

PFG-NMR studies of ATP diffusion in PEG-DA hydrogels and aqueous solutions of PEG-DA polymers

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Abstract

Adenosine triphosphate (ATP) is the major carrier of chemical energy in cells. The diffusion of ATP in hydrogels, which have a structural resemblance to the natural extracellular matrix, is therefore of great importance to understand many biological processes. In continuation of our recent studies of ATP diffusion in poly(ethylene glycol) diacrylate (PEG-DA) hydrogels by pulsed field gradient nuclear magnetic resonance (PFG-NMR), we present precise diffusion measurements of ATP in aqueous solutions of PEG-DA polymers, which are not cross-linked to a three-dimensional network. The dependence of the ATP diffusion on the polymer volume fraction in the hydrogels, ϕ , was found to be consistent with the predictions of a modified obstruction model or the free volume theory in combination with the sieving behavior of the polymer chains. The present measurements of ATP diffusion in aqueous solutions of the polymers revealed that the diffusion coefficient is determined by ϕ only, regardless of whether the polymers are cross-linked or not. These results seem to be inconsistent with the free volume model, according to which voids are formed by a statistical redistribution of surrounding molecules, which is expected to occur more frequently in the case of not cross-linked polymers. The present results indicate that ATP diffusion takes place only in the aqueous regions of the systems, with the volume fraction of the polymers, including a solvating water layer, being blocked for the ATP molecules. The solvating water layer increases the effective volume of the polymers by 66%. This modified obstruction model is most appropriate to correctly describe the ATP diffusion in PEG-DA hydrogels.

Key words: Hydrogels; poly(ethylene glycol) diacrylate (PEG-DA); adenosine triphosphate (ATP); solute diffusion; pulsed field gradient (PFG) NMR; spin echo; stimulated echo



1. Introduction

Hydrogels are semi-solid polymer networks formed by cross-linked hydrophilic polymer chains swollen in water. For the preparation of hydrogels, aqueous solutions of functional polymers are usually reacted in such a way that three-dimensional networks are formed. Their mesh sizes as well as their macroscopic properties such as the stiffness and the equilibrium degree of swelling are mainly determined by the type of polymer used or the concentration and/or the molecular weight of the polymers. Besides their tunable mechanical properties, hydrogels can also form selective barriers that control the diffusive movement of solute molecules (see, e.g., [1] and references therein). A frequently used class of hydrogels is based on poly(ethylene glycol) diacrylate (PEG-DA). PEG-DA hydrogels have a structural resemblance to the natural extracellular matrix. As such, they are ideal model systems for understanding diffusive transport of biological molecules, e.g. in the context of drug delivery and transport of nutrients or vitamins. Thus, a fundamental understanding of the diffusion processes of solute molecules in well-defined PEG-DA hydrogels is important.

A powerful tool to measure the diffusion coefficients of solutes directly, i.e. without the need for a fluorescent label and independent of any diffusion-model assumptions is pulsed field gradient nuclear magnetic resonance (PFG-NMR). Modern PFG-NMR spectrometers can generate magnetic field-gradient pulses up to 35 T/m [2]. In a PFG-NMR measurement the system remains in thermal equilibrium with no gradient in the solute concentration, and, in contrast to permeation experiments, no physical release of the solute is required and the results can thus not be affected by surface properties of the sample.

In a very recent PFG-NMR work, we studied the diffusion of adenosine triphosphate (ATP), the main source of chemical energy for most cellular processes, in PEG-DA hydrogels. By varying the molecular weight of the polymers before cross-linking between $M_n = 700$ and 8000 g/mol, different polymer networks were obtained, which differed in mesh size, water uptake and swelling behavior. Our previous studies of ATP diffusion have been performed on twelve PEG-DA hydrogels with mesh sizes ranging from $\xi = 1.1$ to 12.9 nm and polymer volume fractions between $\phi = 0.03$ and 0.31 [1]. The main aim of that work was to analyze the dependence of the diffusion coefficients on the polymer volume fraction ϕ and the mesh size ξ of the water-swollen hydrogels and to discuss the results in the framework of existing models. The present work is a continuation of those previous studies. The goal of this contribution is to study the reduction in ATP diffusion in aqueous solutions of PEG-DA polymers, which are not cross-linked to a three-dimensional network, and comparing the results with the previous data on ATP diffusion in PEG-DA hydrogels.

