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Poly (*N*-Isopropylacrylamide) Hydrogel Networks and Sieving Characteristics

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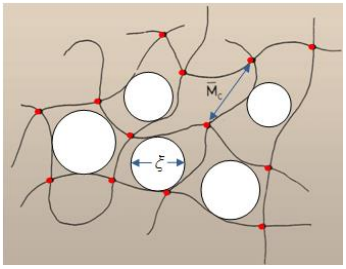
Graphical abstract



Abstract

The three-dimensional of hydrogel networks within nm range can microscopically be considered as "porous"mesh. This feature may imply that hydrogel networks possess sieving characteristics; i.e. exclusion of solutes or molecules based on size. In this study the network and sieving characteristics of poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels were investigated. PNIPAAm hydrogels were prepared via free radical using *N*-isopropylacrylamide (NIPAAm) as main monomers and *N*,*N*'-methylenebisacrylamide (MBAAm) as crosslinkers. As the composition of the hydrogels was varied, the mesh sizes of the resulting hydrogels were in the range of 4.0 to 11.0 nm. These data were obtained from swelling experiments. Dextrans as test solutes with molecular weight in the range of 4 to 2000 kg/mol were used in partitioning experiments to investigate the sieving of the hydrogel networks. The partitioning data indicated that of hydrogel networks excluded the solutes which were bigger than its mesh sizes. The experimental results not only show a good correlation of sieving coefficient on the size basis but also nicely fitted to the partition data estimated from the Ogston model. Undoubtedly, PNIPAAm hydrogel networks possessed sieving characteristics to separate molecules exclusively and selectively as a function of size.

Keywords: PNIPAAm; hydrogels; photopolymerization; rubber elasticity; network structure

1.0 INTRODUCTION

Hydrogels are highly hydrated polymers and built up of three-dimensional macromolecular network architectures [1]. Thus, the study of the hydrogel network is highly fundamental. This is because the network structure of the hydrogel not only that determines their mechanical properties, but also their separation functions.

The hydrogel network can be represented by their intrinsic parameters such as the polymer volume fraction in the swollen state (υ2,s), molecular weight (MW) between two consecutive crosslinks points (M_c) and mesh size (ξ) [2-4] (Figure 1). Then, a characteristic correlation length ξ, serves as an indicator of the screening effect of the network on solvation or solute diffusion. The term ξ is also indicating the maximum size of solutes that can pass through the network. These network parameters are interrelated and can be obtained theoretically or experimentally. It is important to note that, only average values for ξ and the M_c can be calculated because of non-uniformity of the network.

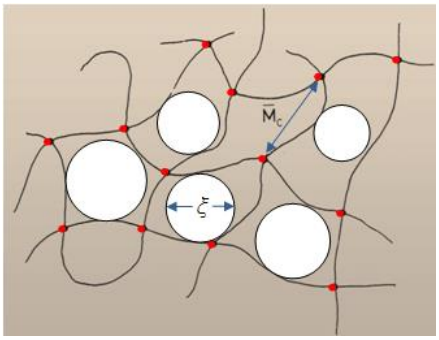


Figure 1 Swollen, crosslinked structure of hydrogel by a thermodynamically compatible liquid, indicating the number average molecular weight between crosslinks, M_c and the average mesh size, ξ (adapted from [2, 3])

The selectivity of hydrogels in separation process depends on two modes; either exclusion based on size and/or charge or sorption mechanism. The size-exclusion effect can be best envisioned by imagining the gel as an expanded mesh. Small molecules can easily be transported through the pores in the mesh, but large solutes cannot enter. On the other hand, Donnan-

exclusion mechanism governs the separation based on charge. The closest example of application based on size-exclusion separation principle is the use of hydrogels as electrophoretic media (for review, c.f. [5]). Polyacrylamide is one of the most studied and used polymer for the development of gel-electrophoresis.

Therefore, the aims of this work were to characterize the separation functions of PNIPAAm hydrogels in terms of sieving properties. PNIPAAm hydrogels with various compositions (c.f. 2.0) were synthesized via free radical photopolymerization. The sieving functions of these gels were assessed based on partitioning of uncharged (dextran) and charged (lysozyme) solutes. Overall, the intrinsic network properties of hydrogel are unique and explorations of its separation functions are highly valuable.

