Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 326 (2006) 7–12
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Monitoring small molecule diffusion into hydrogels at various temperatures by fluorescence technique

Gülşen Akın Evingür [a], Kadir Karslı [a], Önder Pekcan [b,*]

[a] *Istanbul Technical University, Department of Physics, Maslak, 34398 Istanbul, Turkey*
[b] *Isik University, Department of Physics, Kumbaba-Şile, 34980 Istanbul, Turkey*

Received 23 February 2006; received in revised form 23 June 2006; accepted 28 June 2006
Available online 4 July 2006

Abstract

Steady state fluorescence technique was used to study small molecule diffusion into polyacrylamide (PAAm) gels at various temperatures. Pyranine (P_y), dissolved in water was introduced as a probe and fluorescence emission (I_p) from P_y was monitored during diffusion. Scattered light intensities, I_{sc} from PAAm gel was also monitored to observe structural variations during diffusion process. Increase in I_p intensity was attributed to P_y diffusion into PAAm gel. On the other hand decrease in I_{sc} intensity was interpreted as the variation of the spatial heterogeneities in the system. Li-Tanaka and Fickian models were used to quantify the swelling and diffusion experiments and diffusion coefficients were produced in both cases. Related activation energies were also calculated from the corresponding physical processes.

Keywords: Diffusion; Hydrogel; Fluorescence; Temperatures

1. Introduction

The diffusion and swelling kinetics of gels are very important in various fields of industry: in the pharmaceutical industry in designing slow-release devices for drugs, in the agricultural industry for producing storable foods, and in medical industry in developing artificial organs.

The imperfections in the hydrogel structures have been known to influence the solvent permeability, the diffusion of small and large molecules and more indirectly the swelling properties of gels. In the swollen state these imperfections manifest themselves in a non-uniformity of polymer concentration. Considerable work has been done on the characterization of the gel inhomogeneities. It was shown that high permeability of polyacrylamide (PAAm) gels is related to the inhomogeneous cross-link distribution (Weiss et al., 1979). The effect of inhomogeneities of the polymer network on the swollen state of acrylamide gels and on the diffusion of water molecules within the gels were examined (Hsu et al., 1983). When an ionized acrylamide gel is allowed to swell in water, an extremely interesting pattern appears on the surface of the gel and the volume expansion increases by adding some amount of sodium acrylate (Tanaka et al., 1987). If acrylamide gels are swollen in acetone-water mixture, gel aging time plays an important role during collapse of the network (Tanaka, 1978).

The kinetics of swelling of acrylamide gels was studied by light scattering and the cooperative diffusion coefficient of the network was measured (Tanaka and Filmore, 1979; Bastide et al., 1984). Small angle X-ray and dynamic light scattering were used to study the swelling properties and mechanical behavior of acrylamide gels (Ilavsky, 1982; Patel et al., 1989). Photon transmission technique was used to study the existence of frozen blob clusters in acrylamide gels, which caused strong light scattering during swelling (Pekcan et al., 1998). The same technique was used to study gelation and swelling process of PAAm where transmitted light intensity decreased and increased during gel formation and swelling processes, respectively (Kara and Pekcan, 2000; Pekcan and Kara, 2001, 2002). Similar technique was employed to study *N*-isopropylacrylamide gels (Kara et al., 2002; Pekcan and Kara, 2003). On the other hand time resolved and steady state (SSF) fluorescence techniques were employed in our laboratory for studying swelling and diffusion processes in PMMA gels (Erdoğan and Pekcan, 2001; Yılmaz and Pekcan, 1998).

* Corresponding author. Tel.: +90 216 528 7180; fax: +90 216 712 1474.
E-mail address: pekcan@isikun.edu.tr (Ö. Pekcan).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.06.043

In this paper in situ steady state fluorescence experiments (SSF) were reported during the diffusion of water molecules into PAAm gels at various temperatures. Pyranine (P_y) in water was used as a fluorescence probe to monitor diffusion. It was observed that the fluorescence emission intensities, I_p increased continuously as water molecules diffused into gels. In the mean time scattered intensities, I_{sc} from the gel was also monitored to detect the variation in the gel structure during diffusion process. Decreased in scattered light intensity, I_{sc} from the gel was attributed to the spatial inhomogeneities which disappear during swelling process. I_p intensities were measured by SSF technique.