2. Experimental Details

PEG-DA hydrogels were prepared using different volume fractions of polymers before swelling with different molecular weights ($M_n = 700$ g/mol, 3400 g/mol, 6000 g/mol, and 8000 g/mol). The hydrogels were obtained by photo-polymerization of the polymers in an aqueous solution followed by swelling in water to equilibrium. The polymer volume fraction of the water-swollen hydrogels was between $\phi = 0.03$ and 0.31 and the corresponding mesh sizes covered a range from $\xi = 1.1$ to 12.9 nm [1].

For the diffusion studies of the present work, aqueous solutions containing not cross-linked PEG-DA polymers together with ATP were prepared. Whereas the ATP concentration was 10 mg of ATP per milliliter water in all samples, the PEG-DA polymer volume fractions were 0.07 and 0.16 ($M_n = 3400$ g/mol) 0.09 ($M_n = 6000$ g/mol), as well as 0.03 and 0.09 ($M_n = 8000$ g/mol).

^{31}P PFG-NMR was applied to determine the diffusion coefficients D of ATP in PEG-DA hydrogels as well as in aqueous solutions of PEG-DA polymers, which are not cross-linked to a three-dimensional network. Additionally, ^1H PFG-NMR was used for diffusion measurements of water and polymers in one of the aqueous solutions. All diffusion measurements were performed with a Bruker Avance III 400 MHz spectrometer and a Bruker wide bore magnet. Magnetic field gradients were generated using a diff60 diffusion probe and a Great60 gradient amplifier (Bruker Biospin). The diffusion coefficients were measured by the stimulated-echo sequence [3] with spoiler gradients [4]. The shape of the field-gradient pulses was given by a half-sine function with a length of δ_{sine} , which corresponds to an effective length of $\delta_G = \frac{2}{\pi} \delta_{\text{sine}}$ for rectangular gradient pulses of the same integrated area. In this case, the diffusion-induced attenuation of the echo intensity is given by [1, 5–7]

$$M(G) = M(0) \cdot \exp\left(-\gamma^2 G^2 D \left[\delta_G^2 \left(\Delta - \frac{\pi}{8} \delta_G\right)\right]\right). \quad (1)$$

γ denotes the gyromagnetic ratio of the particular nuclei, i.e., ^{31}P or ^1H . Typical operating parameters were a diffusion time of $\Delta = 10$ ms and an effective gradient pulse length of $\delta_G = 1$ ms. The diffusion coefficients were determined from the dependence of the echo attenuation on the amplitude of the applied field-gradient pulses, which were varied in 16 steps between $G = 0.3$ and 10.1 T/m. Signal averaging was performed using 512 scans. The diffusion probe inside the room-temperature bore of the superconducting magnet was cooled with water to a constant temperature of 25 °C. Owing to the good thermal contact, this results in a sample temperature of 25 °C with an accuracy of $\pm 0.2^\circ \text{C}$ without the need for any further temperature controller.

3. Results and Discussion

Here we briefly summarize the main results of our previous diffusion studies of ATP in PEG-DA hydrogels by PFG-NMR. The diffusion coefficients D were compared with the diffusion coefficient D_0 of ATP in pure water at the same temperature ($T = 25^\circ \text{C}$). The value of $D_0 = 3.875 \times 10^{-10} \text{ m}^2/\text{s}$ and the water viscosity at 25 °C from the literature allowed us to determine the hydrodynamic radius $R_h = 0.633$ nm of ATP. The relative diffusivity, i.e., the diffusion coefficient D of ATP in the hydrogel normalized to the value in pure water D_0 , showed a rather strong apparent dependence on the mesh size. It is reasonable to assume that the size of the solute ($R_h = 0.633$ nm) in relation to the size of the openings between polymer chains ξ will have an effect on the movement of the solute. This sieving behavior of the polymer network results in a reduction in the relative diffusivity of the solutes. The probability of a solute of characteristic size R_h to pass through an opening in the network of size ξ is given by [8]

$$p(R_h, \xi) = 1 - \frac{R_h}{\xi}. \quad (2)$$

Based only on this approach, relative diffusivities ranging from $D/D_0 = 0.42$ ($\xi = 1.1$ nm) to $D/D_0 = 0.95$ ($\xi = 12.9$ nm) would be expected for ATP diffusion. However, the experimentally observed relative diffusivities are significantly smaller and showed a more pronounced apparent change with ξ [1]. This has to be ascribed to the correlation between the mesh size ξ and the polymer volume fraction ϕ of the water-swollen hydrogels. The sieving behavior is obviously a relatively minor effect, and the polymer volume fraction ϕ is the dominant factor in determining the diffusion of small solute molecules.

