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ORIGINAL PAPER

Sorption and Desorption of PVA-Pyrene Chains in and out of Agarose Gel

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Abstract In situ steady-state fluorescence (SSF) measurement technique was applied to investigation of pyrene labeled Poly(vinyl alcohol) (PVA-Py) molecules diffusion in and out of agarose gels. Gel samples with four different concentration of agarose were prepared. PVA-Py was synthesized by "click" chemistry method and dissolved in water to use in diffusion experiments. The results were analyzed by using Fickian type diffusion model, and it was found that sorption and desorption processes of PVA-Py molecules in and out of agarose gel have two distinct regions for short and long diffusion times. Sorption and desorption coefficients were measured and it was seen that the diffusion rates were much larger at short times and at lower agarose concentrations.

Keywords Agarose gel · Diffusion · Fluorescence · Poly(vinyl alcohol) · Pyrene

Introduction

The diffusion of small and large molecules through porous hydrogel structure is of great interest because of the large number of pharmaceutical, biological and environmental applications [1]. Based on this, size exclusion chromatography, electrophoretic separation, designing slow-release devices for drugs, developing artificial organs and producing storable foods are the most popular application fields in industry.

Agarose is an important member of polysaccharides family because of its unique properties and large usage fields. Agarose is a marine-polysaccharide extracted from agar which is produced by Rhodophyceae-type red algae. Pharmacology, chromatography, tissue engineering and food industry are some application areas of agarose gels [2, 3]. Molecular structure of agarose is consisting of β-1, linked D-galactose and α-1,4-linked 3,6-anhydro-α-L-galactose [4]. Agarose chains are essentially uncharged, however, it may contain a few ionized sulfate groups which are not exist in idealized neutral form [5, 6]. Agarose forms physically bonded thermoreversible gels when dissolved in water. It can not form a gel structure in organic solvents because of the absence of hydrogen bonding in aqueous solutions. Gelation and thermoreversible phase transitions of biomacromolecules involve intra- and inter-molecular hydrogen bondings, electrostatic and hydrophobic interactions leading to different supramolecular structures, and therefore are of high intrinsic interest [7–9]. As a result of sol–gel transition (<40°C), aqueous solutions of agarose and agarose-like biogels (e.g. carrageenans) generally form rigid and brittle gel structures depending on the polymer concentration and some possible additives such as cations and gums [9]. At higher temperatures (>60°C), a structural destruction occurs due to breaking of molecular bonds, and gel–sol transition takes place [5, 7, 10, 11]. Agarose and carrageenan gel systems show a strong hysteresis behavior on these thermal transitions [5, 12–14]. X-ray diffraction [5, 15], light scattering [15, 16], fluorescence correlation spectroscopy (FCS)

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[17], optical rotation [7, 16], differential scanning calorimeter (DSC) [18], small-angle neutron scattering (SANS) [17, 19, 20], and dynamic viscoelastic measurements [18, 21] are the some major techniques used in the inspection of the agarose gels.

The agarose gels consist of randomly connected polymeric aggregates and pores which entrap the water. As a result, they possess typical characteristics that resemble the living tissues in composition, rheological and relatively neutral nature and water content. It therefore has been widely used to simulate the transportation and release process of small and large molecules [22]. It is hard to apply a certain diffusion model on such heterogeneous structures, and thus, investigation of sorption and desorption processes in such complex systems are still generating intense research interest. There are many studies on determination of pore sizes [3, 23, 24], diffusion coefficients and diffusion models for transportation of macromolecules such as micelles, proteins, and other agents [17, 22, 25–27]. Recently, steady-state fluorescence (SSF) technique was employed in our laboratory for studying thermal phase transitions of agarose [14], and carrageenan gels [28, 29], and also for studying small molecule diffusion into carrageenan [30], and polyacrylamide gels [31].

