Solvent and solute ingress into hydrogels resolved by a combination of imaging techniques

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Using simultaneous neutron, fluorescence, and optical brightfield transmission imaging, the diffusion of solvent, fluorescent dyes, and macromolecules into a crosslinked polyacrylamide hydrogel was investigated. This novel combination of different imaging techniques enables us to distinguish the movements of the solvent and fluorescent molecules. Additionally, the swelling or deswelling of the hydrogels can be monitored. From the sequence of images, dye and solvent concentrations were extracted spatially and temporally resolved. Diffusion equations and different boundary conditions, represented by different models, were used to quantitatively analyze the temporal evolution of these concentration profiles and to determine the diffusion coefficients of solvent and solutes. Solute size and network properties were varied and their effect was investigated. Increasing the crosslinking ratio or partially drying the hydrogel was found to hinder solute diffusion due to the reduced pore size. By contrast, solvent diffusion seemed to be slightly faster if the hydrogel was only partially swollen and hence solvent uptake enhanced. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4950954>]

I. INTRODUCTION

Hydrogels are used in a broad range of applications from chemical sensors,^{1,2} responsive coatings,^{3,4} bioadhesives,⁵ or soft contact lenses^{6,7} to food products,^{8,9} drug delivery applications,^{10–13} and potential scaffolds for artificial tissue and organs.^{14–17} In many of these applications, the diffusion of molecules or particles in and through hydrogels is crucial and it is the focus of numerous experimental and theoretical studies.^{18–29} However, only few studies explicitly monitor the solvent diffusion and its interplay with the solute motion.^{7,30}

We simultaneously followed solvent and solute to investigate their diffusion and their effects on each other as well as on the hydrogel. This was done using a novel setup, which combines neutron radiography (NR), fluorescence imaging (FI), and optical brightfield imaging (OI).³¹ These three imaging techniques monitor different properties of the samples. In the optical images, contrast is generated by differences in the local optical density of the sample. The hydrogel can be distinguished from the solvent and a potential change in hydrogel size due to solvent uptake or loss can be monitored and also defects in the sample can be observed. FI provides information about the local fluorophore concentration and thus enables us to study, e.g., the diffusion of fluorescent molecules independently of solvent diffusion. The fluorophores can be used to label specific molecules, particles, or structures and can additionally report on properties such as pH, temperature, or chemical environment. By contrast, the interaction of neutrons occurs with the nuclei and thus depends strongly on the specific isotope. Hence, isotope exchange can be exploited to change the contrast between different parts of the sample and thus highlight them. In the present experiments, the contrast between light (H₂O) and

heavy (D₂O) water was used to distinguish water initially inside or outside the hydrogel, respectively. The number of transmitted neutrons, i.e., intensity, provides spatially and temporally resolved information about the composition of the sample.^{31–33} This has already successfully been applied to investigate the flux of liquids.^{34–39} Exploiting this effect, this allowed us to monitor solvent diffusion into and out of the hydrogel. The simultaneous application of these techniques thus provides information on the diffusion of fluorescent molecules or macromolecules as well as solvent into and out of the hydrogel, while the hydrogel size is monitored. The diffusion of the different species was studied for different characteristics of the hydrogel. Its pore size was changed by varying the crosslinking ratio and the degree of swelling. Furthermore, different fluorescent molecules and initial solvent compositions were investigated.

II. MATERIALS AND METHODS

A. Samples

Polyacrylamide (PAAm) networks were synthesized by copolymerization of the monomer acrylamide (AAm) with the tetrafunctional crosslinking agent N,N'-methylenebis-(acrylamide) (BIS), using ammonium peroxodisulphate (APDS) and tetramethylethylenediamine (TEMED) as redox initiators. Monomer and crosslinker were both purchased from Sigma-Aldrich, APDS from Roth and TEMED from Merck. All components were used without further purification. AAm, BIS, and APDS were first separately dissolved in deionized, filtered water and cooled down to 4 °C. To slow down the reaction, the individual solutions were then mixed at this low temperature. The crosslinking ratio (molar ratio of crosslinker

to monomer) determines the pore size and the characteristics of the hydrogel; ratios of 1:15 and 1:60 were studied. A 2 ml reaction mixture of 1:15 hydrogel contained 75 mg of AAm, 10.83 mg of BIS, 6 mg of APDS, and 10 μ l of TEMED. The same is applied to 1:60 hydrogel, but the reaction mixture only contained 2.71 mg of BIS. Once mixed with the desired ratio, the reaction mixture was transferred to Teflon molds (3.5 cm $\times$ 3.5 cm $\times$ 0.15 cm) using a glass pipette and allowed to warm up and react at room temperature. After 1–2 h, polymerization was complete and the hydrogel was transferred into a larger container filled with deionized water. At this point, the hydrogels could still take up water and swell. With gravimetric measurements, it was found that all hydrogels used here swelled for three to five days. The hydrogels hence were left in deionized water for one week to ensure that swelling was complete. During this time, the excess water was exchanged every day in order to wash out residual chemicals that had not reacted.^{40,41} Afterwards, discs with a radius $a \approx 0.3$ cm to 0.5 cm were cut from the hydrogels using a stamp. The same preparation protocol was applied for D₂O-hydrogels but in every step, D₂O (Deutero GmbH, Kastellaun, Germany, minimum content of 99.9% D₂O) was used instead of H₂O. All vials with solutions containing D₂O were flushed with nitrogen after mixing in order to reduce H₂O uptake from moisture in the air.

Some hydrogel discs were partially dried before the measurement, leading to a change in hydrogel pore structure. During drying, solvent evaporates from the surface of the hydrogel discs, leading to an inhomogeneous solvent distribution inside the hydrogel. Thus, the dried gels were kept in small, tightly sealed containers for at least three days before the measurement to achieve homogeneity. The degree of swelling, m_{ds} , of every sample was determined directly before the measurements. It is defined via the mass fraction of the hydrogel sample, m_{gel} , with respect to the mass of the fully swollen gel, $m_{gel,fs}$,

$$m_{ds} = \frac{m_{gel}}{m_{gel,fs}}. \quad (1)$$

Five separately prepared pieces of every type of hydrogel were weighed to determine $m_{gel,fs}$, then dried until all solvents had been removed and then again weighed to get the polymer mass, m_p . This allowed us to calculate the polymer volume fraction in the fully swollen state,

$$\phi_{p,fs} = \frac{m_p \rho_s}{m_p \rho_s + m_s \rho_p}, \quad (2)$$

where $\rho_p = 1.30$ g/cm³ is the polymer density, $\rho_s = 1.00$ g/cm³ and $\rho_s = 1.107$ g/cm³ are the solvent densities for H₂O and D₂O, respectively. The polymer volume fraction, ϕ_p , of partially dried hydrogels was calculated from the experimentally observed sample volume (disc radii determined with OI, Sec. II C 2 c) and the known mass of polymer in the sample, m_p . It is assumed that ϕ_p is independent of the position within the hydrogel.

The fluorescent dye fluorescein sodium salt, in the following, is referred to as fluorescein (Fluo) and fluorescein-labeled dextrans with different molar masses; D10, 10 kDa (from Sigma-Aldrich for combined FI and NR and from Invitrogen for separate FI) and D70, 70 kDa (from Sigma-Aldrich) were used. They were dissolved in deionized light (H₂O) or heavy (D₂O) water at concentrations of about 25 μ M, which was found to yield a good fluorescent signal while still being dilute enough to not affect the diffusion coefficients of the fluorescent molecules. The samples that were studied with simultaneous NR, OI, and FI experiments are listed in Table I.

B. Combined imaging experiments

A detailed description of the home-built imaging-apparatus to perform optical transmission imaging (OI) and fluorescence imaging (FI) experiments concurrently with neutron radiography (NR) can be found elsewhere.³¹ The sample was irradiated with a parallel beam of light and a collimated neutron beam at the cold neutron imaging facility ICON at the Paul Scherrer Institute (Villigen, Switzerland) and time series of images were recorded with separate CCD cameras for each imaging technique. The chosen distance of the neutron source aperture, L , and diameter of the aperture, D_{ap} , yielded $L/D_{ap} = 343$. This value, together with the distance between sample and scintillator, l , limits the best possible resolution, u_g , of the neutron radiograph,

$$u_g = \frac{l}{L/D_{ap}}. \quad (3)$$

In these measurements, a 10 μ m thick gadolinium based scintillator (Gd₂SO₂S:Tb) and a cooled charge-coupled device (CCD; Andor Neo sCMOS, 2560 $\times$ 2160 pixels, pixel size: 6.5 μ m) were used to convert transmitted neutrons and record the neutron radiographs, respectively. The effective spatial resolution was found to be better than 100 μ m. Images were collected continuously with an individual measurement time

TABLE I. Compositions of the hydrogel samples and solutions investigated are indicated by a $\times$. The crosslinking ratio cr , solvent in the hydrogel, polymer volume fraction in the fully swollen state, $\phi_{p,fs}$, and the degree of swelling, m_{ds} , of the hydrogel are indicated as well as the different solutes. Samples A and B are discussed in detail in Sec. IV B 1.