1.1 Theory and Background

When, the hydrogel is immersed in water, the swelling behaviour in the polymer hydrogels is observed as a result of diffusion of water molecules ('free water') into the polymer system [2]. This process is governed by the osmotic force and polymer interaction parameter.

Mathematically, swelling ratio, Q can be expressed as follows:

$$Q = \frac{m_s}{m_d} \tag{1}$$

where m_s and m_d are mass of the swollen network and mass of the dry network respectively.

A simplified derivation of this model was introduced by taking NIPAAm and MBAAm as an example [6]. Assuming a statistical copolymerization during the gel synthesis, the M_c can be calculated using Equation 2:

$$M_c = \frac{n(NIPAAm)}{n(MBAAm)} M(NIPAAm) + M(MBAAm) \tag{2}$$

where *M(NIPAAm)* is the molar mass of NIPAAm (NIPAAm as monomer), *M(MBAAm)* is the molar mass of MBAAm (MBAAm as crosslinker), *n(NIPAAm)* and *n(MBAAm)* are the mol of NIPAAm and MBAAm incorporated in the reaction respectively.

Thus, the mesh size of hydrogel is functions of distance and molar mass between two adjacent crosslinks in the swollen network is summarized in Equation 3:

$$\xi = Q^{1/3}\left(\frac{2M_c}{M}\right)^{1/2} C_N^{1/2} l_c \tag{3}$$

where l_c is the length of C-C single bond (= 0.154 nm), M is the MW of monomer; can be calculated from the MW of the two monomers and their ratio in the gel, and C_N is the characteristic ratio; a measure of the extension of polymer chain in a disordered condition (for acrylates = 6.9).

Alternatively, the mesh size of hydrogel can be obtained from rheological measurement. The theory of rubber elasticity is used to relate the modulus of elasticity or storage modulus (G') to the mesh size of the network from the following relationship [7]:

$$\xi = \left(\frac{RT}{G' N_A}\right)^{1/3} \tag{4}$$

where R is the universal gas constant and T is the absolute experimental temperature and N_A is Avogadro's number. This theory is only valid for perfect gel; the plateau G' is independent of the angular frequency.

The size-exclusion can be assessed based on partitioning of solutes in terms of intrinsic properties of hydrogels. Although there are different water structures in hydrogel; i.e. 'free-water', 'freezing bound water', and 'non-freezing bound water', only free water is available for solute partitioning and diffusion within the swollen mesh [8]. The partition coefficient of a solute molecule (K) is defined as:

$$K = \frac{c^{gel}}{c^{sol}} \tag{5}$$

where c^{gel} and c^{sol} are the equilibrium concentration in the gel (based on total gel volume) and the solution concentration, respectively.

In the absence of attractive interactions between the solute and gel networks, $K < 1$; because the volume occupied by the network excludes the solute based on the solute´s size [9]. Thus, reflection or retention coefficient (σ, valid for capillary pores) can be calculated based on the following equation [10]:

$$\sigma = (1-K)^2 \tag{6}$$

K is also correlated to the polymer volume fraction of swollen gel, $\upsilon_{2,s}$, as well as the radius of the solute r_s, and the radius of the polymer fiber r_f (Ogston model) [11].

$$K = exp\left[-\upsilon_{2,s}\left(1+\frac{r_s}{r_f}\right)^2\right] \tag{7}$$

■2.0 EXPERIMENTAL

In a glass vessel with a diameter of ~25 mm, PNIPAAm hydrogels with various compositions (NIPAAm: 10 and 15 wt% → M10, M15; MBAAm: 2, 5 and 10 wt% relative to NIPAAm → DC02, DC05 and DC10) were synthesized using free radical photopolymerization (UVA system, Hönle AG, Germany, $\lambda > 300$ nm, UV time = 15 min). Deionized water (25 ml) was been used in the preparation of the pre gel. Irgagure-2959® (2wt% relative to NIPAAm) was used as a photoinitiator.

For further characterization of the hydrogels, mass of the swollen hydrogel in water (15 to 45°C for 24 h in each individual temperature) and mass of the dry network (60°C, 24 h) was determined for the ratio of the swelling by using Equation 1. The mesh size was calculated by using Equation 3.