Poly(vinyl alcohol) (PVA), a polyhydroxy polymer, is the largest volume, synthetic water-soluble resin produced in the world. The excellent chemical resistance and physical properties of PVA resins have resulted in broad industrial use [32]. PVA can be comparatively easily derivatized via the hydroxyl groups in a manner similar to other polyhydric alcohols. The most common PVA modifications reactions are esterification and the etherification of the hydroxyl groups. The ester bond is, however, easily hydrolyzed, and chemically modification with ether linkages may be an alternative approach. Hilborn and coworkers reported partial functionalization of the PVA hydroxyl groups via carbamate linkages which allowed introduction of different functional groups including those which can be used for the cross-linking of the macromer molecules via “click” chemistry [33]. Recently, we reported a versatile method to synthesize side-chain Py functional PVA directly from the bare PVA using “click” chemistry strategy [34]. Such functionalization has brought about improved solubility in a range of solvents with different polarity and fluorescence properties of the pyrene units incorporated to PVA. The outstanding characteristics of modified PVA led to investigate the potential use of glucose oxidase (GOx) immobilized PVA-Py matrix for practical analysis of glucose as a fluorescent bioprobe [34, 35]. On the scope of this study, a novel antibody based water soluble probe containing both fluorescent and target sites in the structure (poly(vinyl alcohol)-pyrene-anti-metadherin) for in vitro imaging of breast cancer cells was recently reported [36].

The purpose of this paper is to study the diffusion process of pyrene labeled PVA chains in and outward of the swollen agarose gel systems prepared with various concentrations. During the sorption and desorption processes of the diffusing molecules, fluorescence intensities, I_{fl} were measured by using steady-state fluorescence (SSF) technique. Fickian diffusion was used to quantify the experimental findings for each sample and it was found that the diffusion processes of PVA-Pyrene chains into agarose gel possess two distinct diffusion regions. Corresponding diffusion coefficients at short (D_s) and long (D_l) diffusion times were measured.

Experimental

Agarose Gel Preparation

Agarose Type 1-B (Sigma A0576) was used without further purification. The agarose gels with four different concentrations (50, 100, 200 and 300 mg) were prepared as follows: The dry agarose powder was slowly added to a cylinder flask with 10 mL distilled water while being stirred and heated on a magnetic stirrer. During sample preparation, the heated agarose solutions were held at 90°C in a hot water bath and continuously stirred for a period of 15 min. Then 1.8 mL of this hot solution transferred to a 1×1×4.5 cm quartz cell to adjust the top center point height of the gel samples to 15 mm. The hot agarose sol is colorless and transparent until the sol–gel transition takes place at lower temperatures. After this, as a result of heterogeneity and light scattering, the gels become opaque-white depending on the agarose concentrations. All the gel samples were cooled at room temperature for 2 h and stored at 10°C until their use.

Synthesis of PVA Containing Pyrene Side-group (PVA-Py)

Side-chain Py functional PVA was synthesized using a three-step procedure as described previously [34], and shown in Fig. 1. The compositions of the polymers were determined by ^{1}H NMR. The content of acetyl units of the starting PVA was about 2.82% and the Py units in PVA were found to be 10.03%. Functionalization was kept deliberately at a low level to preserve PVA properties. The characterization data are as follows: ^{1}H NMR [δ, ppm from tetramethylsilane (TMS) in dimethyl sulfoxide (DMSO- d_6), 250 MHz]: 8.42–8.12 (m, 9H, Py), 7.56 (s, N-CH=C-), 5.26 (s, triazole-CH_2-O), 4.69–4.16 (t, -CH- and –OH repeating unit of PVA), 4.10 (s, O-CH_2-Py), 1.98 (s, OC-CH_3), 1.78–1.19 (s, -CH_2- repeating unit of PVA); FT-IR (ATR): 3,330–3,350, 2,970, 1,740, 1,660, 1,460, 1,390, 1,250, 1,165 cm^{-1}.