Gel	m_{ds}	1:15, D ₂ O, $\phi_{p,fs} = 0.065$				1:60, D ₂ O, $\phi_{p,fs} = 0.053$					1:60, H ₂ O, $\phi_{p,fs} = 0.038$	
		0.73	0.75	0.8	1.00	0.74	0.75	0.78	0.80	0.81	1.00	
Solute	H ₂ O								$\times$			
	Fluo D ₂ O					$\times$						Sample B
	Fluo H ₂ O		$\times$		$\times$		$\times$		$\times$	$\times$	$\times$	Sample A
	D10 H ₂ O			$\times$			$\times$	$\times$				
	D70 H ₂ O	$\times$				$\times$						

of 20–30 s, which was found to be the best compromise between image quality and time-resolution.

Optical transmission and fluorescence imaging were performed with a camera resolution of 10 μm , a field of view of about 25 mm $\times$ 21 mm, and a spatial resolution of about 20 μm . It was chosen to roughly match the one of the corresponding neutron radiography measurements although a significantly better spatial resolution could be obtained. The wavelength of the optical beam was optimized to excite the fluorophores in the sample (LED lamp with a center wavelength of 490 nm). Optical filters were used in the light path to split the light leaving the sample into attenuated excitation light and emitted fluorescence which are then focused onto the OI and FI cameras, respectively. In NR and OI monochrome cameras were used whereas the FI camera was a color camera.

In a typical experiment, the sample was constrained between two glass plates in a specifically designed sample cell that consisted of an aluminum body and quartz glass windows.³¹ The cell was aligned with respect to the neutron and light beams. As neutron radiography experiments can only be performed at large scale facilities where beam time is severely limited, time series of images were recorded for a maximum of 3–8 h. It was found that for the FI experiments, and especially when the larger dextran molecules were used as diffusants, much longer measurement times would be favorable. Thus, additional longer FI and OI experiments extending over several days were performed with all sample systems indicated in Table I. Several neutron and optical images of the hydrogel were collected before the solution was injected into the sample cell. By using a syringe pump that was controlled from outside the bunker of the neutron imaging instrument, the injection process could be followed and the time, t_0 , when the hydrogel was contacted with the solution determined. After injection of the solution, the solvent and the fluorescent molecules diffused into the hydrogel. Diffusion only occurred through the radial edges while the hydrogel faces were covered by the glass windows. FI measurements were continued beyond the end of the NR measurements, until the slightly activated sample cell could be handled and exchanged.

C. Data reduction

The data were obtained as series of two-dimensional grayscale (OI, NR) or color (FI) images with intensities, $I_{\text{raw}}(x, y, t)$. (Note that the dependence on position, (x, y) , and time since the start of the experiment, t , is not explicitly given in the following equations.) As described elsewhere in more detail,^{31,32} based on I_{raw} , the absolute transmission of the sample, T_S , and then the concentration or composition was determined.

1. Absolute transmission

In the first step of the analysis of the neutron data, complementary dark images (I_D), taken when all shutters in the neutron beamline were closed, and bright images (I_B), taken with the sample removed from the sample position,

were used to apply a pixelwise correction to the images to account for spatial inhomogeneities in the neutron beam and detector efficiency as well as neutron and electronic background, yielding the transmission T ,

$$T = \frac{I_{\text{raw}} - I_D}{I_B - I_D}. \quad (4)$$

On the series of dark and bright images taken before contact with solution, a median filter was applied to remove randomly distributed and fluctuating bright spots that can be caused by high intensity γ -particles directly hitting the CCD chip. Bright spots in the images of the time series were omitted during the averaging process described later.

Additionally, the mean transmission in a reference area, $\langle T_{\text{ref}} \rangle$, where no temporal changes in the image intensity were expected (e.g., a part of the cell body) was calculated for every image,

$$\langle T_{\text{ref}} \rangle = \frac{\langle I_{\text{raw,ref}} \rangle - \langle I_{D,\text{ref}} \rangle}{\langle I_{B,\text{ref}} \rangle - \langle I_{D,\text{ref}} \rangle}. \quad (5)$$

This was used to calculate the transmission of the sample relative to the reference area, T_{rel} , which is thus not only corrected for spatial but also for temporal fluctuations in the neutron beam intensity,

$$T_{\text{rel}} = \frac{T}{\langle T_{\text{ref}} \rangle}. \quad (6)$$

The absolute transmission of the sample, T_S , was obtained by

$$T_S = \frac{T_{\text{rel}}}{T_{\text{rel,EC}}}, \quad (7)$$

where $T_{\text{rel,EC}}$ is the relative transmission of the empty sample cell.

Tests showed that the raw optical and fluorescence images do not need to be corrected for inhomogeneities in illumination, detector efficiency, or background. As fluorescein emits light with a center wavelength of 525 nm, only the green channel, which contained the fluorescence signal, was used for further analysis.

2. Sample composition

a. Solvent concentrations from neutron radiography.

The experimentally determined neutron transmission of the sample, T_S , was used to determine the spatially and temporally resolved sample composition by

$$T_S = e^{-Sd}, \quad (8)$$

where d is the sample thickness and S the specific attenuation coefficient. The investigated samples consisted of up to four components: the polymer, H_2O , D_2O , and the fluorescent molecules with the volume fractions ϕ_p , ϕ_H , ϕ_D , and ϕ_f , respectively. Due to the low volume fraction of the fluorescent molecules ($\phi_f \approx 10^{-6}$), their contribution to the neutron signal can be neglected and thus

$$S = \phi_D S_D + \phi_H S_H + \phi_p S_p, \quad (9)$$

with

$$\phi_D + \phi_H + \phi_p = 1. \quad (10)$$

Using Equations (8)–(10), ϕ_D could be determined from T_S ,

$$\phi_D = \frac{\ln T_S + (1 - \phi_p)S_H d + \phi_p S_p d}{(S_H - S_D)d}, \quad (11)$$

and ϕ_H could be obtained similarly, since ϕ_p was known (Sec. II A).

For every component, i , the attenuation coefficient S_i was calculated using the total neutron cross sections, σ_j , of each kind of nucleus, j , in the material,^{42,43}

$$S_i = \frac{\rho_i}{M_i} N_A \sum_j n_{i,j} \sigma_j, \quad (12)$$

where $n_{i,j}$ is the number of each nucleus j , ρ_i the material density, M_i the molar mass, and N_A the Avogadro's constant. Polyacrylamide consists of repeated units C_3H_5NO of molar mass $M_p = 71.08$ g/mol and has a density $\rho_p = 1.30$ g/cm³. Total neutron cross sections, σ_j , can be found in data libraries:^{42,43} $\sigma_H = 82.36$ b, $\sigma_C = 5.5555$ b, $\sigma_N = 13.41$ b, and $\sigma_O = 4.23219$ b for a neutron wavelength of $\lambda = 0.1798$ nm. This yields an attenuation coefficient $S_{p,H} = 4.913$ cm⁻¹. If the hydrogels are swollen in D₂O instead of H₂O, hydrogen might be replaced by deuterium ($\sigma_D = 7.64052$ b), resulting in $S_{p,D} = 0.799$ cm⁻¹ (if all H are replaced by D). For light and heavy water, $S_H = 5.648$ cm⁻¹ and $S_D = 0.651$ cm⁻¹, respectively, were calculated similarly.

The attenuation coefficient of the polymer, S_p , could not be measured easily. Because the value of S_p has a negligible effect on the values of the determined diffusion coefficients of solvent and solute, the calculated values were used (see the supplementary material).⁶⁹ However, S_D and S_H were also determined experimentally because they strongly depend on the setting of the instrument (e.g., filters and apertures), the wavelength distribution of the neutrons, and also on the sample properties, such as density and possible impurities. Neutron radiographs of a series of H₂O–D₂O mixtures were investigated. Their absolute transmission is (Eqs. (8) and (9))

$$-\ln T_S = (\phi_D S_D + \phi_H S_H)d = (S_D - S_H)d\phi_D + S_H d, \quad (13)$$

with $\phi_H = 1 - \phi_D$. Thus, $-\ln T_S(\phi_D)$ decays linearly with slope $(S_D - S_H)d$ and intercept $S_H d$ (Fig. 1). It was found that $S_H = 4.067$ cm⁻¹ and $S_D = 0.604$ cm⁻¹ best describe the experimental data. Although these values significantly deviate from the values calculated for $\lambda = 0.1798$ nm, they agree with values previously measured at ICON.⁴⁴ Interestingly, the value of the neutron attenuation coefficients S_D , S_H , and S_p was found to not affect the value of the diffusion coefficient determined by a fit to the transmission in the center of the hydrogel (Sec. IV B).