Rheological measurements were carried out using a rheometer model MCR-300 (Anton Paar, Graz, Austria) equipped with a Peltier device for temperature control. The hydrogels were cut into disc-like pieces and immersed in distilled water for several washings. 25 mm diameter upper plate was used, with the desired separation distance that was equivalent to 5 N. The bulk hydrogels were characterized under frequency sweep (ω = 0.1–100 rad/s, 20°C) and strain amplitude, γ = 1 %, was kept constant throughout the experiments. The mesh size of PNIPAAm hydrogels from rheology data was calculated using Equation 4 Solute molecule was calculated by using Equation 7.

■3.0 RESULTS AND DISCUSSION

Figure 2 revealed that the degree of swelling depended both on the temperature and compositions. The monomer and crosslinker contents were varied from 10 to 15 wt% and also from 2 to 10

wt% relative to NIPAAm respectively. In case of conventional hydrogels, higher swelling was observed below the lower critical solution temperature (LCST) for hydrogels with low monomer or crosslinker content. This can be due to the fact that high content of monomer or crosslinker increased the compactness of the hydrogel network and resulted in a mesh size reduction (c.f. Table 1).

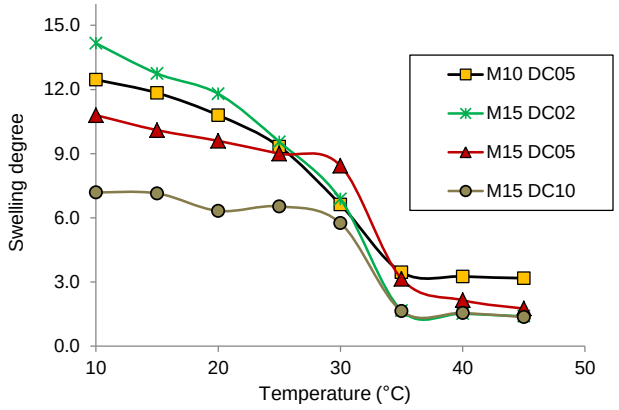


Figure 2 Equilibrium swelling degree of PNIPAAm hydrogels as a function of temperature: influence of compositions (hydrogels were obtained using 2.0 wt% photoinitiator concentration and 15 min irradiation time)

Table 1 Calculated mesh size of PNIPAAm hydrogels prepared via photopolymerization from two independent experiments; (a) at varied UV time (hydrogel composition: M15DC05); and (b) various compositions (obtained at 15 min UV time)

Hydrogels	Average mesh size – swelling [nm]	Average Storage modulus [Pa]	Average mesh size - rheology [nm]
M10DC05	6.6	5800	8.8
M15DC02	10.9	6700	8.5
M15DC05	6.0	29100	5.2
M15DC10	4.0	49800	4.3

The influence of composition on the rheological properties of PNIPAAm hydrogels obtained at 15 min UV time was explored. The plateau value of G' increased systematically from ~ 6000 to ~ 30000 Pa with increasing monomer content from 10 to 15 wt%. Similar trend was also observed for increasing crosslinker concentration from 2 to 10 wt% relative to NIPAAm. Obviously, an increase in monomer or crosslinker content results in formation of hydrogels with denser network [12]. From the rubber elasticity theory, the modulus in the rubbery range is related to the change in entropy. The increasing monomer and/or crosslinker contents will therefore have the same effects as an increase in stiffness or toughness of the network as a result of decrease in chain mobility. This also suggested an enhancement of mechanical properties of such hydrogels.

The microstructure of the classical photo gels was investigated from the partitioning coefficient. The results are expressed in terms of reflection or retention coefficient, σ. The classical PNIPAAm hydrogels are neutral, whereas the dextran molecules are uncharged. Therefore in the absence of electrostatic interactions between test solutes and polymer mesh, the volume exclusion took place. The mesh size of M15DC05 from swelling experiment was 6.0 nm (c.f. Table 1). As can be seen in Figure 3, partitioning of the test solutes in the constant mesh size was clearly a function of solute size (c.f. Table 2). The prediction of σ values from an empirical fit of larger solutes should in an ideal case be exactly 100 %. Figure 3 and Figure 4 show that estimation of K based on Ogston model for the solutes larger than the mesh size were indeed 100 %. The experimental data show slightly lower σ than the ideal case for the bigger solutes size and vice versa. This deviation can be associated to the broader size distribution of dextran comprising either larger or smaller fraction of small solute, respectively.