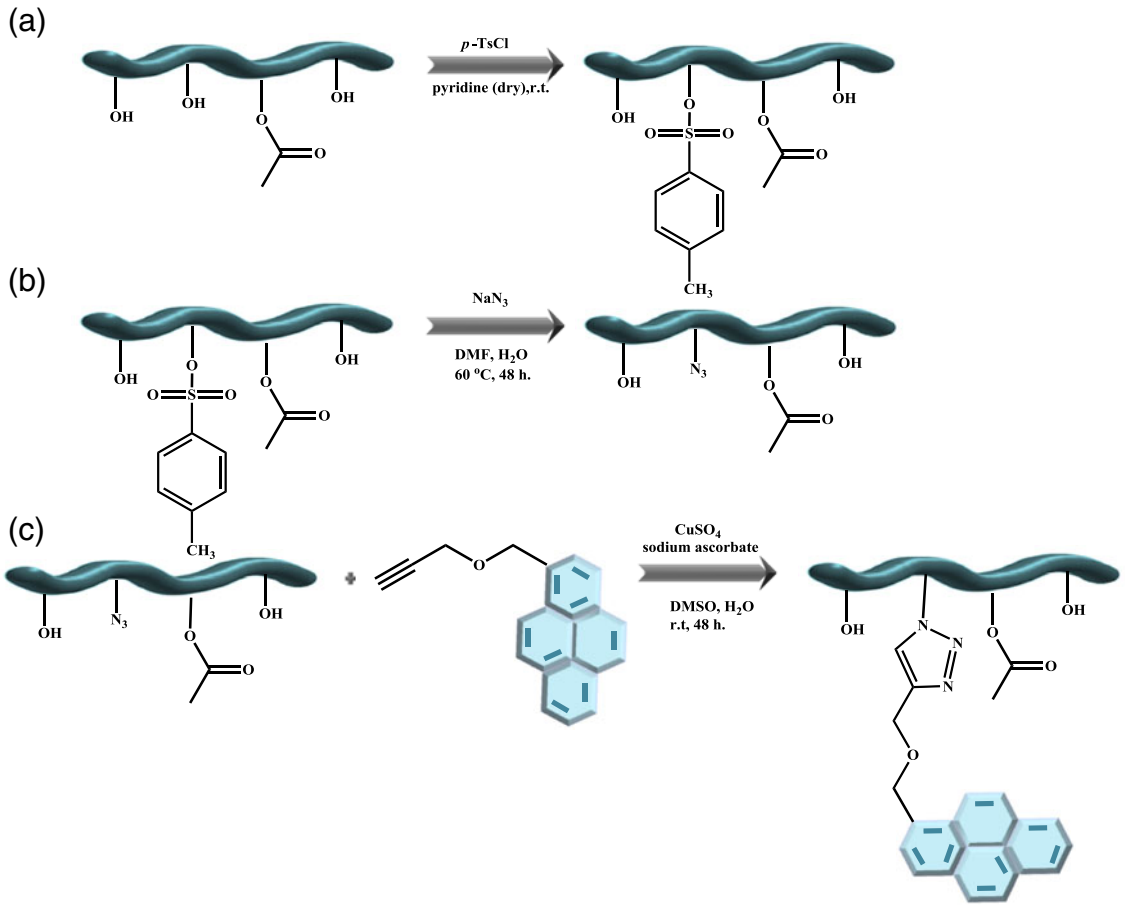

Fig. 1 Synthesis of side-chain Py functional PVA via three-step procedure; **a** Partial tosylation of PVA**, b** Synthesis of azide functional PVA (PVA-N_3)**, c** "Click" reaction of PVA-N_3 with propargyl pyrene

Sorption and Desorption Measurements

A stock solution of PVA-Py was prepared to use in sorption experiments. To do so, 20 mg of the powder of synthesized PVA-Py was dissolved in 30 mL of distilled water at room temperature while being stirred on a magnetic stirrer for 24 h. Just before the sorption measurements were started, 2 mL of this PVA-Py solution was poured into each cell with different agarose concentration. Right after the sorption experiment was finished, the agarose gel samples were gently "washed" by injecting pure distilled water to remove the remaining PVA-Py solution and then pure water was injected to fill the bottoms of the cells to lift up the gel samples before the desorption measurements is started.

The measurements of steady-state fluorescence (SSF) intensities were carried out using a Varian Cary Eclipse Fluorescence Spectrophotometer equipped with a cell changer system. PMT voltage of the spectrometer and the slit widths were set to 750 V and 5 nm, respectively, to obtain the optimum intensities during the diffusion measurements.

Results and Discussion

By taking advantage of fluorescence property and the water solubility of pyrene labeled PVA, the diffusion study in and outward of the swollen agarose gel systems in different concentrations was performed. Figure 2 shows the emission spectra of the PVA-Py solution which was used in the experiments, where the excitation at 340 nm gives characteristic pyrene emission peaks at 375 and 395 nm. During the sorption and desorption experiments, the stronger one at 375 nm was chosen as a reference to follow the variation in fluorescence emission intensities.