The neutron radiographs of the H₂O–D₂O mixtures were recorded in the same sample cell as used in the diffusion experiments, with the optical imaging setup in place and with an expected sample thickness of $d = 0.101$ cm. The sample cell was not moved between the measurements but rinsed with an appropriate amount of the mixture to be measured next to match the experimental conditions to those of the actual experiments. This was important because for H₂O the measured absolute transmission decreases with increasing distance of the sample from the scintillator due to incoherent scattering.^{45,46} Furthermore, the sample thickness, d , might

FIG. 1. Absolute neutron transmission, T_S , of mixtures of D₂O and H₂O with their volume fractions, ϕ_D and ϕ_H , respectively. The mixtures were contained in the same sample cell as used for the other experiments. The transmission, T_S , was averaged over an area of 40×40 pixels in the top, center, and the bottom of the cell (as indicated). A linear fit (Eq. (13)) was applied to the individual data sets (lines of corresponding colors) and their average (red line).

slightly vary with sample position although care was taken when the sample cells were manually screwed together. The variation in d was estimated by determining the transmissions at the top, center, and bottom of the cell separately (Fig. 1). Indeed, the transmission T_S slightly depends on the location as does the slope of $-\ln T_S$ versus the H₂O–D₂O composition. We found that the thickness at the top and bottom differed by about -3% and $+2\%$ from that at the center, respectively; thus, $d = 0.101 \pm 0.003$ cm. Hence, in the data analysis, the sample thickness d has to be considered carefully. This is particularly important for large H₂O content, where T_S shows a stronger variation due to the larger total neutron cross section of hydrogen.

In general, hydrogen in H₂O mainly attenuates the neutron beam by incoherent scattering, which influences the measured transmission. This can lead to blurry radiographs and an underestimated H₂O content. It was previously shown that incoherent scattering from H₂O samples plays a dominant role for sample thicknesses greater than 0.2 cm.^{47,48} As our samples are thinner and we observe a very sharp interface between the reservoir edge and the sample cell in the neutron radiographs³¹ (not shown), these effects are neglected in the present experiments.

b. Fluorophore concentrations from fluorescence imaging. For all fluorescent molecules studied here, a linear relation between fluorescence intensity and concentration was found in the concentration range of 0–50 μ M. Thus, the intensity can directly be related to the concentration, ϕ_f . However, the absolute concentration could not be deduced from the intensity as it is affected by the illumination and the alignment of the sample cell as well as photobleaching, which might also have occurred before the measurement. The absolute detected intensities between repeat measurements performed under the same experimental conditions varied by less than 15%. A normalization of the raw intensities with the initial and final values of the intensity inside the hydrogel, I_i and I_∞ , respectively, was performed to obtain a normalized volume fraction of fluorescent molecules,

$$\phi_f = (I - I_i)/(I_\infty - I_i). \quad (14)$$

This resulted in a very good agreement of ϕ_f between different measurements and hence indicates good reproducibility.

c. Hydrogel radius from optical imaging. For every time step, the hydrogel radius was determined from the optical transmission images. The area, $A(t)$, taken up by the hydrogel was determined by applying a reasonable threshold and using the image processing software ImageJ. Then the radius of a circle with the equivalent area, $a(t) = \sqrt{A(t)/\pi}$, was calculated.⁴⁹

3. Spatial averaging

The time dependences of the detected signals, i.e., of the absolute neutron transmission, $T_S(x, y, t)$, and the fluorescence intensity, $I(x, y, t)$, were analyzed in two ways. First, the signal measured in a limited region of the sample, here the center of the hydrogel, was considered as a function of time. To improve statistics, the average over an area of 20×20 pixels, which is small compared to the hydrogel disc radius ($a \approx 350$ pixels), was determined for each image.

In a second approach, intensity profiles were calculated for each time step. An image recorded shortly before (NR) or immediately after (FI) the hydrogel was contacted with solution was used to define the initial hydrogel-solution interface. This was used to calculate for each pixel its shortest distance, r^* , to the initial interface using a Danielsson algorithm.⁵⁰ By averaging all signals at positions with the same r^* , profiles were calculated. An upper threshold was applied in the averaging process to remove bright spots in the neutron radiographs that are caused by, e.g., high intensity γ -particles. Due to the macroscopic sample size, this approach yielded very good statistics except in the center of the hydrogel disc. In the following, we use the normalized distance to the center of the hydrogel, $r/a = 1 - r^*/a$.

4. Photobleaching correction

Even though the excitation light was not focused into the sample and its intensity was minimized, photobleaching of the fluorescent dyes was observed especially for long measurements of several hours. This made a photobleaching correction necessary.^{51,52}

We considered a region in the reservoir around the hydrogel, where for long times the decrease of the fluorescence intensity is mainly governed by bleaching rather than dilution. The time-dependence of the fluorescence intensity of this region was fitted by an exponential decay, which yielded the time-dependent bleach parameter, $\xi(t)$. Then the intensities, $I(x, y, t)$, or equivalently, the intensity profiles, $I(r, t)$, were corrected by dividing through $\xi(t)$.

It was found that the bleaching behavior in the reservoir and inside the hydrogel was not necessarily identical. Thus, a long measurement was performed. The intensity in the center of a fully swollen 1:60 hydrogel is expected to initially increase and then saturate when the equilibrium concentration is reached. However, for long times, the intensity decreases due to photobleaching (Fig. 2). The long-time behavior again was fitted by an exponential decay, yielding $\xi(t)$, which was then

FIG. 2. Normalized fluorescein concentration, $\phi_{Fluo}(0, t)$, in the center ($r = 0$) of a hydrogel (sample A) with radius a (Table II) as a function of time, t , without (black squares) and with (blue circles) correction for photobleaching. Photobleaching is assumed to lead to an exponential decay of the intensity (red line). The magenta crosses indicate $\phi_{Fluo}(0, t)$ obtained at very few times, t , thus avoiding photobleaching.

used for the photobleaching correction inside the hydrogel. The procedure was tested by performing measurements during which photobleaching was avoided by illuminating the sample only occasionally, yielding very similar results (Fig. 2).

III. DIFFUSION — THEORETICAL MODELS

The cylindrical hydrogels were sandwiched between two parallel glass plates, which prevent flux through the faces of the hydrogels. Thus, the hydrogels can be described as infinitely long cylinders with radial diffusion only. These cylinders are immersed in a finite unstirred reservoir. To our knowledge, there is no model that considers such a boundary condition and different diffusion coefficients in the cylinder and reservoir. Thus, different solutions for the cylindrical geometry with similar, although not identical, boundary conditions were compared and used to interpret the data.

A. Models 1a and 1b: Cylinder in a stirred, infinite, or finite reservoir

The first model describes radial diffusion into a cylinder of radius a and volume V_{cyl} immersed in a stirred reservoir of volume $V_{res} = \beta V_{cyl}$. This situation is identical to an unstirred reservoir with an (infinitely) large diffusion coefficient in the reservoir. Due to stirring, the concentration in the reservoir, $\phi_{res}(t)$, is uniform and only depends on time and the initial concentration $\phi_{res,i}$. The initial and final concentrations inside the cylinder are denoted as ϕ_i and ϕ_∞ , respectively. Mass conservation implies $\phi_\infty = V_{res} \phi_{res,i} / (V_{res} + V_{cyl})$ in the cylinder and reservoir at equilibrium. Thus, $\phi_{res}(t)$ decreases with time. These boundary conditions lead to the following expression for the time and position dependent concentration in the cylinder,⁵³

$$\frac{\phi(r, t) - \phi_i}{\phi_\infty - \phi_i} = 1 + \sum_{n=1}^{\infty} \frac{(4 + 4\beta) \exp(-Dq_n^2 t/a^2) J_0(q_n r/a)}{(4 + 4\beta + \beta^2 q_n^2) J_0(q_n)}, \quad (15)$$

where D is the diffusion coefficient, q_n are the positive roots of $\beta q_n J_0(q_n) + 2J_1(q_n) = 0$, J_0 is the Bessel function of the first kind of order zero, and J_1 is the Bessel function of the first order. In the following, this will be called model 1b.

