Investigation of Molecular Diffusion in Hydrogel by Electronic Speckle Pattern Interferometry

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The technique of real-time electronic speckle pattern interferometry (ESPI) is proposed to study the diffusion process of surfactants and proteins in gels. The diffusion coefficient is simply and directly determined from the interferograms. The experiments were carried out on a series of surfactants as well as on a myoglobin diffusion in agarose gels. The agreement of the diffusion coefficient values obtained from ESPI with those obtained by electrical conductance measurement or the reported data demonstrates the usefulness of the method. Moreover, spatial distribution of refractive index change in the gel can be determined by phase evaluation. Fick's law for diffusion is utilized to analyze the spatial distribution of the diffuser concentration curves in gel, which are compared with experimental data. We find that the agarose gel in surfactant solution shrinks slightly, which increases the density of the gel and leads to the additional refractive index change. The effect of gel shrinking to surfactant diffusion is discussed, and the distribution of density variation of the gel with time is evaluated.

Introduction

The study of surfactant and protein diffusion in hydrogels is crucial both for gaining a better understanding of solute−gel interactions and for the design of novel applications of such materials.[1,2] Conventionally, diffusion coefficients in gels are measured by monitoring the concentration of the diffusing solute in the solution outside the gel.[3,4] Holographic interferometry technique offers a powerful tool for noncontact measurement. It has been used to measure diffusion coefficients in binary liquid systems in the past.[5−9] Recently, this technique was employed for liquid−gel or gel−gel systems.[10−12] This method has several clear advantages over other conventional techniques, since it is the direct method, which avoids sampling and analysis of the liquid solution outside the gel. The diffusion coefficients in gels could be conveniently determined from the fringes in interferograms on the basis of Fick's law.

However, in most cases the density of hydrogel changes in the course of diffusion process as a result of the solute−gel interaction, which results in additional refractive index change in the interferometric measurements and which decays the precision of diffusion coefficients. On the other hand, the density distribution of hydrogel due to shrinking could be determined if the concentration distribution of the surfactant was known. It will provide a microscopic view of the kinetic process and a better understanding of solute−gel interactions.

In an effort to improve the experimental simplicity and accuracy, and to provide better treatment of the information, we introduce an electronic speckle pattern interferometry (ESPI) to represent the holographic methods. The ESPI technique is also known as TV holography. A CCD camera is used as the recording device. The main advantages of ESPI are the ease of recording the holographic interferograms and analyzing the data and the possibility of observing the object in near real time. Furthermore, quantitative phase information can be obtained with phase measuring algorithms, and the distribution of refractive index change can be accurately determined as shown in this work.

In this paper, the diffusion process of surfactants and myoglobin in agarose gels are monitored by ESPI. The diffusion coefficients of surfactants and myoglobin in agarose gel have been measured by this method. We use a phase evaluation method to map the refractive index changes when the surfactants diffuse into gels. The relationship of refractive index variation to surfactant concentration and the refractive index variation to agarose concentration are measured. We find that there exists an additional refractive index variation between the experimental data and the theoretical prediction for diffusion in terms of Fick's law. From the discussion about the diffusion in gel, we think the change of diffusion coefficients due to gel shrinking in surfactant solution is negligible and the distribution of surfactant concentration can be considered to obey Fick's law. Therefore, the distribution of density of agarose gels in surfactant solution is obtained.

Principle

In ESPI, the intensity distribution of the dual-illumination speckle images I_{t1} and I_{t2} taken at different diffusion time t_1 and t_2, respectively, can be shown mathematically,[13,14]

$$I_{t1} = I[1 + \gamma \cos(\theta)] \quad (1)$$

$$I_{t2} = I[1 + \gamma \cos(\theta + \varphi)] \quad (2)$$

where $I = I_1 + I_2$, I_1, and I_2 are the intensities of the object

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beam and the reference beam, θ is the initial phase difference between the two interferometric beams, γ is the visibility, and φ is the change in phase difference caused by surfactant diffusion into hydrogel and gel shrinking. By subtracting two images and taking the modulus, we can obtain the fringes of the system, which can be written as