A photo of the samples which are used in the sorption measurements is shown in Fig. 3a. These four cells were placed in the spectrometer cell changer compartment, and then the measurements of the fluorescence intensities were started. The spectrometer was programmed to measure the fluorescence intensities with a period of 2 h for total of 140 h sorption time at 25°C. Desorption measurement is presented in Fig. 3b where, the pathway of the light beam passes from the center of the water-filled part of

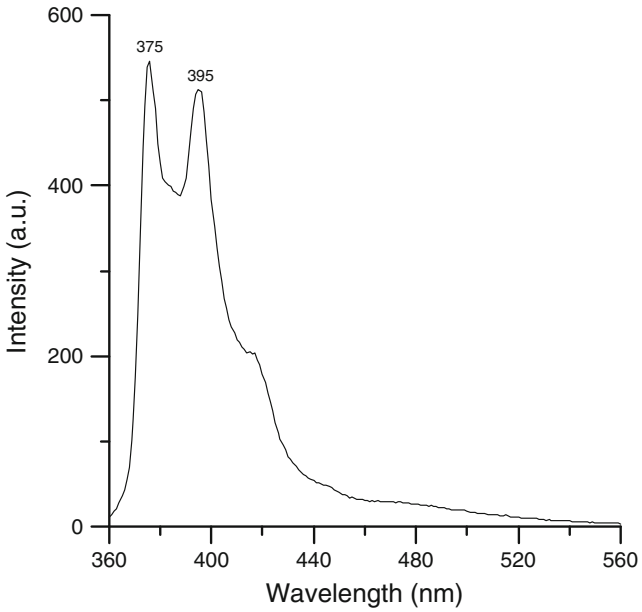

Fig. 2 Emission spectra of the PVA-Py solution excited with 340 nm light

the cell. Then, the desorptions of PVA-Py molecules from the gel to the water were monitored on the spectrometer for 90 h.

The fluorescence intensities which were measured at every 2 h are shown in Fig. 4 for two different agarose content gels, here gel samples were excited at 340 nm and PVA-Py spectra were monitored during sorption measurements. Figure 4a and b present the fluorescence spectra of PVA-Py absorbing by the gel prepared with 50 and 300 mg agarose contents respectively. It is seen that the fluorescence intensity increases and almost saturates, indicating that PVA-Py chains are absorbed by agarose gel as sorption time is increased.

Figure 5 presents the fluorescence intensity variation of the highest emission peak at 375 nm versus sorption time, where the data were normalized according to the highest intensity level at longest time. The curves in Fig. 5 show purely Fickian behavior. Under this picture, the diffusion process can be treated using the Fickian diffusion model.

According to Fick's law, the equation for diffusion in one dimension, when the diffusion coefficient D is constant, is expressed as [37],

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D(c)\frac{\partial c}{\partial x}\right) = D\frac{\partial^2 c}{\partial x^2} \tag{1}$$

where c is the concentration of diffusing species at time, t. For a plane sheet geometry and keeping the initial concentration of water constant, the solution of the Fick's equation is given by the following equation;

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left(-\frac{D(2n+1)^2\pi^2 t}{d^2}\right) \tag{2}$$

where d is the thickness of the specimen and M_t and M_∞ are the amount of fluorescing probes sorbed or

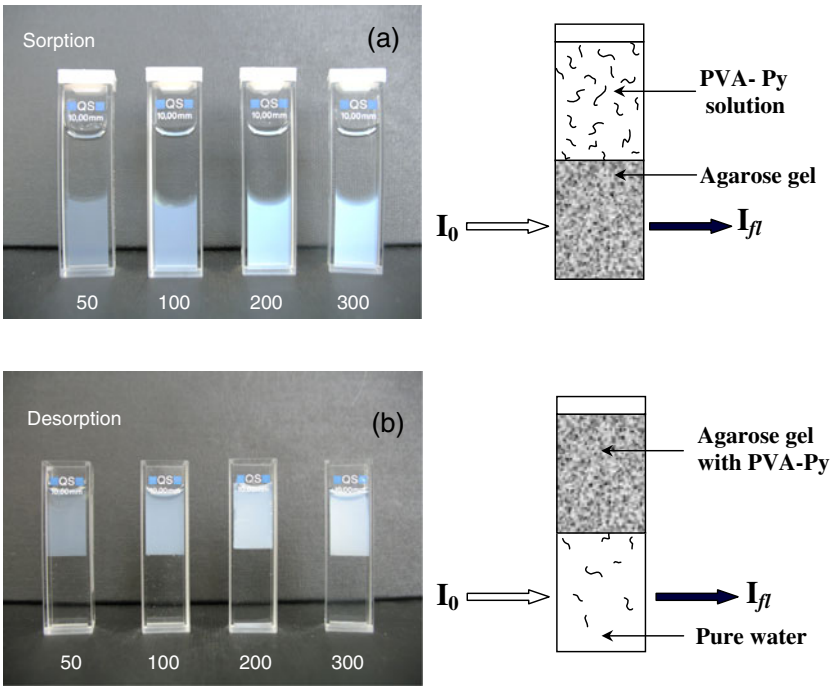

Fig. 3 The photos of the agarose gel samples just before (**a**) sorption, and (**b**) desorption experiments. The numbers represent the amount of agarose content for each sample