2. Theoretical considerations

2.1. Kinetics of swelling

Swelling experiments of disc shaped gels have shown that the relative changes of diameter and thickness are the same, indicating that the gel swelling processes are not pure diffusional processes. In fact the equality of the relative changes of diameter thickness stems from the non-zero shear modulus, μ which results; the change of the total shear energy in response to any small change in shape that maintains constant volume element within the gel should be zero. The high friction coefficient, f, between the network and the solvent overdamps the motion of the network, resulting in a diffusion like relaxation. The equation of the motion of a network element during the swelling can be given by (Li and Tanaka, 1990):

$$\frac{\partial \vec{u}}{\partial t} = D_c \vec{\nabla}^2 \vec{u} \tag{1}$$

where $\vec{u}$ is the displacement vector measured from the final equilibrium location after the gel is fully swollen ($u=0$ at $t=\infty$). $D_c = (K+4\mu/3)/f$ is the collective diffusion coefficient. Here t denotes the time and K is the bulk modulus. Eq. (1) has been used with some success to study the swelling of gels (Yılmaz and Pekcan, 1998). However, these studies did not properly treat the shear deformation that occurs within a gel during swelling, and, hence, cannot explain, for example, the isotropic swelling of a cylindrical gel. This shortcoming was due to the shear modulus of the network keeping the system in shape by minimizing the non-isotropic deformation. For a disc shaped gel, any change in diameter is coupled to a change in thickness. The total energy of a gel can be separated into a bulk energy and a shear energy. The bulk energy is related to the volume change, which is controlled by diffusion. The shear energy, F_{sh}, on the other hand, can be minimized by readjusting the shape of the gel (Li and Tanaka, 1990):

$$\delta F_{sh} = 0 \tag{2}$$

Each small diffusion process determined by Eq. (1) must couple to a small shear process given by Eq. (2) producing the following relation for a disc shaped gel:

$$\frac{u_r(r,t)}{r} = \frac{u_z(a,t)}{a} \tag{3}$$

where r is the radius and a is the half thickness of the disc gel. Eq. (3) indicates that the relative change in shape of the gel is isotropic, i.e. the swelling rates of a disc in the axial (z) and radial (r) directions are the same.

Simultaneous solution of Eqs. (1) and (2) produces the following equations for the swelling of a gel disc in axial and radial directions (Li and Tanaka, 1990):

$$u_z(z,t) = u_z(z,\infty)\sum_n B_n \exp\left(-\frac{t}{\tau_n}\right) \tag{4a}$$

$$u_r(r,t) = u_r(r,\infty)\sum_n B_n \exp\left(-\frac{t}{\tau_n}\right) \tag{4b}$$

where the axial and the radial displacements are expressed as series of components, each of them decaying exponentially with a time constant, τ_n. The first terms of the expressions are dominant at large t that is at the last stage of swelling. Eq. (4) can also be written in terms of vapor and solvent uptakes W and W_∞ at time t and at equilibrium, respectively, as follows:

$$\frac{W_\infty - W}{W_\infty} = \sum_{n=1}^{\infty} B_n \exp\left(-\frac{t}{\tau_n}\right) \tag{5}$$

In the limit of large t, or if τ_c is much larger than the rest of τ_n, all higher terms ($n \geq 2$) in Eq. (5) can be omitted and the swelling kinetics is given by the following relation:

$$\left(1 - \frac{W}{W_\infty}\right) = B_1 \exp\left(-\frac{t_s}{\tau_c}\right) \tag{6}$$

It should be noted from Eq. (5) that $\sum B_n = 1$, therefore B_1 should be less than 1. B_1 is related to the ratio of the shear modulus, μ and longitudinal osmotic modulus, $M=(K+4\mu/3)$. Hence, once the value of B_1 is obtained, one can determine the value of $R=\mu/M$. Here we have to note that Eq. (6) can also be obtained by using the theoretical results (Tanaka and Filmore, 1979), in the case of $R \to 3/4$ ($\mu/K \to \infty$), time constant $\tau_c \approx (3/4-R)^{-1}$ goes to infinity and all B_n's go to zero except B_x, which goes to unity. The dependence of B_1 on R for a disc can be found in the literature (Erdoğan and Pekcan, 2001). τ_c is related to the collective diffusion coefficient D_c at the surface of a gel disc by:

$$D_c = \frac{3a^2}{\tau_c \alpha_1^2} \tag{7}$$

where α_1 is a function R only and is given the literature (Tanaka and Filmore, 1979), and a stands for the half thickness of the gel in the final equilibrium state. Hence, D_c can be calculated, if one obtains τ_c values from the swelling experiments.