$$I_{sub} = |2I\gamma \sin(\theta + \varphi/2) \sin(\varphi/2)| \quad (3)$$

For one dimension systems, the intensity data contained in the pixels are averaged over a certain number of adjacent pixels along the direction perpendicular to the diffusion. The averaging is done over the same pixels for the I_{sub} image. As the phase value φ is constant along the direction perpendicular to the diffusion, this averaging can be expressed as

$$<I_{sub}> = (2I\gamma/N)|\sin(\varphi/2)|\sum_{i}^{N}|\sin(\theta_i + \varphi/2)| \quad (4)$$

where N is the number of the pixels involved in the averaging. If N is sufficiently large, the values of $\sum_i^N|\sin(\theta_i + \varphi/2)|$ approach to a constant, because the random phases θ are uniformly distributed over all values between 0 and 2π. Therefore, the fringe image reflects the refractive index variation in hydrogel due to surfactant diffusion.

The diffusion process is ruled by Fick's law, which for 1-D diffusion can be expressed as[15]

$$\partial C/\partial t = D(\partial^2 C/\partial x^2) \quad (5)$$

where D, the diffusion coefficient, is independent of the concentration in the interval of concentrations considered. The boundary condition in a solute−gel system in a cell could be expressed as

$$D_g \frac{\partial C_g}{\partial x} = D_s \frac{\partial C_s}{\partial x} \qquad x = 0$$
$$C_g = kC_s \qquad x = 0 \quad (6)$$

where D_g and D_s are diffusion coefficients in gel and in solution, respectively, and k is the partition coefficient between gel and solution. Here we define the interface between gel and solution at $x = 0$, and the region of gel as $x > 0$.

If we consider this as an infinite system, the solution of this equation, in the case of two media initially separated at the point $x = 0$ and with concentrations C_{0s} and C_{0g} is

$$C(x,t) = (C_{0s} + C_{0g})/2 + [(C_{0s} - C_{0g})\pi/2\sqrt{2}] \int_0^{x/2\sqrt{Dt}} \exp(-\eta^2)d\eta \quad (7)$$

As a result of the diffusion process which is taking place in the cell, the concentration gradient changes as a function of time. If the refractive index varies linearly with the concentration, we can express the change of the index of refraction between times t_1 and t_2 as

$$n(x,t_1) - n(x,t_2) = F[C(x,t_1) - C(x,t_2)] \quad (8)$$

where F is a constant.

When the fringe image is formed, a series of interference fringes appears superimposed on the image of the cell whenever the following condition is satisfied,

$$n(x,t_1) - n(x,t_2) = (2m + 1)\lambda/2d \quad (9)$$

where m is the interference order, λ is the wavelength of the light used, and d is the thickness of the gel that the light goes through. Thus, for the fringe of the pth order that appears in position x_1, and for the qth order fringe that appears in position x_2, we may obtain,

$$(2p + 1)\lambda/2d = F[C(x_1,t_1) - C(x_1,t_2)] \quad (10)$$

$$(2q + 1)\lambda/2d = F[C(x_2,t_1) - C(x_2,t_2)] \quad (11)$$

Combining eqs 10 and 11 we have

$$[C(x_1,t_1) - C(x_1,t_2)]/(2p + 1) = [C(x_2,t_1) - C(x_2,t_2)]/(2q + 1) \quad (12)$$

the diffusion coefficient being that value which meets eq 12.