Three fundamentally different approaches to describe the ϕ dependence of the solute diffusion in hydrogels have been developed in the past decades [9]: (i) The hydrodynamic

model, which takes into account the change in the hydrodynamic drag experienced by the solute, (ii) the obstruction model, in which the polymer chains have been proposed to act as physical obstructions, thereby increasing the effective path length of the solute, and (iii) the free volume model, which considers that solute movement requires the formation of sufficiently large voids by a statistical redistribution of the molecules adjacent to the solute. A general feature of all model is that D/D_0 decreases with increasing ϕ ; however, the strength of this decrease varies across the different models.

The relative diffusivity D/D_0 of ATP in the hydrogels is plotted as a function of the polymer volume fraction ϕ in Fig. 1. In the hydrodynamic model the solute molecules are assumed to move as hard spheres at a constant velocity in a system consisting of water and polymer chains. Their movement is resisted by frictional drag, which can be determined by summing the frictional contribution of each polymer chain. The result can be expressed in terms of an effective viscosity of the hydrogel, which can then be related to the viscosity of pure water. With this approach, the relative diffusivity

$$\frac{D}{D_0} = \exp(-BR_h\sqrt{\phi}) \quad (3)$$

of solutes in water-swollen hydrogels is obtained, where B is a fitting parameter [10]. An attempt to fit Eq. (3) to the dependence of the diffusivity on ϕ , as shown by a black dashed line in Fig. 1, does not yield a reasonable agreement. Obviously, this model is not capable of correctly describing the solute diffusion as a function of the hydrogel volume fraction.

In the obstruction model the solute diffusion is described as taking place in a heterogeneous system, consisting of the water phase and the polymer chains of the water-swollen hydrogel. The polymer chains act as obstructions for the diffusion, which results in an increase in the effective length of the diffusion path. Under the assumption that the solute diffuses on a simple cubic lattice and ϕ represents the probability that a given lattice site is occupied and thus blocked for the solute, the following expression for the reduced diffusivity can be derived [11]:

$$\frac{D}{D_0} = \left(\frac{1-\phi}{1+\phi}\right)^2. \quad (4)$$

In this model, the nature of the interaction between solute and hydrogel is not taken into consideration, besides the assumption that lattice sites occupied by hydrogel are blocked. The variation of the relative diffusivity D/D_0 with ϕ expected from the obstruction model, which works without a fitting parameter, follows the general trend of the experimental data (*cf.* blue dotted line in Fig. 1). However, the experimentally observed reduction in the relative diffusivity D/D_0 is significantly stronger than that predicted by Eq. (4).

In the framework of the free volume model, the solute molecules are again assumed to move as hard spheres in the water-polymer system. The basic concept is that a statistical redistribution of “free volume” in this system opens up voids of sufficient volume to accommodate a solute molecule. It is assumed that the voids are formed by a general withdrawal of the surrounding molecules due to random thermal motion, which occurs without any change in energy. Occasionally, the density fluctuations form voids, which are large enough to permit a considerable displacement of a solute molecule. Such a displacement may give rise to diffusive motion, and the diffusion coefficient is thus dependent on the probability that a void is formed adjacent to the solute. The sieving behavior of the polymer chains has also been incorporated into the free volume theory. The relative diffusivity of the solute in a water-polymer system at infinite dilution is then expressed as [8]

$$\frac{D}{D_0} = \left(1 - \frac{R_h}{\xi}\right) \exp\left(-Y \frac{\phi}{1-\phi}\right). \quad (5)$$

The result of this fit is shown by the red solid line in Fig. 1, and corresponds to a fit parameter of $Y = 4.7$. This parameter Y represents a scale factor for the ratio of the critical volume required for a successful diffusional jump to the average free volume per molecule of the solvent, which is expected to be of the order of unity.