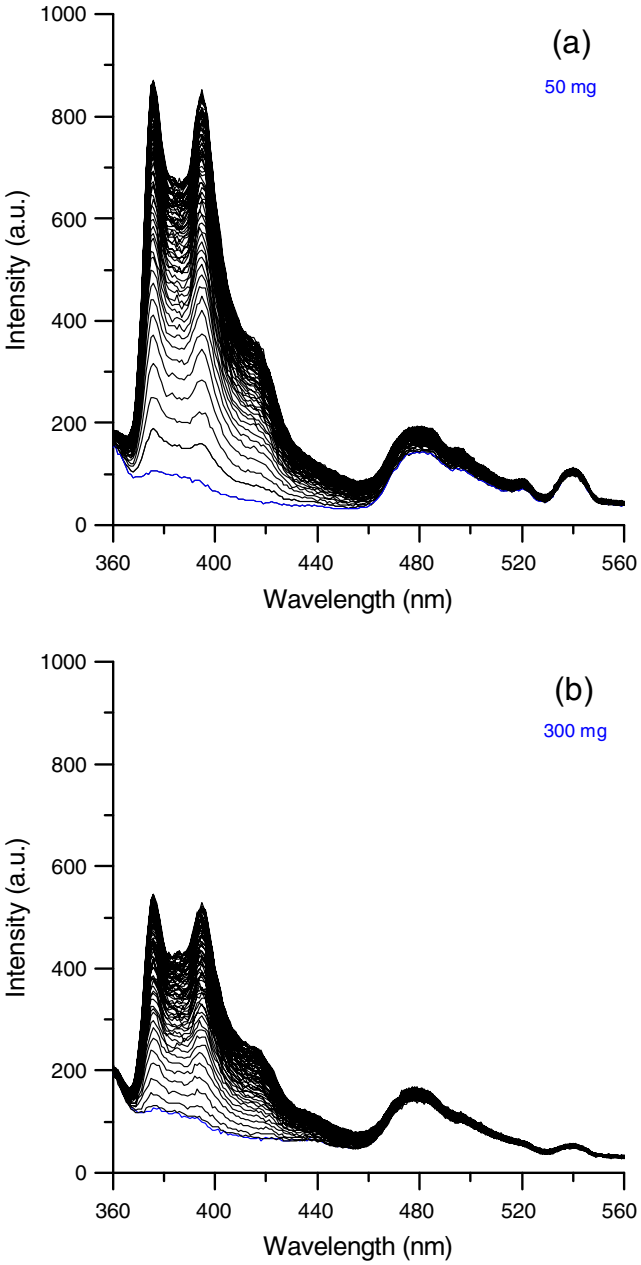

Fig. 4 The fluorescence intensities of the agarose gel samples excited at 340 nm during the sorption of PVA-Py solution for (**a**) 50 and (**b**) 300 mg agarose concentrations

desorbed at time t and at time infinity (thermodynamic equilibrium), respectively. The emission intensities, I_{fl} and $I_{fl\infty}$ from the absorbed or desorbed PVA-Py molecules at times t and ∞ (i.e., when the intensity approached a constant value) were obtained by using fluorescence emission intensity measurements. The relation between the fraction M_t/M_∞ and fluorescence intensities can be simply expressed as $M_t/M_\infty \approx I_{fl}/I_{fl\infty}$ at a given time.

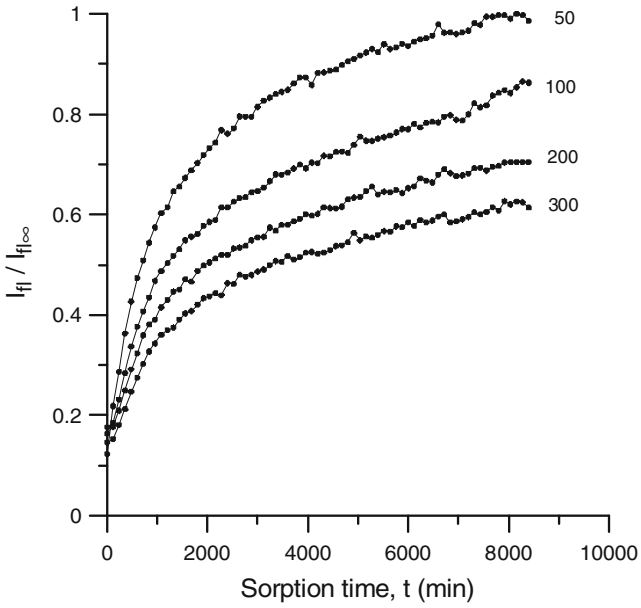

Fig. 5 Normalized intensities of the peaks at 375 nm in the Fig. 4 versus sorption time for the sorption process