2.2. Fickian diffusion

According to Fick's law, the equation for diffusion in one dimension, when the diffusion coefficient D is constant, is expressed (Crank and Park, 1968) as

$$\frac{\partial c}{\partial t} = \frac{\partial}{\partial x}\left(D(c)\frac{\partial c}{\partial x}\right) = D\frac{\partial^2 c}{\partial x^2} \tag{8}$$

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:

$$\frac{M}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left(-\frac{D(2n+1)^2\pi^2 t}{d^2}\right) \quad (9)$$

where d is the thickness of the specimen and M and M_∞ are the masses of water sorbed or desorbed at times t and ∞, respectively.

3. Materials and methods

Each gel was prepared by using 0.71×10^{-3} kg (AAm) and 0.008×10^{-3} kg (APS) as an initiator by dissolving them in 5×10^{-3} l of water in which 8 μl of tetramethylethylenediamine was added as an accelerator. N'-Methylenebis(acrylamide) (Bis) (0.01×10^{-3} kg) was used as a cross-linking agent. This stock solution was deoxygenated by bubbling nitrogen through it for 10 min just before the polymerization process. It was transferred into round glass tubes of 0.99×10^{-2} m internal diameter. Free radical cross-linking copolymerization (FCC) was performed (for each sample) at room temperature.

Steady-state fluorescence measurements were carried out using a Perkin-Elmer Model LS-50 spectrometer, equipped with temperature controller. All measurements were made at the 90° position and slit widths were kept at 5 nm. In situ swelling and diffusion experiments were performed in round glass tubes of 0.99×10^{-2} m internal diameter at various temperatures. Cylindrical gels obtained from FCC were placed at the bottom of the glass tube. Pyranine (P_y) solution (10^{-4} M) was then poured on top of the gel sample, as shown in Fig. 1a. The glass tube then was placed in the spectrometer and fluorescence emission was monitored at a 90° angle so that during swelling and diffusion experiment only the gel was illuminated by the excitation light. Here the fluorescence emission from P_y molecules which are diffused into the swelling gels was monitored. Fig. 1a and b present the fluorescence cell and the gel positions before and after the diffusion process has started, respectively.

During the swelling and diffusion experiments the cell was illuminated with 340 nm. excitation light and P_y intensity, I_p was monitored at 512 nm. In these experiments no shift was observed in the wavelength of maximum intensity of P_y. Transparencies of the gels were detected by monitoring the scattered light intensity, I_{sc} of the excitation during swelling and diffusion experiments. Typical spectra from a gel, after started and the end of swelling and diffusion processes are presented in Fig. 2a and b, respectively. In swelling experiments continuous volume transition is expected which should result continuous decrease in I_{sc} due to disappearance of lattice heterogeneities during swelling. On the other hand, during diffusion experiments one should expect an increase in I_p, due to increasing amount of P_y molecules in the gel. Swelling and diffusion experiments were performed separately at 20, 30, 40, 50, and 60 °C temperatures.

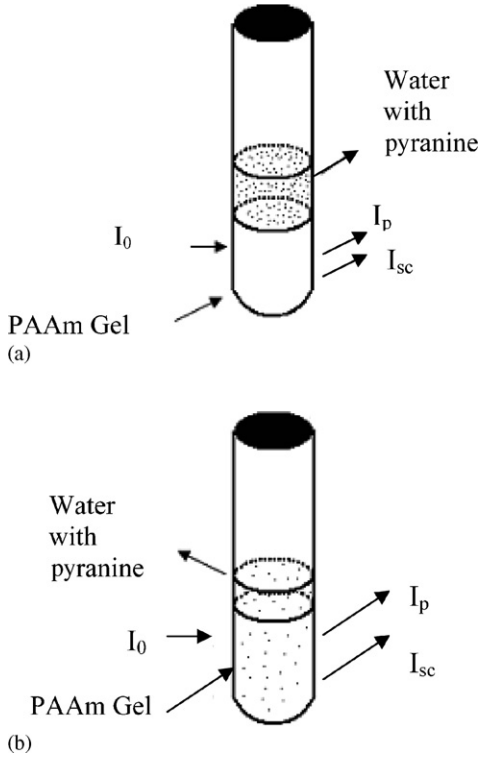

Fig. 1. Fluorescence cell and the gel positions (a) before and (b) after, the diffusion process has started. I_0 and I_p represent the excitation and emission intensities, respectively, I_{sc} is the scattered light intensity.