In the case of an infinite reservoir ($\beta \rightarrow \infty$, model 1a), $\phi_{\text{res}}(t)$ remains constant and the concentration in the cylinder, $\phi(r, t)$, becomes⁵³

$$\frac{\phi(r, t) - \phi_i}{\phi_\infty - \phi_i} = 1 - \frac{2}{a} \sum_{n=1}^{\infty} \frac{\exp(-D\alpha_n^2 t) J_0(r\alpha_n)}{\alpha_n J_1(a\alpha_n)}, \quad (16)$$

where α_n are the positive roots of $J_0(a\alpha_n) = 0$.

Although this model assumes a stirred reservoir, for an unstirred reservoir, it becomes a good approximation if diffusion outside the cylinder is very fast compared to diffusion inside the cylinder, which results in an approximately uniform concentration in the reservoir. However, if the experimentally observed concentration profile outside the cylinder is not constant, it cannot be described by this model.

B. Model 2: Cylinder in an unstirred, infinite reservoir

Since in this model the reservoir is not stirred, a concentration profile develops inside and outside the cylinder. Inside the cylinder, the concentration increases from the initial uniform concentration ϕ_i to the uniform equilibrium concentration ϕ_∞ . By contrast, in the reservoir, the concentration is initially uniform, $\phi_{\text{res},i}$, then decreases toward the cylinder to eventually rise again towards the initial, uniform reservoir concentration, $\phi_{\text{res},i}$, due to the infinite reservoir volume. The concentration profiles can be calculated inside and outside the cylinder,⁵³

$$\phi(r, t) = \phi_\infty + \frac{\phi_i - \phi_\infty}{2Dt} e^{-r^2/4Dt} \int_0^a e^{-r'^2/4Dt} I_0\left(\frac{rr'}{2Dt}\right) r' dr', \quad (17)$$

with I_0 , the modified Bessel function of the first kind of order zero. In the center of the cylinder ($r = 0$), a much simpler expression is found,

$$\phi(0, t) = \phi_i + (\phi_\infty - \phi_i) e^{-a^2/4Dt}. \quad (18)$$

In both models, the diffusion coefficient is assumed to be independent of concentrations of the diffusing molecules. Although in our experiments the local concentrations change over the course of a measurement, their effect on the diffusion coefficient is considered small enough to be neglected. Furthermore, the concentration profile covers the entire hydrogel and a large part of the reservoir, but only the profile in the hydrogel is considered. Thus, the reservoir only enters through the concentration at the interface of the cylinder which was fitted. Emphasis is given to the diffusion in the hydrogel and the diffusion coefficient in the reservoir is assumed to be identical to the one in the hydrogel to reduce the number of free parameters. The above models are compared for typical values of our experiments in the supplementary material.⁶⁹

IV. RESULTS AND DISCUSSION

A. Images and profiles

Applying OI, FI, and NR simultaneously, the evolution of the systems was followed in order to study the diffusion of solvent and solute into polymer hydrogels. The obtained data are illustrated for the case of a 1:60 hydrogel swollen in D₂O with a degree of swelling $m_{\text{ds}} = 0.75$ that was contacted with fluorescein in H₂O (Fig. 3). OI indicates that the size of the hydrogel remained about constant, i.e., the hydrogel did not swell or shrink significantly (Fig. 3(a)). A slight change in contrast due to solvent and solute ingress can be observed in the OI images. Additionally, small defects, such as an air bubble in the hydrogel, can be identified in the brightfield images and these areas were omitted in the averaging process of the other imaging techniques. As observed by FI, the fluorescence intensity and thus the dye concentration inside the hydrogel increased with time and the front broadened (Fig. 3(b)). The same small air bubble is observed with FI as in OI. NR shows that the neutron transmission inside the hydrogel decreased with time starting at the hydrogel-solution interface, which also becomes blurred (Fig. 3(c)). Due to the significantly higher neutron attenuation coefficient of H₂O compared to D₂O, this implies that H₂O diffused into the hydrogel. Thus, mixing of the solvents inside and outside the hydrogel can be captured by NR. In NR the air bubble is better visible at later times as the contrast between air and D₂O is much smaller than between air and H₂O.

The images were quantitatively analyzed to yield the time-evolutions of the concentration profiles and of the concentration in the center of the hydrogel (Sec. II C 3). Fig. 4 shows profiles of the fluorescence intensity, $I(r, t)$, and hence volume fraction of the fluorescent molecules, $\phi_f(r, t)$, as well as neutron transmission, $T_S(r, t)$, and hence H₂O volume fraction, $\phi_H(r, t)$, for different times. The data were obtained from four simultaneous FI (top row) and NR (middle and bottom row) experiments, with different hydrogels and diffusants. The effect of the crosslinking ratio (Figs. 4(a) and 4(b)) as well as the molar mass and hence the size of the diffusant, namely, fluorescein, 10 kDa, and 70 kDa dextrans (Figs. 4(b)-4(d)) is illustrated. The profiles depicted in Fig. 4(b) correspond to the images shown in Fig. 3.

The fluorescence intensity profiles, $I(r, t)$, were normalized to 0 inside the initial hydrogel and 1 in the reservoir close to the hydrogel (Eq. (14)). They can directly be related to the concentration of fluorescent molecules, $\phi_f(r, t)$ (Sec. II C 2 b). With time, $I(r, t)$ increases inside the hydrogel and reaches further into the hydrogel while it decreases outside the hydrogel. The profiles hence reflect the ingress of fluorophores. The changes in the concentration profiles occur at a slower rate as the size of the diffusing molecules increases. By contrast, a decreasing crosslinking ratio within the range studied does not seem to influence the dye diffusion very strongly.

For some samples, a particularly high fluorescence intensity was observed close to the hydrogel-solution interface. This can be seen as a peak in $I(r, t)$ that becomes more pronounced with time (Fig. 4(a)). It is

FIG. 3. Series of simultaneously recorded (a) optical brightfield images, (b) fluorescence images, and (c) normalized neutron radiographs of a 1:60 hydrogel swollen in D_2O with a degree of swelling $m_{\text{ds}} \approx 0.75$, contacted with fluorescein in H_2O at times since contact as indicated.

FIG. 4. (top) Intensity profiles, $I(r, t)$, determined by FI and (middle) transmission profiles, $T_s(r, t)$, and (bottom) H_2O volume fraction profiles, $\phi_{\text{H}}(r, t)$, both based on NR as a function of the normalized distance to the hydrogel cylinder, r/a , at different times, t , after contact with solution (as indicated). The samples consisted of D_2O -hydrogels with different crosslinking ratios and a degree of swelling, $m_{\text{ds}} \approx 0.75$ (as indicated), that were contacted with H_2O solutions containing different fluorescent molecules (fluorescein, 10 kDa dextran, 70 kDa dextran as indicated).

attributed to scattering at the interface which may neither be smooth nor perpendicular to the illumination, or different fluorescence intensities inside, in the vicinity or outside the hydrogel. This is supported by the finding that no systematic behavior was observed. In the present system, a higher intensity inside the hydrogel compared to the reservoir is not caused by an increased fluorophore concentration due to attractive interactions between the hydrogel and the diffusing molecules.²⁹ In many measurements, the intensities inside and outside the hydrogel were identical after equilibration. A similar fluorescence intensity peak just inside the hydrogel was previously seen when concentration profiles of fluorescent macromolecules diffusing into HEMA/MAA hydrogel slabs were observed by confocal microscopy.²⁰ In those experiments, the hydrogel slabs were immersed in fluorescent macromolecule solution and removed for taking the confocal images at predefined times. Thus the concentration profiles could not be measured *in situ*. The observed intensity peaks were attributed to a locally dried hydrogel surface due to the measurement procedure and neglected in their analysis. Interestingly, the authors found that the measured concentration profiles did not change whether the reservoir was stirred between taking the confocal images or not.²⁰

The neutron transmission was converted to H₂O volume fraction profiles, $\phi_H(r,t)$, using Eq. (11). They reflect the solvent ingress into the hydrogel and the increasing amount of H₂O in the hydrogel. At the same time, the H₂O content in the reservoir slightly decreases. Solvent ingress occurs much faster than solute ingress due to the smaller size of the solvent molecules compared to the size of fluorescein.