In the diffusion process, the agarose gels are assumed to be as thin slabs, then the logarithmic form of Eq. 2 for $n=0$ can be given as follows:

$$ln\left(1-\frac{I_{fl}}{I_{fl\infty}}\right) = ln\left(\frac{8}{\pi^2}\right) - \frac{D\pi^2}{d^2}t \qquad (3)$$

The fit of the Eq. 3 to the data in Fig. 5 are given in Fig. 6. It is seen in Fig. 6 that diffusion of the PVA-Py molecules has two distinct regions, namely short and long times, produces two different diffusion coefficients as D_s and D_l, respectively. By taking the thickness of the samples, d as 15 mm, the diffusion coefficients were calculated and are presented in Table 1. It is seen from this table that the sorption is faster at short times, compare to the sorption at longer times. Most probably swelling front moves faster into the gel at early times, however later at longer times, swelling slows down due to the over swollen gel which does not allow more water molecules into the gel system. In other words, high elasticity of highly swollen gel does not allow PVA-Py molecules into the gel system. On the other hand, the increase in the concentration of agarose, increases the gel density which leads to more compact and tight gel network. As a result, the penetration of the PVA-Py molecules into the gel becomes slower compare to the loose gel system.

Similar results were performed for desorption processes of the same samples after their sorption processes were completed. Figure 7 shows the increase in intensity caused by desorption of PVA-Py molecules from gel into water for

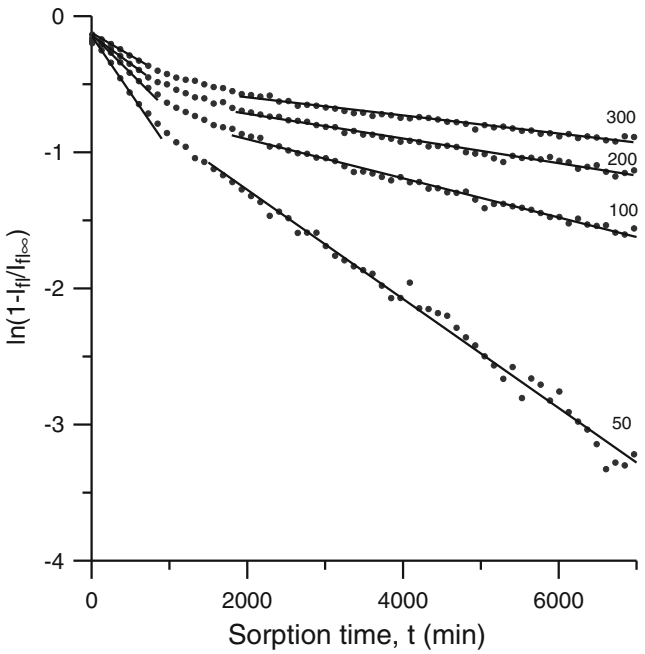

Fig. 6 Linear regression of the data in Fig. 5. The slopes of the straight lines produce *D* values for sorption process