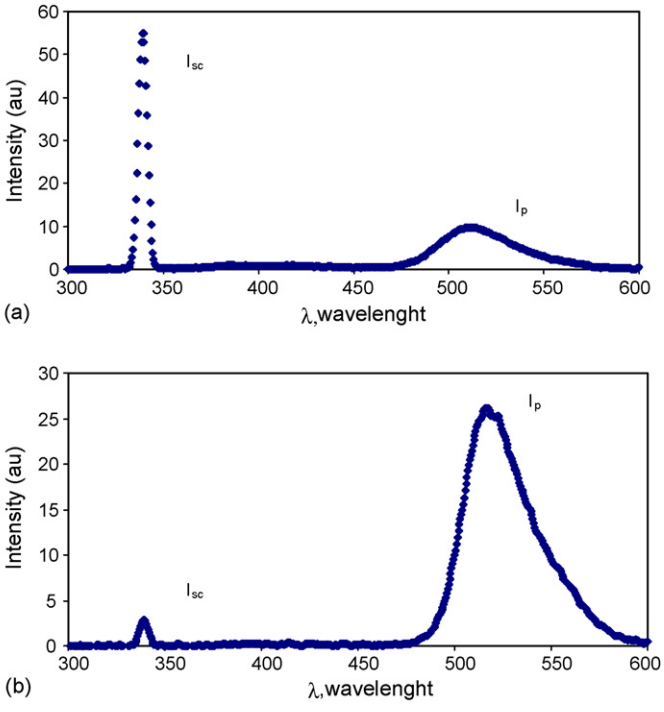

Fig. 2. Fluorescence emission I_p and scattered light, I_{sc} spectra of PAAm gel (a) after started and (b) the end of swelling and diffusion process have completed.

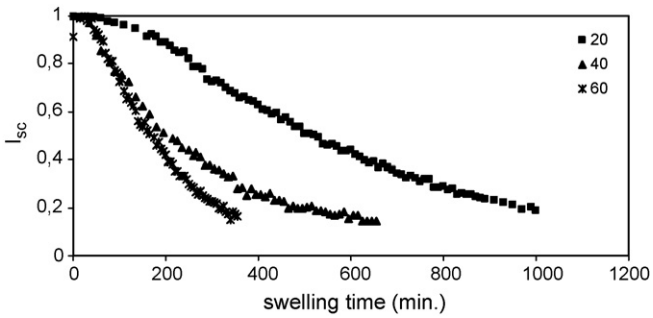

Fig. 3. Scattered light intensity, I_{sc} curves against swelling time, t, for the gels kept at 20, 40 and 60 °C temperatures.

4. Results and discussion

4.1. Swelling

Scattering intensity, I_{sc} versus swelling time, t is plotted in Fig. 3 for the gels swell at various temperatures. The nos. in Fig. 3 corresponds to temperatures in °C. It is seen in Fig. 3 that, I_{sc} decreases by increasing swelling time, indicating that lattice heterogeneities disappear during water uptake of the gel under consideration. It should be noted that water uptake is much faster in gels at high temperatures. These results can be quantified by assuming that the water uptake, W is proportional to the transmitted light intensity, $I_{tr} = 1 - I_{sc}$. During swelling, as more water molecules enter into the gel, structural heterogeneities are disappeared as a result I_{sc} decreases as W increases. Under this assumption Eq. (6) can be written in terms of I_{sc} in the logarithmic form, i.e.

$$\ln(I_{sc}) = \ln B_1 - \frac{t}{\tau_c} \tag{10}$$

Linear regression of curves in Fig. 4a–c provides us with B_1 and τ_c values from Eq. (10). Fits are shown in Fig. 4. Taking into account the dependence of B_1 on R one obtains R values and from α_1–R dependence, α_1, values were produced (Li and Tanaka, 1990). Then using Eq. (7) cooperative diffusion coefficients, D_c were determined for PAAm gels. Experimentally obtained parameters, τ_1 and B_1 together with a and D_c values are summarized in Table 1 for various temperatures. Here one should have noticed that measured D_c values present larger numbers at high temperature. This result is expected, since swelling process is much faster at higher temperatures. Fig. 5 shows the Arrhenius treatment of D_c from which the activation energies, ΔE_1 and ΔE_2 at low and high temperature regions for gel swelling are calculated, and found to be 24.69 and 2.29 kJ mol^{-1}. These values of activation energies are much smaller than the produced activation energy for PMMA in chloroform in the same temperature region (Erdoğan and Pekcan, 2001). From here one may

Table 1
Experimentally produced swelling time constants τ_c, preexperimental factors B, and collective diffusion coefficients D_c

T (°C)	τ_1 (s)	B	D_c ($\times 10^{-8}$ m^2 s^{-1})	a ($\times 10^{-2}$ m)
20	526.31	0.98	0.864	0.51
30	434.78	0.98	0.905	0.55
40	322.5	0.97	0.918	0.56
50	285.71	0.96	1.045	0.62
60	169.49	0.94	1.378	0.625

a is the half thickness of PAAm gels at the final equilibrium state.