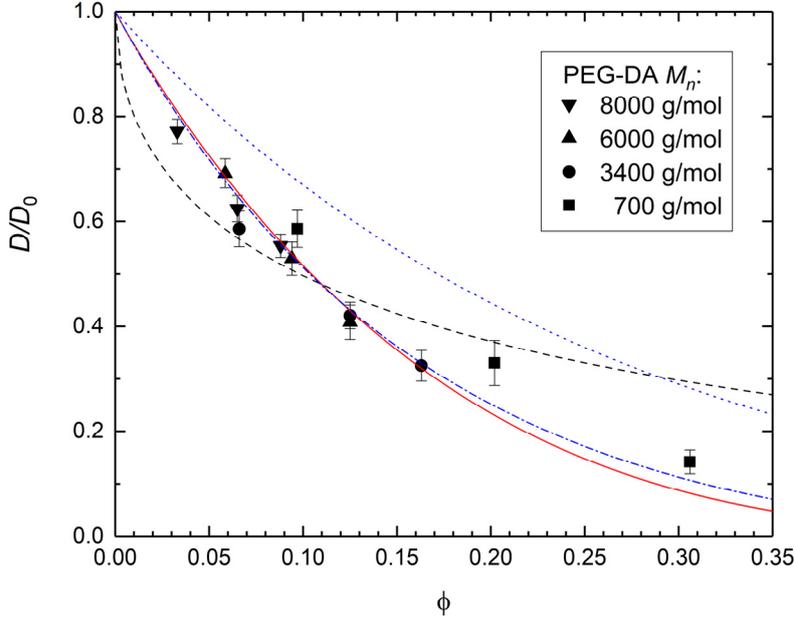


Fig. 1: Dependence of the relative diffusivity D/D_0 of ATP on the polymer volume fraction ϕ of the water-swollen PEG-DA hydrogels ($D_0 = 3.875 \times 10^{-10} \text{ m}^2/\text{s}$). The symbols indicate the molecular weights M_n of the polymers. The lines are obtained by fits of different diffusion models: Hydrodynamic model (black dashed line), obstruction model (blue dotted line), free volume model (red line), and the modified obstruction model with $\alpha = 1.66$ (blue dash-dotted line). Reproduced from “Günter Majer and Alexander Southan, *J. Chem. Phys.* **146**, 225101 (2017)”, with the permission of AIP Publishing.

It is evident from Fig. 1 that the dependence of the relative diffusivity D/D_0 on the hydrogel volume fraction ϕ , as predicted by the free volume model together with the sieving behavior of the polymer chains, is in much better agreement with the experimental results for ATP diffusion in PEG-DA hydrogels than is either the hydrodynamic model or the simple obstruction model. On the other hand, the hydrodynamic radius of ATP ($R_h = 0.633 \text{ nm}$) is much smaller than the typical mesh sizes of the hydrogels ($\xi = 1.1$ to 12.9 nm). One may therefore ask, whether it is really the free volume that determines the diffusion of ATP in PEG-DA hydrogels, or whether the obstruction model would not be more appropriate. It is evident from Fig. 1 that the obstruction model, as it is published in the literature, predicts a weaker reduction in the diffusivity as observed experimentally. However, it may be expected that hydrophilic polymers such as PEG-DA include temporarily associated water molecules that on average increase their volume by a constant factor α . In order to account for this effect, we parameterized Eq. (4) by putting a single fitting factor α in front of each instance of ϕ [1]:

$$\frac{D}{D_0} = \left(\frac{1 - \alpha \cdot \phi}{1 + \alpha \cdot \phi} \right)^2. \quad (6)$$

The modified obstruction model obtained this way (Eq. (6)) is capable of describing the experimental data very well (*cf.* blue dash-dotted line in Fig. 1). The corresponding fit parameter of $\alpha = 1.66$ indicates that the polymer volume fraction including the solvating water layer in the swollen hydrogels is actually 66 % higher than the value determined on the basis of the chain mass fraction only. Assuming that the densities of PEG and water in the solvated state are similar to the densities of PEG and water itself (1.12 g cm^{-3} and 1.0 g cm^{-3} , respectively), this corresponds to an average number of 1.4 water molecules associated with a PEG repeating unit with a molecular weight of 44.05 g/mol . This result is in the same range as other reported values [12, 13]. The modified obstruction model is just as consistent with the experimental data as the free volume model. It seems reasonable to assume that neither the

mesh size nor the statistical formation of free volume has a strong influence on the ATP diffusion in PEG-DA hydrogels. Nevertheless, the free volume model cannot be excluded on the basis of the present data only.