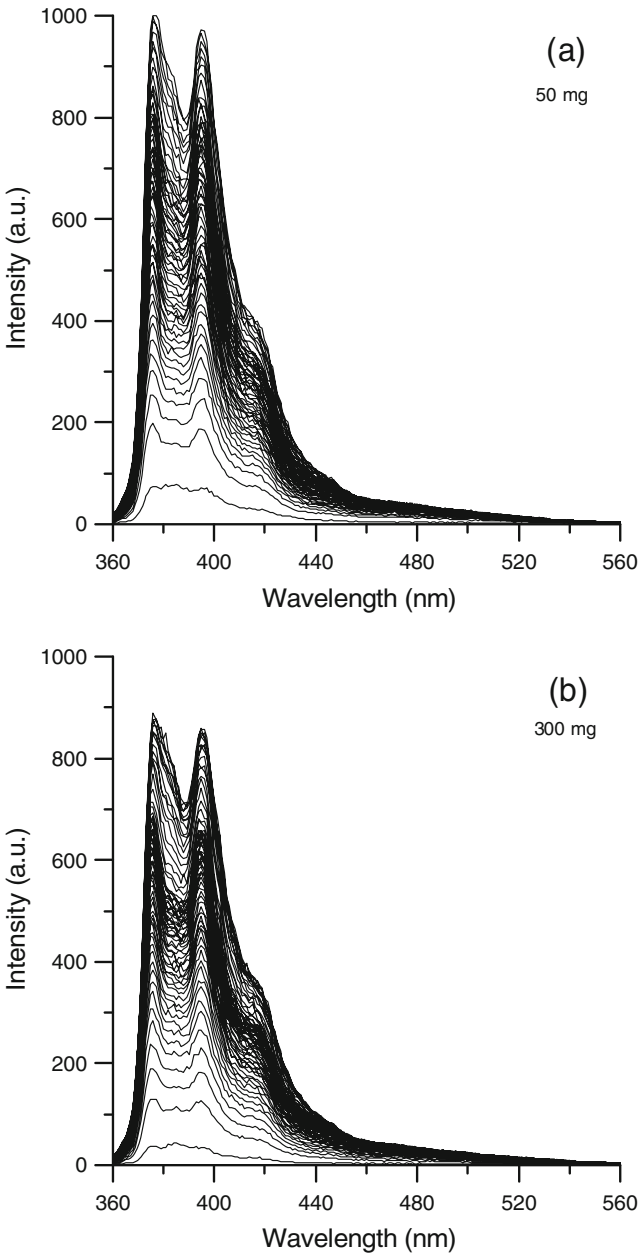

Fig. 7 The fluorescence intensities of the agarose gel samples excited at 340 nm during the desorption of PVA-Py for (**a**) 50 and (**b**) 300 mg agarose concentrations

50 and 300 mg content gel samples. Each curve was measured with a period of 1 h. This time was extended to 2 h, after being approached to the saturation region. Figure 8 presents the variations in the normalized maximum peak heights versus desorption time for all the gel samples. The logarithmic fits of the data in Fig. 8 to Eq. 3 are shown in Fig. 9. The produced D_s and D_l diffusion coefficients are listed in the Table 2. Again, it is observed that desorption processes have two distinct regions, namely short and long times, similar to sorption processes. At early times, PVA-Py chains at the gel front move immediately into water, however at longer times, desorption slows down due to the moving front of the highly swollen gel system. When the Tables 1 and 2 are compared, it is seen that the desorption coefficients are in general much larger than the sorption coefficients, indicating that once the gel is swollen, due to high elasticity, it is much easier for PVA-Py molecules to be released from the gel system. On the other hand penetration of PVA-Py molecules into the swollen gel is much slower due to high restraining forces, own by the gel system.

Our result can be compared with the results of direct energy transfer experiments between polymer-bound naphthalene (N) and free pyrene (P) molecules in chloroform–heptane mixture, where free P molecules desorbed from swelling PMMA glass into polymer solution prior to dissolution [38]. The strobe technique was used to study

Table 1 The calculated diffusion coefficients for sorption process

Agarose content (mg)	D_s (short time) ($\times 10^{-6}$) $cm^2 \cdot s^{-1}$	D_l (long time) ($\times 10^{-6}$) $cm^2 \cdot s^{-1}$
50	3,19	1,52
100	2,17	0,55
200	1,59	0,35
300	1,26	0,25

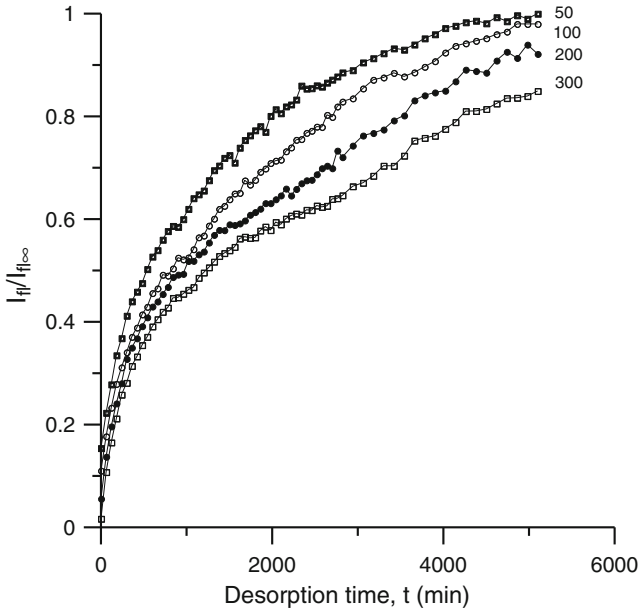

Fig. 8 Normalized intensities of the peaks at 375 nm in the Fig. 7 versus desorption time for the desorption process