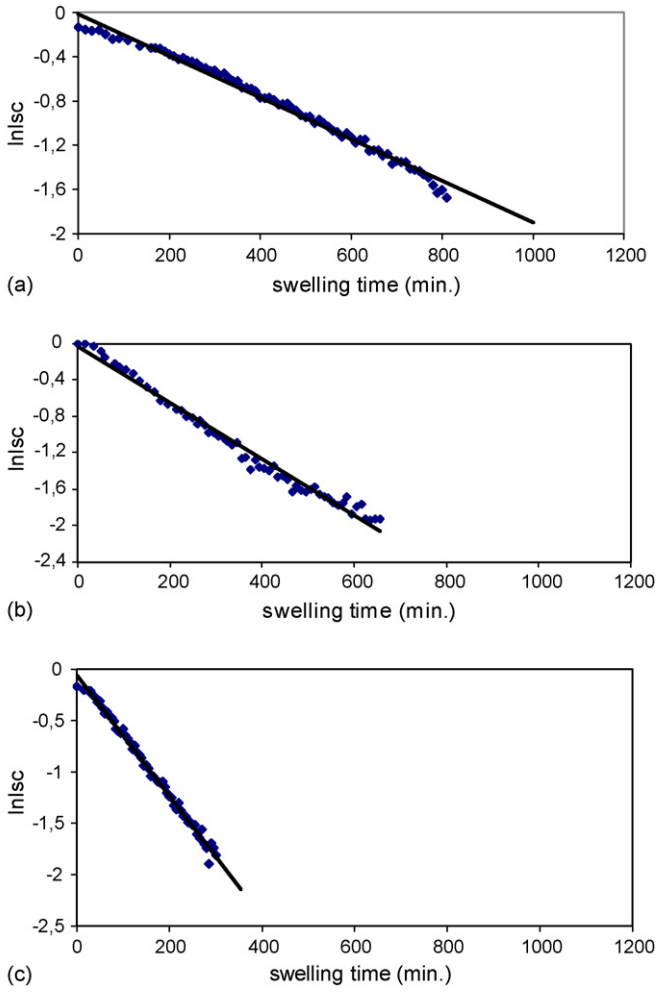

Fig. 4. Linear regression of the curves given in Fig. 3. The intercept and the slope of the curves produce B_1 and τ_1 parameters.

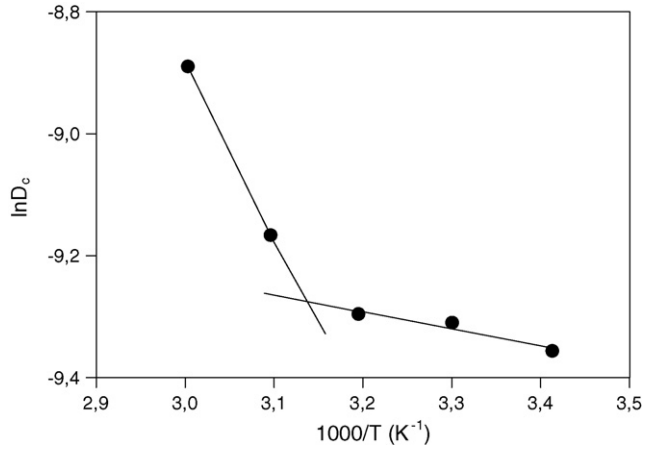

Fig. 5. Arrhenius treatment of D_c values. The slopes of the straight lines produce two different activation energies, namely ΔE_1 and ΔE_2 for gel swelling.