In contrast to the FI results, the neutron transmission, $T_S(r,t)$, does not show an increased intensity at the interface. The observed neutron transmission is less affected by scattering since the same structures, here the hydrogel-solution interface, scatter at smaller scattering angles due to the smaller wavelength of neutrons compared to light. Neutrons scattered by relatively large structures are scattered in forward direction and thus arrive in (about) the same pixel as the transmitted neutrons. Thus, scattering remains largely unnoticed.

The increase in H₂O volume fraction, $\phi_H(0,t)$, in the center of the hydrogels (Fig. 5) provides the time scale and thus the diffusion coefficient of the influx and also facilitates the comparison of different measurements more directly. The addition of small fluorescent molecules or larger macromolecules as well as the crosslinking ratio of the hydrogel does not influence the diffusion behavior of the solvent very strongly. This is consistent with the similarity of the solvent profiles of the four measurements shown in Fig. 4 (bottom). Nevertheless, with increasing solute size, $\phi_H(0,t)$ appears to be very slightly shifted to longer times, indicating slightly slower diffusion of the solvent in the presence of larger molecules. This is supported by the additional observation that the presence of fluorescein appears to slow down solvent diffusion compared to its absence, i.e., pure water (Fig. 5, inset). This might be due to the hydration of the solutes, which slows down a small part of the solvent molecules.

FIG. 5. Normalized H₂O volume fraction, $\phi_H(0,t)$, in the center of the hydrogel with radius a as a function of time t after the D₂O-hydrogel has been contacted with H₂O solutions for different sample compositions, i.e., crosslinking ratios, degree of swelling $m_{ds} \approx 0.75$, and fluorescent molecules (fluorescein, 10 kDa dextran, 70 kDa dextran as indicated). The inset shows $\phi_H(0,t)$ at short times and includes a hydrogel contacted with pure H₂O. Small differences in the initial disc radii, a , are accounted for by normalizing the time t with the squared radius, a^2 .

B. Quantitative analysis and interpretation

The sequence of images (e.g., Fig. 3), profiles (e.g., Fig. 4), and data at the center (e.g., Fig. 5) is quantitatively interpreted using the models presented above (Sec. III). Due to their specific features, all four combinations of hydrogel solvent (H₂O or D₂O) and contacting solvent (H₂O or D₂O) can provide information on different aspects of the diffusion of solvent and solute. Thus, they are presented and discussed in turn.

1. H₂O-hydrogels contacted with D₂O solutions

Two different hydrogels with a crosslinking ratio of 1:60, fully (sample A) and partially (sample B) swollen in light water (H₂O) were contacted with D₂O containing fluorescein (Table II).

a. Hydrogel size from optical imaging. Optical bright-field transmission imaging allows us to follow the changes in the hydrogel. Slight decreases in the hydrogel radii of 4% and 0.4% were observed for samples A and B, respectively (Table II). Initially the polymer network was swollen in H₂O, while the D₂O-content increased during the experiments. Polyacrylamide hydrogels are known to exhibit a different degree of swelling depending on the solvent.^{54,55} This is confirmed by the dependence of the polymer volume fraction in the fully swollen hydrogels, $\phi_{p,fs}$, on the solvent: $\phi_{p,fs} = 0.053$ and $\phi_{p,fs} = 0.038$ for D₂O and H₂O, respectively (Table I). Fully swollen H₂O-hydrogels were found to shrink when contacted with D₂O and D₂O-hydrogels to swell when contacted with H₂O. However, some H₂O-hydrogels were also observed to shrink after contact with H₂O, indicating that factors other than solvent quality can also induce size changes. Due to the very small fluorophore concentration, osmotic deswelling of the hydrogels is not expected. This is confirmed by the observation that equilibrated, fully swollen hydrogels did not change their size within two weeks after

TABLE II. Sample composition and size for measurements of H₂O-hydrogels contacted with D₂O solution containing fluorescein; crosslinking ratio cr , degree of swelling m_{ds} , initial polymer and H₂O volume fractions, $\phi_{p,i}$ and $\phi_{H,i}$, respectively, initial and final hydrogel radii, a_i and a_{∞} , respectively, and initial sample thickness, d_i . The final D₂O volume fraction, $\phi_{D,\infty}$, was calculated based on the composition and fitted using models 1a, 1b, and 2. The diffusion coefficient of D₂O was obtained by fitting models 1a, 1b, and 2 to the data in the center. The whole profile and the FI data were analyzed based on model 1a only. Values in brackets correspond to the average values of three to five additional long FI measurements during which equilibration of the hydrogel and reservoir was achieved.

	Sample A	Sample B	Sample C
cr	1:60	1:60	1:60
m_{ds}	1.00	0.64	1.00
$\phi_{p,i}$	0.038	0.060	0.038
$\phi_{H,i}$	0.962	0.940	0.962
a_i [cm]	0.310	0.489	0.341
a_{∞} [cm]	0.298	0.487	0.325
d_i [cm]	0.146	0.098	...
	(0.150)	(0.100)	(0.100)
Final D ₂ O volume fraction $\phi_{D,\infty}$			
Expected	0.875	0.745	...
Models 1a & b	0.880	0.753	...
Model 2	1.250	1.100	...
D ₂ O diffusion coefficient D_D [10^{-6} cm ² /s]			
1a, profile	17.5 ± 0.2	19.8 ± 0.2	...
1a, center	17.5 ± 0.1	19.8 ± 0.2	...
1b, center	17.1 ± 0.1	19.1 ± 0.2	...
2, center	19.2 ± 0.6	22.9 ± 0.5	...
Fluorescein diffusion coefficient D_{Fluo} [10^{-6} cm ² /s]			
1a, profile	4.09 ± 0.02	3.71 ± 0.03	...
	(4.01 ± 0.02)	(3.68 ± 0.02)	(3.89 ± 0.02)
1a, center	4.07 ± 0.03	3.60 ± 0.03	...
	(4.02 ± 0.01)	(3.62 ± 0.02)	(3.85 ± 0.01)

contact with solutions with even higher dye concentrations. Moreover, deswelling was occasionally seen after hydrogels were contacted with pure water. An apparent shrinking of the hydrogel might also be caused by a slight expansion of the sample cell.

b. Diffusion of solvent into the hydrogel. Neutron radiography allows us to follow the diffusion of solvent. In a first step, only the center of the hydrogel disc is considered. Figure 6(a) shows the time dependence of the D₂O volume fraction in the center of the hydrogels, $\phi_D(0,t)$, for both samples. Since $\phi_D(0,t)$ depends on the radius, a , of the hydrogel, the time axis is scaled as t/a^2 (Fig. 6(b)). This almost leads to an overlap of the two NR curves, indicating that the diffusion of D₂O hardly depends on the initial degree of swelling. All three models have been fitted to the data (Table II and Fig. 6(a)). Models 1a and b both describe the data very well. By contrast, model 2 fails to fit the long time behavior. If the final volume fraction, $\phi_{D,\infty}$, is fixed to an unrealistically high value, at least the initial rise in $\phi_D(0,t)$ can be fitted. In the fully swollen hydrogel A, the diffusion coefficient, D_D , is very close to the bulk diffusion coefficient of D₂O (1.7×10^{-5} cm²/s).^{56–58} For the partially

FIG. 6. Normalized volume fractions of D₂O, $\phi_D(0,t)$, and fluorescein, $\phi_{Fluo}(0,t)$, in the center of the H₂O-hydrogels with radius a (samples A and B, Table II) as a function of (a) time, t , after contact with D₂O solution, (b) normalized time, t/a^2 , and (c) $\tau = Dt/a^2$ with D the diffusion coefficient as determined based on model 1a (Table II) by simultaneous FI and NR measurements. The FI data in (c) were complemented by long repeated measurements of both samples. Lines in (a) represent fits based on models 1a (red), 1b (blue), and 2 (green).

swollen hydrogel B, D_D , as determined using model 1 is about 12% larger (Sec. IV B 1 d). The corresponding fitted $\phi_{D,\infty}$ are only slightly higher than expected (Table II).

Figure 7 shows the experimentally determined D₂O volume fraction profiles, $\phi_D(r,t)$, for sample B. To take hydrogel swelling into account, for each time step, the actual radius, $a(t)$, was determined from the corresponding brightfield image and used in the model calculations. The D₂O volume fraction decreases outside the hydrogel, but at the interface ($r = a$) the concentration is essentially constant. Thus, model 1b, which predicts a decreasing concentration at the interface, is not appropriate and especially fails close to the hydrogel border. By contrast, model 1a describes the profiles inside the hydrogel very well although it assumes a homogeneous reservoir concentration. In the following, model 1a will be used to interpret the data. The fitted values of the diffusion coefficients (Table II) are identical to the values obtained by considering the center only. On the other hand, the decrease of $\phi_D(r,t)$ in the reservoir can be fitted by model 1b, yielding a diffusion coefficient about 6% larger than derived from the data in the center.