To get the distribution of refractive index change, the phase data are evaluated by image processing. Adding I_{t1} and I_{t2}, and subtracting the result by from $2(I_1 + I_2)$ to eliminate the dc term, we can obtain the modified fringe system, which can be written as

$$I_{add} = |2I\gamma \cos(\theta + \varphi/2) \cos(\varphi/2)| \quad (13)$$

By the same averaging processing as that for subtracting the fringe system, we can obtain,

$$<I_{add}> = (2I\gamma/N)|\cos(\varphi/2)|\sum_{i}^{N}|\cos(\theta_i + \varphi/2)| \quad (14)$$

The values of $\sum_i^N|\cos(\theta + \varphi/2)|$ and $\sum_i^N|\sin(\theta_i + \varphi/2)|$ in eq 4 approach each other. Therefore, the phase $\varphi/2$ can be evaluated as

$$\varphi/2 = \tan^{-1}(<I_{sub}>/<I_{add}>) \quad (15)$$

From eq 15 and the orders of fringes, the phase change along the diffusion direction can be determined continually. In the experiment, the phase change relates to the refractive index change and can be written as

$$\varphi = 2\pi\Delta n(x)l/\lambda \quad (16)$$

where Δn, the refractive index change of gel, is a function of position x, l is the optical path of diffusion cell, and λ is the wavelength of laser in a vacuum. With the use of eqs 15 and 16 we can evaluate the refractive index change of gel in the diffusion process.

Experiments

Apparatus and Procedure. Figure 1 shows a diagram of our ESPI apparatus. A continuous wave He−Ne laser (model 127, Spectra-Physics Lasers, Inc.) emitting coherent light at 632.8 nm is used as the light source. The laser beam is divided into a reference beam and an object beam by a beam splitter. Each beam is focused through a pinhole spatial filter by a 25× microscope objective, and then passes through collimating lenses. The object beam traverses the diffusion cell, and the reference beam is reflected in the same way as the object beam. The two beams impinge on the CCD array. In holographic interferometry, the photosensitive element (photographic plate) must be able to resolve spatial frequencies in the range of the thousands of lines/mm. For CCD detectors, this condition is impossible to be met with the present technological constraints. It is necessary to introduce a ground glass plate as speckle source

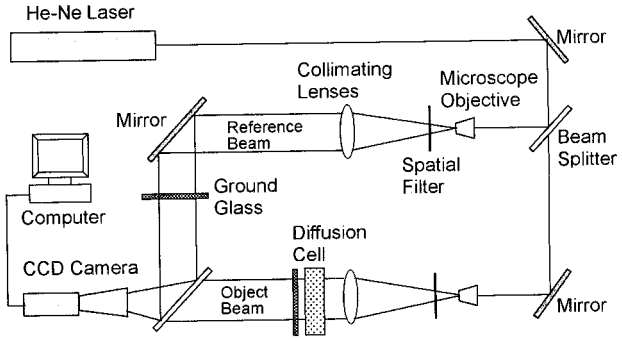

Figure 1. Experimental arrangement for measurement of diffusion in hydrogels by ESPI.

into the object beam to produce a speckle pattern to match the CCD resolution. To achieve quantitative and precise results, the quality of the speckle interference fringes is very important. The most favorable speckle can be obtained when the ground glass is located on the viewing side of diffusion cell and the objective focusing on it, because the refractive index variation of gel simply acts on the optical path. Therefore, at the ground glass level, only the phase varies, and the speckle pattern remains at the same place, favoring a good correlation factor. The interference fringes are recorded with a cooled CCD camera (C4742−95, Hamamatsu Co., Japan). The total image area contains 1280 × 1024 pixels. The diffusion cell is a 1.0 × 4.5 cm spectrophotometric cuvette with a 5 mm light path.

The measurement for diffusion concentration distribution is performed in two steps after the diffusion cell is fixed on the sample holder. In the first step, speckle images illuminated by the object beam I_1 and by the reference beam I_2 and the interference image I_0 illuminated by both beams simultaneously are taken by the CCD camera and stored on a hard disk. In the second step, the surfactant solution or myoglobin solution is put into the diffusion cell, and the dual-illumination images are sequentially taken and stored on the hard disk with an interval time of 5 min. Only the second step is performed when the measurement is for diffusion coefficients. The background noise is subtracted from all of the images taken in the image acquisition step.