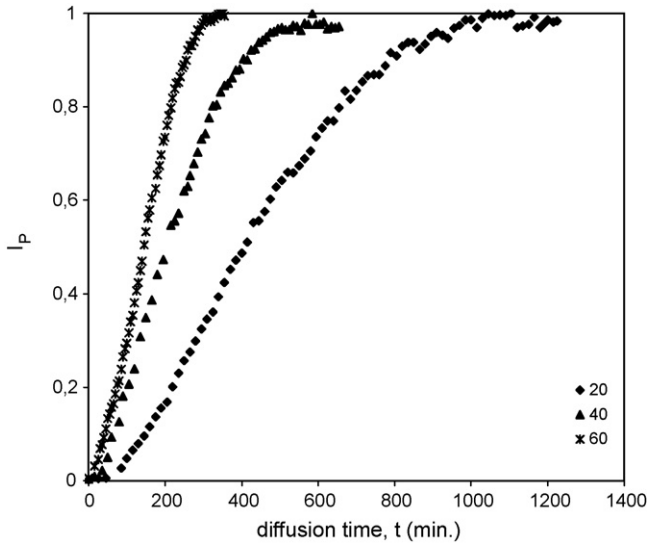

Fig. 6. The behavior of P_y intensity, I_p vs. time of P_y diffusion into PAAm gels, kept at 20, 40 and 60 °C temperatures.

conclude that the energy need for hydrogel swelling is very small compared to the organic solvent base systems. This behavior of PAAm gel may be explained with its low T_g values. Two different values of activation energies may indicate two different regimes of swelling process. At low temperature region swelling needs much lower energy than at high temperature region, as expected.

Table 2
Experimentally produced diffusion coefficients D_s and D_l

T (°C)	D_s ($\times 10^{-8}$ m^2 s^{-1})	D_l ($\times 10^{-8}$ m^2 s^{-1})	d ($\times 10^{-2}$ m)
20	1.37	4.01	1.02
30	1.87	4.87	1.11
40	3.94	10.45	1.12
50	4.05	14.65	1.24
60	7.28	28.84	1.25

d is the thickness of PAAm gels at 20, 30, 40, 50 and 60 °C.

4.2. Diffusion

Fig. 6 presents the results of the diffusion experiments where P_y intensity, I_p increases as diffusion time increased for the all gel samples. Here nos. represents the temperatures. Since I_p is directly proportional to the number of P_y molecules enter into the gel, the behavior of the intensity curves in Fig. 6 suggest that P_y molecules are absorbed much faster at higher temperatures. Under this picture the diffusion process can be treated using the Fickian diffusion model. Curves in Fig. 6 also support this suggestion where they show purely Fickian behavior. Here PAAm gels are assumed to be as thin slabs, then the logarithmic form of Eq. (9) for $n=0$ can be given as follows:

$$\ln\left(1-\frac{I_p}{I_{p\infty}}\right)=\ln\left(\frac{8}{\pi^2}\right)-\frac{D\pi^2}{d^2}t \tag{11}$$

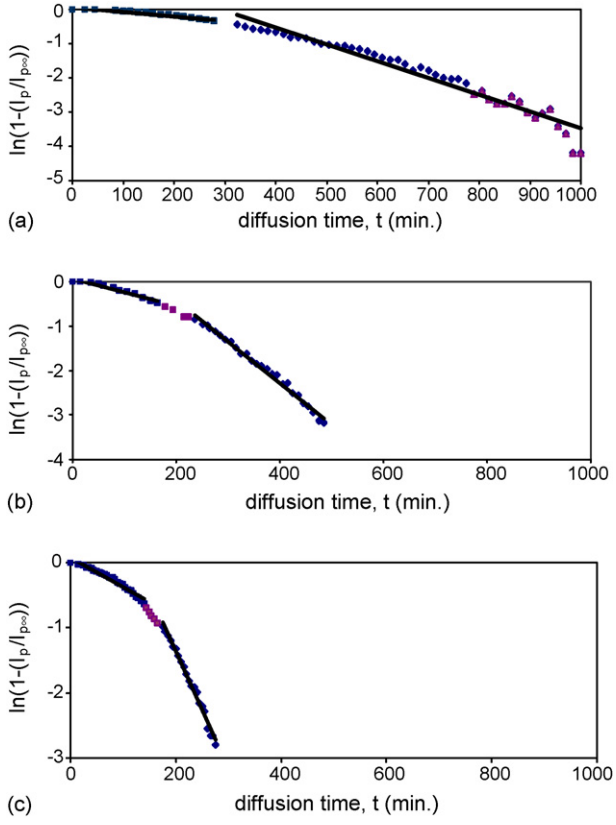

Fig. 7. Linear regression of the data in Fig. 6. The slope of the straight lines produce D values.