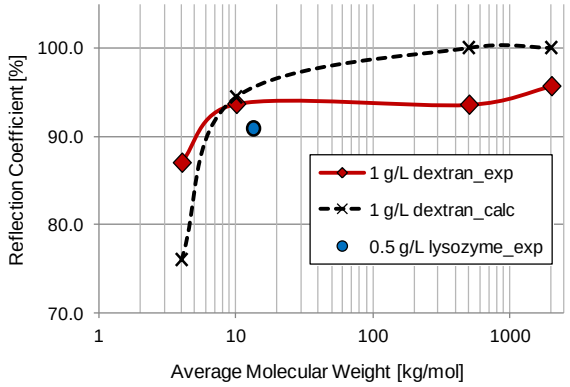


Figure 3 The comparison between experimental (solid line) and calculated data (dashed-line) of the reflection coefficient of test solutes: (i) dextran from various molecular weights, and (ii) lysozyme, in M15DC05 gel

Table 2 Test solutes for partition and sorption experiments of PNIPAAm hydrogels

Test Solutes	Molecular weight [kg/mol]	Isoelectric point	Diameter [nm]
Dextran	4	--	*3
	10	--	*4.6
	500	--	*27.6
	2000	--	*54
Lysozyme	14.6	10.8	**4

The partitioning of charged macromolecules; i.e., protein in the neutral gel give rise to the volume exclusion on the size basis. Figure 3 revealed lower reflection coefficient for lysozyme as compared to the dextran. This can be explained by the chain conformation of macromolecules; lysozyme is more compact than dextran with same MW. Overall, the σ values derived from estimation of K (Equation 7) were not too far from those experimentally determined (Equation 5). This implied that the Ogston model provides good estimation for K and σ values.

The reflection coefficients of 4 and 2000 kg/mol dextrans in various gel compositions are shown in Figure 4. The estimation of σ based on Ogston model for larger solute than the mesh size of the gels (mesh size of hydrogels from various compositions; c.f. Table 1) gave rise to the 100 % exclusion. Hydrogels with the smallest mesh size (M15DC02) showed the highest reflection coefficient and vice versa. Indeed the partition data estimated from the Ogston model fit to the physical nature of the gel matrix and the solute quite closely.

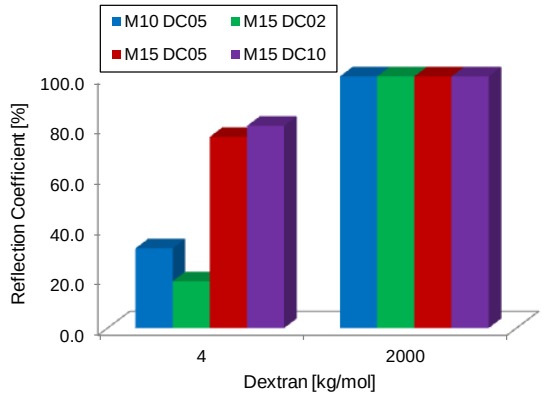


Figure 4 The reflection coefficient of dextran from various gel compositions (calculated data)

4.0 CONCLUSION

The mesh size determination from rheology experiments fitted very well with those derived from equilibrium swelling data. Surprisingly, the estimation of mesh size from rheological measurements based on rubber elasticity theory was in a good agreement to those obtained from swelling (Table 1). The range of mesh size obtained suggested that this type of hydrogel can be potentially employed as a material for the sieving of macromolecules based on their size. The reflection coefficients of 4 and 2000 kg/mol dextrans in various gel compositions are shown in Figure 3. The estimation of σ based on Ogston model for larger solute than the mesh size of the gels (mesh size of hydrogels from various compositions gave rise to the 100 % exclusion. Hydrogels with the smallest mesh size (M15DC02) showed the highest reflection coefficient and vice versa and solute that larger than mesh size will 100% excluded. In conclusion, characterization of mesh size of hydrogels is important because it can be used to deduce sieving property of the hydrogel network.

Acknowledgement

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