FIG. 7. Normalized D₂O volume fraction profiles, $\phi_D(r,t)$, for different times (as indicated) after the H₂O-hydrogel (sample B) has been contacted with D₂O solution containing fluorescein as obtained by NR. Fits based on models 1a (red lines) and 1b (blue lines) as well as the data are corrected for the time dependence of the hydrogel radius, $a(t)$.

c. Diffusion of fluorescent dye into the hydrogel. The increase of the fluorophore concentration in the center of the hydrogel was followed (Fig. 6(a)). The restriction to the center of the hydrogel avoids effects of the hydrogel-solution interface like scattering (Sec. IV A). However, in the center equilibrium was not reached during the measurement time due to the slower diffusion compared to that of the solvent (and the limited neutron beam time). Thus the equilibrium values of the fluorescein concentration, $\phi_{\text{Fluo},\infty}$, which are required to normalize the data, were extracted from a region closer to the hydrogel border, where saturation was reached earlier. In contrast to the time dependence of the D_2O volume fraction, $\phi_{\text{D}}(0,t)$, scaling of the time t with a^2 does not lead to an overlap of the FI data (Fig. 6(b)). This indicates a smaller diffusion coefficient of fluorescein in the partially swollen sample B than in the fully swollen sample A. Analysis with model 1a yields diffusion coefficients $D_{\text{Fluo},\text{A}} = 0.407 \times 10^{-5} \text{ cm}^2/\text{s}$ and $D_{\text{Fluo},\text{B}} = 0.360 \times 10^{-5} \text{ cm}^2/\text{s}$. If the normalized concentration curves are plotted as a function of the dimensionless time $\tau = Dt/a^2$, all four curves of samples A and B agree (Fig. 6(c)). They also agree with longer FI measurements, which were performed outside the neutron beamline.

In the experimental data, the initial plateau in the concentration trace, which represents the time needed for the first fluorescent molecules to reach the hydrogel center, is not always as pronounced as expected. The reason for this is a not perfectly perpendicular and smooth hydrogel border leading to an already initially broadened concentration profile.

The normalized fluorescein concentration profiles, $\phi_{\text{Fluo}}(r,t)$, were also fitted by model 1a. The fit parameter $\phi_{\text{Fluo},\infty}$ was adjusted for each profile individually such that it matched the experimental value at the hydrogel-solvent interface. This accounts for the effect of photobleaching and for potential changes in sample thickness.

As already discussed in Section IV A, in some samples a particularly high fluorescence intensity close to the hydrogel-solvent interface was found, which was interpreted as an artifact caused by scattering from the interface. The corresponding peak in the concentration profiles propagates further into the hydrogel with increasing time (as seen in Fig. 8) and leads to an inflection point in the profiles that cannot be fitted using model 1a. For short and intermediate times, the fit works nicely, if the region close to the hydrogel-solution interface is ignored. The validity of this fitting procedure was proven by many additional measurements (outside the neutron beamline), where this artifact was not seen and which yielded comparable diffusion coefficients. This approach was also used previously by another group.²⁰

For all FI measurements, three to five additional long measurements, during which equilibration of the hydrogel and reservoir was achieved, were performed and the results averaged (values in brackets in Table II). The results of the individual measurements and analysis of the center intensities and complete concentration profiles show good agreement (Table II). Thus, rather short measurement times appear sufficient, which is favorable for the combined imaging experiments requiring central facilities.

d. Dependence of diffusion coefficients on hydrogel properties. The diffusion of solvent and fluorophore was

FIG. 8. Normalized fluorescein concentration profiles, $\phi_{\text{Fluo}}(r,t)$, for different times (as indicated) after the H_2O -hydrogel (sample B) has been contacted with D_2O solution containing fluorescein as obtained by FI. Fits based on model 1a (red lines) as well as the data are corrected for the time dependence of the hydrogel radius, $a(t)$.

found to depend on the hydrogel studied (samples A and B). These differed in thickness and their degree of swelling, which affects the pore size and influx of solvent. To disentangle the effects of these two parameters, we performed FI measurements with a third sample, which had the degree of swelling of sample A and the thickness of sample B (sample C, Table II).

The diffusion coefficient of fluorescein, D_{Fluo} , increases from sample B to C to A. The reduction of the mean pore size by partial drying of sample B thus leads to a slower fluorescein diffusion. As compression to $d = 0.1 \text{ cm}$ (sample C) also influences the solute diffusion, it appears to affect the pore structure within the hydrogels. With $d = 0.15 \text{ cm}$, the fully swollen hydrogel discs (sample A) are compressed as little as possible to still guarantee contact with both glass plates. This is hence expected to result in the largest mean pore size and indeed yields the largest diffusion coefficient. Thus the diffusion coefficients reflect the obstruction by pores of different sizes. This is consistent with previous observations on various hydrogels and solutes^{24,26,59–61} as well as theoretical predictions.^{18,27,62}

The diffusion coefficient of D_2O decreases from sample B to A. The diffusion of the much smaller D_2O molecules is expected to be less affected by the pore size, which points to another mechanism increasing its mobility. Even though H_2O is a better solvent than D_2O , the partially swollen H_2O -hydrogel with $\phi_{\text{p,i}} = 0.06$ (Table II) contains more polymer than a fully swollen D_2O -hydrogel ($\phi_{\text{p,fs}} = 0.053$, Table I) and is thus expected to take up solvent. This seems to speed up the D_2O influx in order to reach equilibrium.^{63–66} Since this follows similar time-dependencies as diffusion, it cannot be disentangled. This effect is expected to be even more pronounced for D_2O -gels contacted with H_2O , which will be discussed in Section IV B 3.

2. D_2O -hydrogels contacted with D_2O solutions

During the handling of D_2O -hydrogel samples the uptake of moisture (H_2O) has to be avoided. To validate the sample preparation and storage procedure, one short combined

OI, FI, and NR measurement was performed. A hydrogel partially swollen in D₂O with $m_{ds} = 0.75$ and a crosslinking ratio of 1:60 was contacted with D₂O solution containing fluorescein. Although no change in the neutron transmission was expected, a slight increase in transmission inside the hydrogel was monitored (data not shown). This suggests that some H₂O was contained in the initial D₂O-hydrogel. To determine the fraction of H₂O in the initial hydrogel, the sample thickness, d , is required. Its determination from the neutron transmission of the initial hydrogel is not possible due to the uncertainty in the sample composition. Instead, the transmissions of four regions in the reservoir around the hydrogel right after injection of the D₂O solution were used to determine the sample thickness, $d = 0.098$ cm, which is close to the expected value (0.101 cm). With this value, the initial composition of the sample was calculated (Eq. (11)): $\phi_{p,i} = 0.072$, $\phi_{D,i} = 0.768$, and $\phi_{H,i} = 0.160$. The initial D₂O-hydrogels hence may contain a significant amount of H₂O that originates from moisture during sample preparation and storage. Therefore, the possible contamination with H₂O has been taken into account in the analysis of all measurements with D₂O-hydrogels (Sec. IV B 3).

Having determined the initial fraction of H₂O, not only the diffusion coefficient of fluorescein but also of D₂O was determined. The D₂O volume fraction inside the hydrogel increased from $\phi_{D,i} = 0.768$ to $\phi_{D,\infty} = 0.890$ in equilibrium. This relatively small increase and the short measurement time rendered an analysis based on the data in the center of the hydrogel unreliable. Additionally, the measurement time was not long enough for the fluorescein concentration to approach saturation. Thus, an analysis of the data in the center was not performed. However, the volume fraction profiles were analyzed using model 1a, which resulted in $D_D = 1.89 \times 10^{-5}$ cm²/s and $D_{Fluo} = 0.369 \times 10^{-5}$ cm²/s, consistent with the above results (Table II). The other models also adequately described the NR data and yield the same D_D . This is due to the small absolute change in D₂O volume fraction, which renders the different boundary conditions less crucial (see the supplementary material).⁶⁹ This finding also supports the notion that, for short times, the models are essentially indistinguishable.