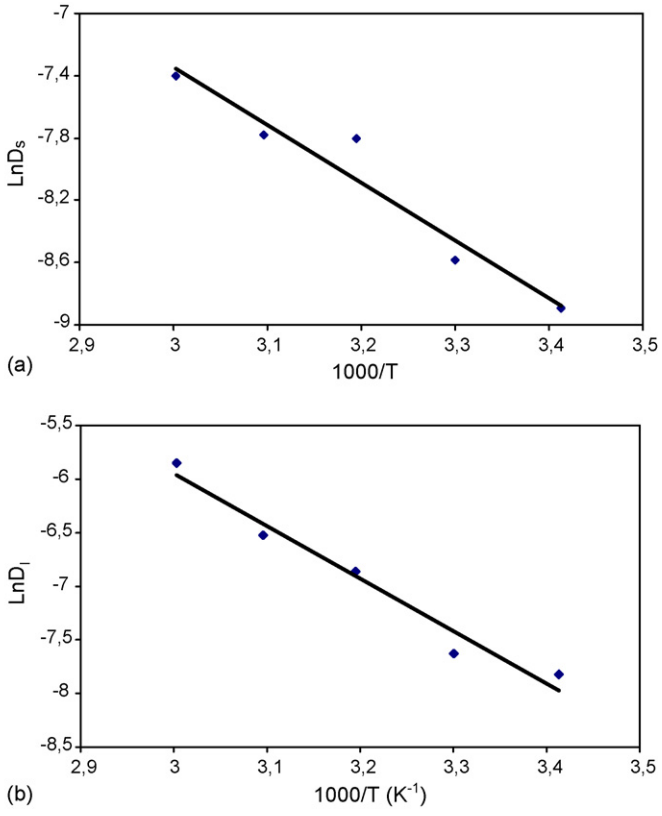

Fig. 8. Arrhenius treatment of (a) short and (b) long time diffusion coefficients, which produced ΔE_s and ΔE_l activation energies, respectively.

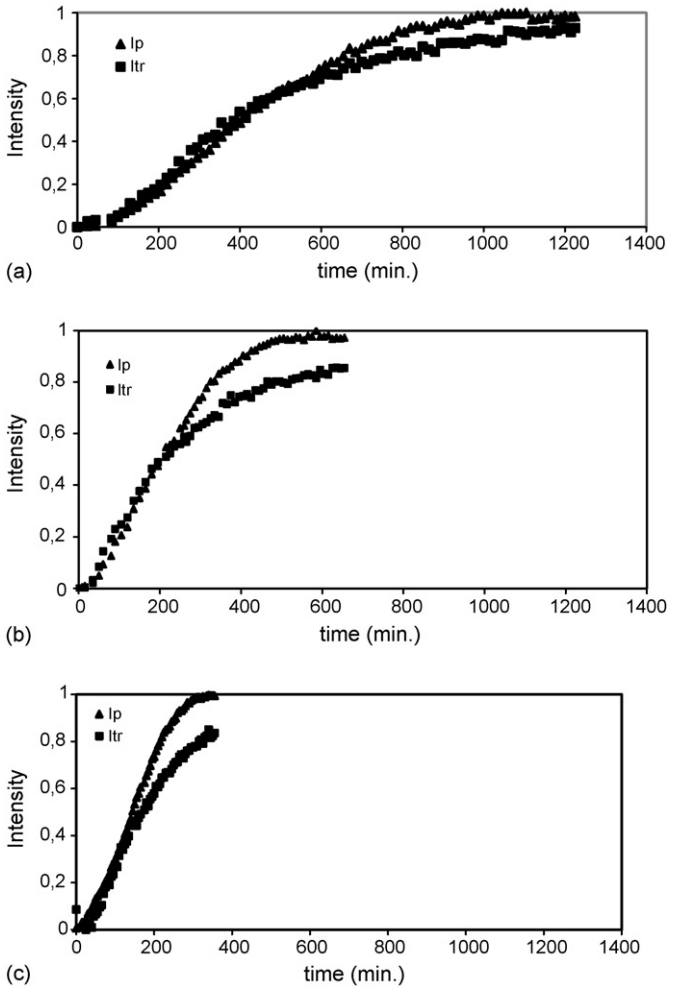

Fig. 9. Comparison of swelling ($I_{tr}(I_{sc})$) and diffusion (I_p) curves of PAAm gels kept at 20, 40 and 60 °C temperatures.