In order to investigate which of the modified obstruction model and the free volume model with their corresponding model assumptions is more suitable for describing the diffusion mechanism of ATP in PEG-DA hydrogels, we studied ATP diffusion in aqueous solutions of PEG-DA polymers, which are not cross-linked to a three-dimensional network. The corresponding diffusion coefficients are summarized in Table I. In the case of a sample with $M_n = 6000$ g/mol and $\phi = 0.09$ we obtained a value of $D_{\text{ATP}} = 2.12 \times 10^{-10}$ m²/s for the diffusion coefficient of ATP at 25 °C. In the same sample, we also measured the diffusion coefficient of the polymers as well as the self-diffusion of water, both by ¹H PFG-NMR at 25 °C. It is interesting to note that the diffusion coefficient of the polymers, $D_{\text{Polymer}} = 3.74 \times 10^{-11}$ m²/s, is about one order of magnitude lower than that of the ATP molecules. On the time scale of ATP diffusion, the polymers can therefore be considered as effectively immobile, even if they are not cross-linked. Furthermore, the diffusion coefficient of the water molecules in the aqueous polymer solution, $D_{\text{H}_2\text{O}} = 1.28 \times 10^{-9}$ m²/s, is reduced by a factor of 0.56 compared to the self-diffusion coefficient of pure water at 25 °C ($D_{0,\text{H}_2\text{O}} = 2.30 \times 10^{-9}$ m²/s, [14]). This value is very close to the relative diffusivity $D/D_0 = 0.55$ of ATP in the same sample. For a diffusion process that requires the formation of sufficiently large voids to permit a considerable displacement of a solute molecule, a strong dependence of the relative diffusivity on the size of the solute molecule would be expected. The observation that the polymers cause a comparable reduction in the diffusivity of ATP and water molecules indicates that the diffusion process is not determined by the formation of free volume. The polymers seem to act rather as physical obstructions for the diffusion of both, the water molecules and the ATP molecules. An increase in ϕ results in an increase in the effective length of the diffusion path and therefore in a reduction of the diffusion coefficient.

TABLE I. Summary of the diffusion coefficients D of ATP (10 mg/ml) in aqueous solutions of PEG-DA polymers measured at 25 °C by PFG-NMR. The not cross-linked solutions were prepared from polymers with different molecular weights M_n and with different polymer volume fractions ϕ . The relative diffusivity D/D_0 is obtained by normalizing D to the ATP diffusion coefficient in pure water ($D_0 = 3.875 \times 10^{-10}$ m²/s).

Sample	M_n [g/mol]	ϕ	D of ATP [10^{-10} m ² /s]	D/D_0
Polymer solution 3400-7%	3400	0.07	2.47 ± 0.04	0.64
Polymer solution 3400-16%	3400	0.16	1.51 ± 0.03	0.39
Polymer solution 6000-9%	6000	0.09	2.12 ± 0.03	0.55
Polymer solution 8000-3%	8000	0.03	3.09 ± 0.04	0.80
Polymer solution 8000-9%	8000	0.09	2.15 ± 0.03	0.56

The red symbols in Fig. 2 show the relative diffusivities D/D_0 ($D_0 = 3.875 \times 10^{-10}$ m²/s) of ATP in the aqueous solutions of PEG-DA polymers at different polymer volume fractions ϕ . Surprisingly, these data coincide with the diffusivities of ATP in hydrogels with the same polymer volume fractions. We would like to emphasize that essentially the same variation of the relative diffusivity with the polymer volume fraction is observed for all samples, independent of the molecular weight of the polymers and also independent of whether the polymers are cross-linked or not. In our opinion, this result again contradicts the basic concept of the free volume model. According to the free volume model, voids sufficiently large to accommodate solutes are formed by a statistical redistribution of surrounding molecules [8],

which should occur more frequently in the case of not cross-linked polymers. In the framework of the modified obstruction model, on the other hand, neither formation of free volume nor sieving behavior of the polymers is taken into consideration. According to this model, solute diffusion takes place only in the aqueous regions of the systems, with the volume fraction of the polymers including the solvating water layer ($\alpha \cdot \phi$) being blocked for the solute molecules. In this case, an increase in the volume fraction of the polymers increases the effective length of the diffusion path and so reduces the diffusion coefficient.