3. D₂O-hydrogels contacted with H₂O solutions

Imaging experiments on different D₂O-hydrogels contacted with a selection of fluorescent molecules solved in H₂O (Table I) were performed. The measurements and data analysis are illustrated for one D₂O-hydrogel with a crosslinking ratio of 1:60. This hydrogel was partially swollen, $m_{ds} = 0.78$, corresponding to an initial polymer volume fraction, $\phi_{p,i} = 0.068$, and was contacted with H₂O solution containing fluorescent 10 kDa dextran (Figs. 9–11). The same analysis procedure was applied to the data from the other D₂O-hydrogels contacted with H₂O solutions and the results are summarized in Table III.

a. Initial and equilibrium composition. From the initial neutron transmission of the solution around the hydrogel, $T_{S,i}^{sol} = 0.679$, the sample thickness was determined to be $d_i = 0.095$ cm. Using this value, $\phi_{p,i} = 0.068$, and the neutron

FIG. 9. Transmission, $T_S(0, t)$, and corresponding normalized H₂O volume fraction, $\phi_H(0, t)$, in the center of a partially swollen D₂O-hydrogel with crosslinking ratio of 1:60 as a function of time, t , after contact with H₂O solution containing fluorescent 10 kDa dextran calculated assuming different polymer volume fractions, ϕ_p , and sample thicknesses, d (as indicated). Lines are fits based on model 1a (see text for details).

transmission of the hydrogel before contact, $T_{S,i}^{gel} = 0.901$ (Fig. 9), $\phi_{D,i} = 0.793$, and $\phi_{H,i} = 0.139$ were determined. They are close to the initial D₂O–H₂O composition found for D₂O-hydrogels contacted with D₂O solutions (Sec. IV B 2).

Having determined the initial composition, we turn to the final, equilibrium composition after contact with the H₂O solution. During the measurement, the hydrogel radius increased from $a_i = 0.558$ cm to $a_\infty = 0.582$ cm as determined by OI. Based on a constant sample thickness, d , the final polymer volume fraction $\phi_{p,\infty} = 0.063$ was calculated. Moreover, $T_{S,\infty}^{gel} = 0.707$ (Fig. 9) implies a final H₂O volume fraction $\phi_{H,\infty} = 0.876$ (Eq. (11)). This value is significantly larger than expected based on the sample and reservoir volumes: $\phi_{H,EQ} = 0.723$. This is in contrast to findings with H₂O-hydrogels contacted with D₂O solutions (Sec. IV B 1), where expected and fitted equilibrium values were very similar. This could be due to the relatively large amount of H₂O in the sample, which might result in more scattering and hence might change the measured transmission. Furthermore, a possibly small deviation in d leads to a larger uncertainty in measured transmission (Fig. 1).

FIG. 10. H₂O volume fraction profiles, $\phi_H(r, t)$, for different times (as indicated) after the D₂O-hydrogel has been contacted with H₂O solution containing fluorescent 10 kDa dextran. The two limiting parameter sets were used for the calculations: (a) $\phi_{p,i} = 0.068$ and $d_i = 0.095$ cm and (b) $\phi_{p,\infty} = 0.054$ and $d_\infty = 0.110$ cm. Fits based on model 1a (red lines) and the data are corrected for the time dependence of the hydrogel radius, $a(t)$.

FIG. 11. Normalized fluorescent molecule volume fraction profiles, $\phi_f(r, t)$, for different times (as indicated) after the D₂O-hydrogel has been contacted with H₂O solution containing fluorescent 10 kDa dextran. Fits based on model 1a (red lines) as well as the data are corrected for the time dependence of the hydrogel radius, $a(t)$.

Thus, we do not assume a constant sample thickness, d , but calculate the apparent final sample thickness, d_∞ , based on the expected final H₂O–D₂O sample composition, $\phi_{H,EQ}/\phi_{D,EQ} = 3.83$, the transmission, $T_{S,\infty} = 0.707$, as well as the sample geometry, i.e., d_i , a_i , and a_∞ as given above. With Equations (10) and (11) and $\phi_{p,\infty} = (a_i^2 d_i / a_\infty^2 d_\infty) \phi_{p,i}$, this yields $\phi_{H,EQ} = 0.730$, $\phi_{D,EQ} = 0.216$, $\phi_{p,\infty} = 0.0539$, and $d_\infty = 0.110$ cm. These values appear more consistent and will thus be used in the following. The same analysis was performed for the other measurements where equilibration between H₂O and D₂O was reached. This resulted in very similar values for d_i , d_∞ , and $\phi_{p,\infty}$.

Thus, D₂O-hydrogels contacted with H₂O appear to swell towards the equilibrium state. The swelling appears to slightly change the sample thickness, d , which has to be considered carefully in the data analysis. In fact, the small mismatch between expected and fitted equilibrium sample compositions for H₂O-hydrogels contacted with D₂O solutions, which deswelled during the experiments (Sec. IV B 1) could be explained by a slight decrease in sample thickness: from

$d_i = 0.146$ cm to $d_\infty = 0.143$ cm for sample A and from $d_i = 0.098$ cm to $d_\infty = 0.096$ cm for sample B. This possible small change in d does not, however, affect the fitted values of the diffusion coefficients but only the absolute values of the volume fractions (see the supplementary material).⁶⁹ Similarly, a small variation of the polymer volume fraction, ϕ_p , is found to be negligible (see the supplementary material).⁶⁹

b. Diffusion of solvent into the hydrogel. Based on the limiting sets of parameters, the initial values $\phi_{p,i} = 0.068$ and $d_i = 0.095$ cm and the equilibrium values $\phi_{p,\infty} = 0.054$ and $d_\infty = 0.110$ cm, respectively, two limiting cases for the increase in H₂O volume fraction, $\phi_H(0, t)$, were calculated (Fig. 9). The two data sets represent the initial ($t < 3000$ s) and later behavior with the corresponding radii of the hydrogel. Fits based on model 1a and using $\phi_{H,i} = 0.139$ and the corresponding $\phi_{H,\infty} = 0.876$ (blue) and $\phi_{H,\infty} = 0.730$ (green) both yielded $D_H = 2.11 \times 10^{-5}$ cm²/s. The diffusion coefficient of H₂O, D_H , is slightly larger than that of D₂O, $D_D = 1.98 \times 10^{-5}$ cm²/s (Table II). This is consistent with previous findings in bulk.⁵⁷ Moreover, in the present situation, the difference might be enhanced since H₂O is the better solvent and hence preferentially enters the hydrogel.

With both parameter sets, concentration profiles for distinct time steps were calculated (Fig. 10). For short times ($t \lesssim 3000$ s) using the parameters describing the initial hydrogel and for long times ($t \gtrsim 3000$ s) using the parameters describing the final hydrogel, a fit yielded the same diffusion coefficient as the analysis based on the hydrogel center only. This supports the findings above and the interpretation that the sample thickness increased during the measurement.

c. Diffusion of fluorescent molecules into the hydrogel. For the same experiment, FI yielded concentration profiles of the fluorescent molecules, $\phi_f(r, t)$ (Fig. 11). FI is not sensitive to the isotope, and hence, the initial H₂O contamination of the D₂O-hydrogels did not have to be considered and the data could be analyzed as described in Section IV B 1. The normalized D10 volume fraction profiles, $\phi_f(r, t)$, were

TABLE III. Diffusion coefficients for H₂O, D_H , and fluorescent molecules, D_f , obtained by combined NR and FI for hydrogels with a different degree of swelling, m_{ds} , polymer volume fraction, $\phi_{p,i}$, crosslinking ratio, cr , initial sample thickness, d_i , and initial H₂O volume fraction, $\phi_{H,i}$. Additional longer FI measurements were performed and the listed diffusion coefficients, D_f , are the mean values of three to five measurements.