The fit of the Eq. (11) to the data in Fig. 6 are given in Fig. 7, from where D values are produced and are listed Table 2. As expected, D values for the gels at high temperature are found to be much larger than they are at low temperature. Here it is seen in Fig. 7, that diffusion of P_y molecules has two distinct regions, namely short and long times, produces two different diffusion coefficients as D_s and D_l, respectively. Arrhenius treatment of the diffusion coefficients produced two different diffusion activation energies as shown in Fig. 8a and b from where ΔE_s and ΔE_l values were obtained and found to be 30.9 and 40.6 kJ mol^{-1}, respectively. Here one may suggest that P_y diffusion into PAAm gel is a two stage mechanism. At short times, diffusion is slow and needs less energy; however, at longer times diffusion speeds up with higher energy need. In both cases diffusion activation energies are much higher than swelling activation energies indicating that these two processes have different origins.

On the other hand if one compares the swelling $I_{tr}(I_{sc})$ and diffusion I_p curves of the gels at a given temperature, it can be seen that there is a delay between these processes. Fig. 9 shows that swelling process takes places latter than diffusion i.e. there is a delay between diffusion and swelling processes. In other words diffusion process is much faster than swelling process and takes place at early times, which then need higher energy to be performed.

5. Conclusion

These preliminary results have shown that the direct fluorescence method can be used for real time monitoring of the swelling and diffusion processes. In this novel method in situ fluorescence experiments are easy to performed and provide us quite sensitive results to measure the swelling and diffusion parameters.

References

Bastide, J., Duoplessix, R., Picot, C., Candau, S., 1984. Small angle neutron scattering and light spectroscopy investigation of polystyrene gels under osmotic swelling. Macromolecules 17, 83–93.

Crank, J., Park, G.S., 1968. Diffusion in Polymer. Academic Press, London.

Erdoğan, M., Pekcan, Ö., 2001. Temperature effect on gel swelling: a fast transient fluorescence study. Polymer 42, 4973–4979.

Hsu, T.P., Ma, D.S., Cohen, C., 1983. Effects of inhomogeneties in polyacrylamide gels on thermodynamic and transport properties. Polymer 24, 1273–1278.

Ilavsky, M., 1982. Phase transition in swollen gels. 2. Effect of charge concentration on the collapse and mechanical behavior of polyacrylamide networks. Macromolecules 15, 782–788.

Kara, S., Pekcan, Ö., 2000. Photon transmission technique for monitoring free radical cross-linking copolymerization in various cross-linker contents. Polymer 41, 3093–3097.

Kara, S., Okay, O., Pekcan, Ö., 2002. Real time temperature and photon transmission measurements for monitoring phase separation during the formation of poly(*N*-isoproylacrylamide) gels. J. Appl. Polym. Sci. 86, 3589–3595.

Li, Y., Tanaka, T., 1990. Kinetics of swelling and shrinking of gels. J. Chem. Phys. 92, 1365–1371.

Patel, S.K., Rodiguez, F., Cohen, C., 1989. Mechanical and swelling properties of polycrylamide gel spheres. Polymer 30, 2198–2203.

Pekcan, Ö., Çatalgiz-Giz, H., Cahşkan, M., 1998. In situ photon transmission technique for studying ageing in acrylamide gels due to multiple swelling. Polymer 39, 4453–4456.

Pekcan, Ö., Kara, S., 2001. In situ monitoring of energy release in real time during gelation of acrylamide (AAm). Polymer 42, 7411–7417.

Pekcan, Ö., Kara, S., 2002. Photon transmission technique for monitoring formation and swelling of polyacrylamide gels. Polym. Plast. Technol. Eng. 41, 573–588.

Pekcan, Ö., Kara, S., 2003. The effect of preparation temperature on phase transitions of *N*-isopropylacrylamide gel. Phase Transit. 76, 601–609.

Tanaka, T., 1978. Collapse of gels and the critical endpoint. Phys. Rev. Lett 40, 820–823.

Tanaka, T., Filmore, D., 1979. Kinetics of swelling gels. J. Chem. Phys. 20, 1214–1218.

Tanaka, T., Sun, S.T., Hirokawa, Y., Katayama, S., Kucera, J., Hirose, Y., Amiya, T., 1987. Mechanical instability of gels at the phase transition. Nature 325, 796–798.

Weiss, M., Van Vliet, T., Silberberg, A., 1979. Permeability of heterogeneous gels. J. Polym. Sci. Polym. Phys. Ed. 17, 2229–2240.

Yılmaz, Y., Pekcan, Ö., 1998. In situ fluorescence experiments to study swelling and slow release kinetics of disc-shaped poly(methyl methacrylate) gels made at various cross-linker densities. Polymer 39, 5351–5357.