desorption of pyrenes from swelling of PMMA glass prepared by free radical polymerization. Desorption coefficients, D_d of pyrene (P) desorbing from the swelling PMMA glass prior to dissolution were measured and found to be 8.3×10^{-8} and 1.3×10^{-5} $cm^2{\cdot}s^{-1}$ in short and long time regions, respectively. It is seen that the desorption

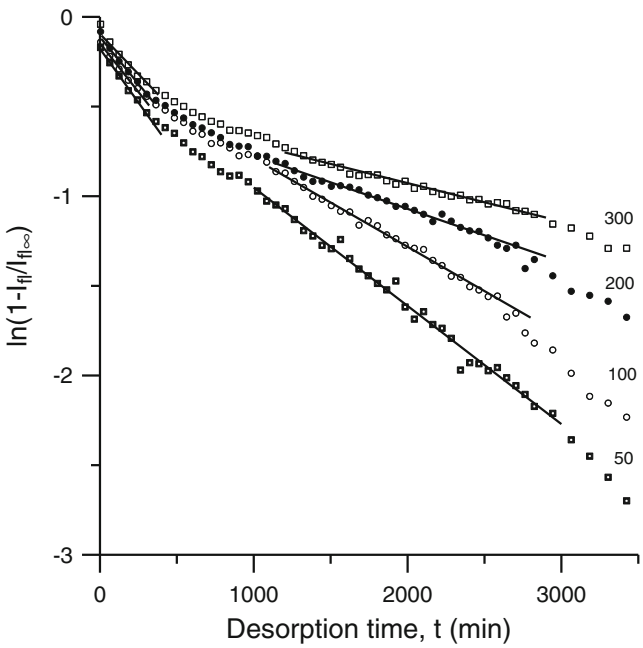

Fig. 9 Linear regression of the data in Fig. 8. The slopes of the straight lines produce *D* values for desorption process

Table 2 The calculated diffusion coefficients for desorption process

Agarose content (mg)	D_s (short time) ($\times 10^{-6}$) $cm^2{\cdot}s^{-1}$	D_l (long time) ($\times 10^{-6}$) $cm^2{\cdot}s^{-1}$
50	4,61	2,49
100	4,12	1,88
200	3,96	1,12
300	3,39	0,81

coefficient observed above work are quite different than the results of our present study, at short times, desorption from PMMA glass is much slower than our gel system which is expected. However, at long times free pyrenes desorb much easier from swollen PMMA glass than the desorption of bounded pyrenes from the agarose gel system. Another work related to small molecule sorption and desorption in and out of iota-carrageenan was studied by using steady-state fluorescence (SSF) technique [30]. Pyranine dissolved in water used as fluorescence probe and fluorescence emission intensity, I_{fl} from pyranine was monitored for studying sorption and desorption processes at various temperatures. The Fickian model was applied to produce sorption, D_s, early desorption, D_{ed}, and desorption, D_d coefficients. Experimentally produced D_s, D_{ed} and D_d coefficients at various temperatures were found to be (10^{-5} $cm^2{\cdot}s^{-1}$), (10^{-6} $cm^2{\cdot}s^{-1}$) and (10^{-5} $cm^2{\cdot}s^{-1}$) respectively, which are quite similar to our findings as expected for similar gel systems. Order of magnitude difference in diffusion coefficients between two gel systems may be explained with the diffusion of free pyranine and bounded pyrene.

Conclusion

This work has presented the kinetic parameters for pyrene labeled PVA molecules diffusing in and out of agarose gels, which may find many applications in cosmetics, pharmaceutical and personal care industries. These parameters were obtained by using a very simple and inexpensive fluorescence method to measure sorption and desorption coefficients for pyrene labeled PVA chains in and out of agarose gels. It has to be mentioned that fluorescence technique monitors the events at a molecular level, which produces highly sensitive results. Fickian diffusion kinetics was considered and employed to produce these coefficients. Here in both the sorption and desorption cases, the two distinct regions were observed for the diffusion coefficients and it was understood that the diffusion coefficients decrease as the agarose content is increased, indicating slower sorption and desorption processes takes place for the more dense gels.

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