m_{ds}	$\phi_{p,i}$	cr	d_i (cm)	$\phi_{H,i}$	Fluorescent molecule	D_H (10^{-5} cm ² /s)	D_f (10^{-5} cm ² /s)
0.80	0.0664	1:60	0.095	0.139	...	2.13 ± 0.01	...
1.00	0.0530	1:60	0.099	0.005	Fluo	2.03 ± 0.02	0.382 ± 0.002
1.00	0.0650	1:15	0.096	0.010	Fluo	2.05 ± 0.02	0.360 ± 0.003
0.81	0.0656	1:60	0.097	0.089	Fluo	2.07 ± 0.02	0.359 ± 0.002
0.80	0.0664	1:60	0.099	0.120	Fluo	2.10 ± 0.02	0.352 ± 0.002
0.75	0.0709	1:60	0.102	0.159	Fluo	2.11 ± 0.02	0.341 ± 0.003
0.75	0.0870	1:15	0.096	0.173	Fluo	2.13 ± 0.02	0.295 ± 0.003
0.78	0.0681	1:60	0.095	0.139	D10	2.11 ± 0.01	0.076 ± 0.003
0.75	0.0709	1:60	0.101	0.164	D10	2.11 ± 0.02	0.071 ± 0.002
0.80	0.0815	1:15	0.090	0.138	D10	2.13 ± 0.01	0.070 ± 0.003
1.00	0.0530	1:60	0.100	...	D70	...	0.017 ± 0.003
0.74	0.0718	1:60	0.095	0.187	D70	2.05 ± 0.02	0.015 ± 0.004
0.73	0.0894	1:15	0.098	0.194	D70	2.08 ± 0.02	0.011 ± 0.004

fitted by model 1a (Fig. 11), yielding the diffusion coefficient of fluorescent 10 kDa dextran, $D_{D10} = 0.085 \times 10^{-5} \text{ cm}^2/\text{s}$. As this value is slightly higher than a published bulk value ($0.083 \pm 0.008 \times 10^{-5} \text{ cm}^2/\text{s}$),²² longer separate FI experiments were performed with D10 from another supplier (Invitrogen as in Ref. 22). These measurements yielded qualitatively consistent results, but slightly different (about 15% lower) diffusion coefficients (Table III). This is attributed to the different statistics and duration of the experiments as well as possibly, e.g., different average molar mass and polydispersity of the dextrans. In all measurements where a D₂O-hydrogel was contacted with H₂O solution containing fluorescent 10 kDa dextran, the fluorescence intensity inside and outside the hydrogel was identical after equilibration was reached. In addition, no enhanced intensity close to the hydrogel-solution interface was observed (Fig. 11).

d. Dependence of diffusion coefficients on hydrogel properties. The diffusion of different molecules into various hydrogels with different properties was studied. For all samples, the same analysis procedure as described above was performed. The initial sample thickness, d_i , and H₂O volume fraction, $\phi_{H,i}$, were determined. Then, the H₂O increase in the sample center, $\phi_H(0,t)$, and concentration profiles, $\phi_H(r,t)$, were calculated with the two limiting parameter sets and the diffusion coefficients, D_H , were deduced by fitting model 1a to the data. Due to the need to use central facilities, D_H was obtained from a single measurement only and thus is associated with a relatively large uncertainty. Nevertheless, we found good agreement between the values obtained by considering the center concentrations and profiles, respectively. With FI and OI, three to five additional longer measurements were performed and the concentration profiles of the fluorescent molecules, $\phi_f(r,t)$, were analyzed based on model 1a. For the largest and hence slowest dextran (D70), equilibrium was only reached after about 14 days (data not shown). Due to the very long measurement time, a very stable setup was required and at later times images were recorded only once per day to reduce photobleaching. The obtained diffusion coefficient $D_{D70} = 0.017 \times 10^{-5} \text{ cm}^2/\text{s}$ is consistent with the ones obtained in shorter measurements (Table III). Hence, again, short measurements seem to provide reliable results, as in the case of H₂O-hydrogels and D₂O solutions (Sec. IV B 1). The results for all samples are summarized in Table III.

The solvent diffusion coefficient is comparable to previous findings^{56–58} and slightly increases upon partial drying of the hydrogel. This is in line with the results presented in Section IV B 1 and might be related to the solvent uptake during swelling. By contrast, the fluorescent molecule diffusivity decreases with the reduced swelling of the initial hydrogel and hence with the decreasing pore size. In the case of D10, the decrease from the bulk value ($0.083 \pm 0.008 \times 10^{-5} \text{ cm}^2/\text{s}$)²² is less pronounced than for the larger D70 ($0.030 \pm 0.002 \times 10^{-5} \text{ cm}^2/\text{s}$)²² which drops very significantly (Table III). The decrease of the diffusion coefficient of Fluo is more pronounced than D10 and less than D70. A decrease of diffusion coefficients with decreasing pore size is due to the increasing obstruction by the hydrogel and consistent with previous findings in similar systems^{23,24,26,59,67}

and from theoretical considerations.^{18,27,62} By contrast, the larger drop in the case of Fluo compared to D10 might be due to specific interactions.²⁹

Furthermore, the H₂O diffusion coefficient, D_H , tends to very slightly drop with increasing size of the solute, consistent with the observations described above (Fig. 5).

4. H₂O-hydrogels contacted with H₂O solutions

For H₂O-hydrogels contacted with H₂O solutions, neutron radiography cannot provide information on solvent diffusion due to the missing contrast and hence was not performed. However, FI and OI experiments are able to yield information and were conducted. Figure 12 shows typical FI intensity profiles of fluorescein in H₂O diffusing into a H₂O swollen hydrogel.

Compared to the previously discussed results, a qualitatively different behavior is observed. The concentration profiles of the fluorescent molecules presented in Secs. IV B 1–IV B 3 (Figs. 4, 8, and 11) show an essentially constant concentration in the reservoir except close to the hydrogel. Here, however, for short and intermediate times, a pronounced intensity profile is developed outside the hydrogel. Furthermore, in the equilibrated state, the intensity of the fluorescent molecules in the hydrogel was higher than that in the solvent reservoir. The reasons for these effects are not clear. Optical effects might play a role, e.g., scattering from the hydrogel-reservoir interface or an enhanced fluorescence of molecules in a H₂O-swollen hydrogel compared to the same molecule in solution. Again, for measurements with fluorescent 10 kDa dextran, the same fluorescence intensity in the hydrogel and in the reservoir was observed after equilibration. While the concentration profiles well inside a H₂O-swollen hydrogel in H₂O solution and in other porous materials have been reported,^{20,21,68} we are not aware of any experimental study, where concentration profiles inside as well as outside a hydrogel have been measured and reported. Thus, we restricted our analysis to the concentration profiles inside the hydrogel. We again applied model 1a to quantitatively

FIG. 12. Normalized fluorescein concentration profiles, $\phi_{\text{Fluo}}(r,t)$, for different times (as indicated) after a H₂O-hydrogel with crosslinking ratio 1:60, a degree of swelling $m_{ds}=0.80$, and sample thickness $d_i=0.100 \text{ cm}$ have been contacted with H₂O solution containing fluorescein as obtained by FI. Fits based on model 1a (red lines) as well as the data are corrected for the time dependence of the hydrogel radius, $a(t)$.

analyze the data. If the region around the hydrogel-solution interface is ignored, this provided reasonable fits (Fig. 12) and a fluorescein diffusion coefficient $D_{\text{Fluo}} = 0.355 \times 10^{-5} \text{ cm}^2/\text{s}$.

The complete profiles, except the region around the hydrogel edge, qualitatively resemble profiles predicted by model 2. In a previous study, we used the dye alexa488 instead of fluorescein (at a slightly smaller concentration of up to 10 μM), while the hydrogels studied and measurement procedure were the same.²⁹ Interestingly, for those samples, the long time behavior could not be explained with model 1a, but with model 2. With fluorescence correlation spectroscopy measurements, we found evidence for adsorption of alexa488 to the polymer, which leads to its continued diffusion towards and enrichment inside the hydrogel. Hence, in the case of an adsorbing solute, the assumption of an infinite reservoir becomes more realistic, while for the present system, model 1a seems appropriate.

V. CONCLUSIONS

Using simultaneous neutron, fluorescence, and optical transmission imaging, we were able to distinguish solvent and solute diffusion into a polymer hydrogel and, at the same time, monitor macroscopic hydrogel swelling or shrinking. Polyacrylamide hydrogel discs, swollen in either H_2O or D_2O , were contacted with different sized fluorescent molecules, fluorescein, and fluorescein-labeled dextrans, dissolved in H_2O or D_2O . Based on the neutron and fluorescence images, solvent and solute concentration profiles, respectively, were determined. They were quantitatively interpreted using different models describing diffusion. The data were best described when the hydrogel was modeled as a cylinder in a stirred infinite reservoir or, equivalently, with a much larger diffusion coefficient outside than inside the hydrogel. Diffusion markedly slows down with increasing molecule size and also depends on the hydrogel properties as expected based on previous studies on similar systems.^{23,24,26,59,67} Increasing the crosslinking ratio or partially drying the hydrogel both reduce the pore size and were found to slow down solute diffusion, in particular, of the large solutes.

The determination of quantitative results required the careful and precise consideration of several experimental parameters. The sample thickness might change over the course of the experiment, the initial D_2O – H_2O composition in the hydrogels might be affected by moisture, and scattering from the hydrogel-reservoir interface can affect the results. If these effects are taken into account, the methods and procedures presented provide detailed quantitative information on the motion of the different species simultaneously. With additional filters, it would furthermore be possible to distinguish different fluorophore species and simultaneously study the diffusion of different macromolecules and their effect on each other.

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