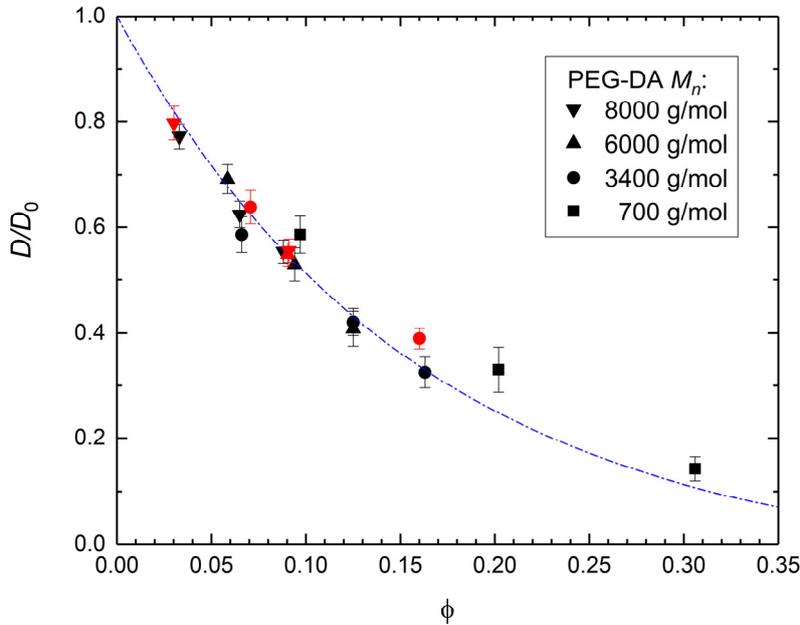


Fig. 2: Relative diffusivity D/D_0 of ATP in PEG-DA hydrogels (black symbols) and in aqueous solutions of not cross-linked PEG-DA polymers (red symbols). The shape of the symbols indicates the molecular weight M_n of the polymers. The blue dash-dotted line represents the modified version of the obstruction model as taken from Fig. 1.

The present results allow us to conclude that the modified obstruction model is the most appropriate to correctly describe the diffusion of ATP, and probably also that of other small solute molecules, in PEG-DA hydrogels. It remains to be elucidated whether a different diffusion mechanism dominates the diffusive behavior of larger solute molecules, such as, e.g., vitamins, insulin or dextran.

3. Summary and conclusion

The present paper reports on diffusion studies of ATP in well-defined PEG-DA based hydrogels as well as aqueous solutions of PEG-DA polymers, performed by pulsed field gradient nuclear magnetic resonance (PFG-NMR). This technique permits determining the diffusion coefficients directly, i.e. without the need for a fluorescent label and independent of any diffusion-model assumptions. Hydrogels with mesh sizes ranging from $\xi = 1.1$ to 12.9 nm and polymer volume fractions between $\phi = 0.03$ and 0.31 were prepared using polymers with different molecular masses ($M_n = 700, 3400, 6000, \text{ and } 8000$ g/mol). Aqueous solutions of not cross-linked PEG-DA polymers were prepared using polymers with $M_n = 3400$ g/mol ($\phi = 0.07$ and 0.16), $M_n = 6000$ g/mol ($\phi = 0.09$), and $M_n = 8000$ g/mol ($\phi = 0.03$ and 0.09). The results of the diffusion studies of ATP in the PEG-DA hydrogels have been published recently [1]. Those diffusion data have been compared with the predictions of various mathematical expressions developed under different model assumptions. The results were found to be in good agreement with the predictions of two models, the free volume theory and a modified version of the obstruction model. The present diffusion studies on ATP in aqueous solutions of PEG-DA polymers, which are not cross-linked, revealed that the diffusion coefficient is determined

only by the polymer volume fraction. This result suggests that the modified obstruction model is the correct approach to describe the ATP diffusion in PEG-DA hydrogels. The corresponding solvating water layer in PEG-DA hydrogels increases the effective volume fraction of the polymers by a factor of 1.66, which corresponds to an average of 1.4 water molecules associated with each repeating unit of the PEG-DA polymers. It remains to be elucidated whether this modified obstruction model describes the general diffusion process of small solute molecules in hydrogels. Another open question is whether a different diffusion mechanism dominates the diffusive behavior of larger solute molecules. Investigations of these topics are currently in preparation.

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