly negative intercept values, as observed for dextran, are possibly caused by the polymer free-volume [45] which has been considered negligable for solute diffusion in the Yasuda analysis. Negative intercept values might also be caused by a decrease in specific free-volume of water in the gel compared to free water due to polymer–water interactions. For uranine, the additional deviation from a zero intercept value

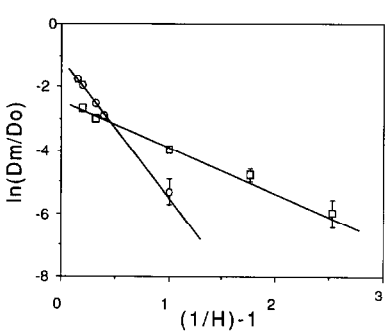

Fig. 6. Logarithm of normalized diffusion coefficients of uranine (□) and dextran 4,4K (○) in crosslinked poly(NIPAAm-co-BMA, 95:5 mol%) membrane as function of inverse membrane hydration.

(intercept value about −2.5, compared to only −1 for the dextran) is possibly due to a value of D_0 of uranine lower than calculated from eqn. (7). This causes the curve of uranine to be shifted downwards. A lower value of D_0 of uranine than expected might be caused by its charge which causes strong hydration and by its platelike rather than spherical structure as assumed in eqn. (7).

The derivation of the free-volume equation (eqn. 1), as it was derived in similar form by Yasuda [16], assumes that the partitioning of solutes is proportional to membrane hydration. The solute partitioning in this study is not proportional to membrane hydration, however. Still, the results of the diffusion data of the solutes show that the free-volume relationship still applies for the membrane and solutes used in this study and is not disturbed by the observed deviation from proportionality between solute partitioning and membrane hydration. This is probably due to the fact that the deviation is relatively not very high (the highest deviation observed is for uranine at 26°C, where $K_d=3$ and $H=0.5$) considering the partition coefficients of more hydrophobic solutes in similar hydrogels which can be as high as 35 or more [46].

The absence of a screening effect for uranine and dextran 4,4K and the validity of the free-volume equation (eqn. 1) cause the diffusion of the solutes to be only controlled by the swelling level of the membrane. This makes separation of the three solutes possible by controlling temperature.

Conclusions

It is possible to separate uranine, dextran 4,4K and dextran 150K by a new method of separation which uses a thermosensitive hydrogel membrane. The separation is based on free-volume theory considerations and makes use of accurate control of the membrane swell-

ing by temperature. The separation achieved by a crosslinked p(NIPAAm–co-BMA, 95:5 mol%)-membrane showed high purity and high recovery of the separated compounds. The diffusion of the compounds in the membrane was shown to follow the free-volume theory of solute diffusion in swollen polymer networks.

List of symbols

D_m	solute diffusion coefficient in membrane (cm^2/sec)
D_0	solute diffusion coefficient in water (cm^2/sec)
ϕ_2	screening factor
B	proportionality factor (Å)
V_f	free-volume in hydrogel ($Å^3$)
H	volume fraction of water in hydrogel
q_2	cross-sectional area of solute ($Å^2$)
P	permeability of membrane (cm^2/sec)
K_d	partitioning coefficient of solute
S	equilibrium swelling ratio
V_p	volume of dried polymer (cm^3)
V_w	volume of water in hydrogel (cm^3)
$(\Delta c)_{t_x}$	concentration difference between donor and receiver at time t_x (mol/l)
A	membrane surface area (cm^2)
V	chamber volume (cm^3)
h	membrane thickness (cm)
r	diameter of equivalent sphere for solute (Å)
ξ	mesh size (Å)
ν_e^*	effective crosslink density (mol/cm^3)
$\phi_{P,0}$	polymer volume fraction in relaxed state
ϕ_P	polymer volume fraction in swollen gel
α	linear deformation factor
τ	stress (kPa)
$\bar{M}_c$	average molecular weight between crosslinks (g/mol)
$\bar{v}$	specific volume of dried polymer (cm^3/g)
γ	linear expansion factor
n	average number of C–C bonds between crosslinks
l	C–C bond length (Å)
c_n	Flory's rigidity factor
$\bar{r}_g$	radius of gyration (Å)
$\bar{r}_0$	average end-to-end distance of polymer chain under unperturbed conditions (Å)
k	Boltzmann constant (J/K)
d	hydrodynamic radius of solute (Å)
η	viscosity of solvent (g/m-sec)
s	sedimentation coefficient (sec)
ρ	density solvent (kg/m^3)
ρ_2	density solute (kg/m^3)
N_A	Avogadro's number (mol^{-1})
X	association parameter
M	molecular weight solvent (g/mol)
V_b	molar volume solute (cm^3)

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