Materials. Agarose (Agarose I, No. 346-00072) was obtained from Dojindo Laboratories Co., Ltd. and used without further purification. The gelation temperature of agarose is approximately 43 °C. To prepare the gel phases for diffusion measurements, an agarose aqueous solution at a prescribed weight percentage (0.5 to 4 wt %) was prepared by mixing agarose powder and deionized water and slowly heating to the solution boiling temperature. The solution was kept at this temperature until the agarose was completely dissolved. Then it was cooled to approximately 80 °C, being stirred until it appears homogeneous. After that, it was transferred to a glass spectrophotometric cuvette using a syringe. The cuvette was then cooled to the room temperature for at least an hour to ensure complete gelation. A piece of rectangular plastic was inserted into the cell to keep the up surface of the gel flat. The gel length in the cell was about 1.8 cm.

The anionic surfactants, methylsulfonic acid sodium salt (SMS) (No. M-0433), 1-butanesulfonic acid sodium salt (SBS) (No. B-0379), and 1-octanesulfonic acid sodium salt (SOS) (No. O-0123) were obtained from Tokyo Kasei Kogyou Co., Ltd. and used as received. The critical micelle concentration for SOS in water is 0.08 mol/L, and the surfactant was used below the cmc. There is no cmc for SMS and SBS.

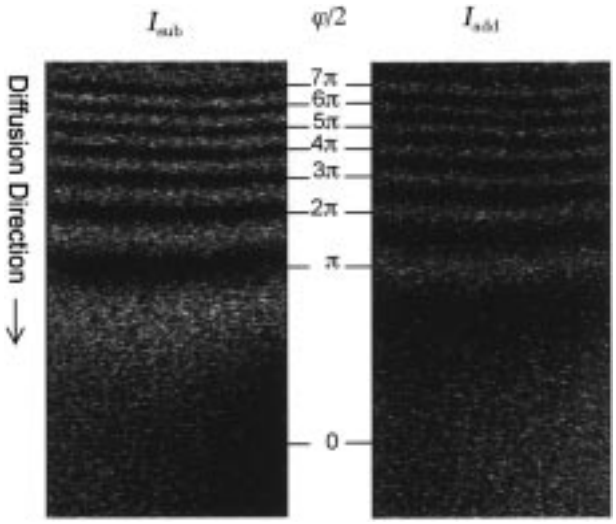

Figure 2. The subtraction and addition fringe patterns and the phase variation data at the diffusion time of 40 min.

Myoglobin from horse muscle (No. M-0630) was obtained from SIGMA (St. Louis, MO) and used without further purification. The molecular weight of the myoglobin molecule $M = 17600$ and its isoelectric point pI = 6.73. The myogobin aqueous solution was prepared by dissolving the myoglobin (5 wt %) in deionized water. The pH of the solution is 7.6 and the myoglobin has a negative net charge. As the agarose gel is nonionic, no charge effect is present in the system.

Conductivity Measurement. The conductivities of surfactant solutions was measured by an LCZ meter using a standard cell. The cell was calibrated beforehand using KCl solutions to determine the cell constant.

The equivalent (molar) conductivity Λ (S cm^2 mol^{-1}) was calculated from the conductivity κ and the surfactant concentration C by the relation of $\Lambda = 1000\kappa/C$.

The detailed experimental setup and the procedure of measurement are described elsewhere.[16]

Refractive Index Measurement. The relationship between the surfactant concentration and the refractive index was measured using a differential refractometer (DRM-1021, Otsuka Electronics Co., Japan), and that of agarose gel was measured using an Abbe refractometer (Atago Co. Ltd., Japan).

Results and Discussion

Diffusion Constant. Figure 2 shows images displaying I_{add} and I_{sub} fringe patterns obtained at $t_1 = 0$ and $t_1 = 40$ minutes. Both images represent parallel fringes, which are consistent with 1-D diffusion systems. The absolute value of $\varphi/2$ for each fringe can be determined, as shown in the middle of Figure 2. It is clearly seen that the fringes of I_{sub} are located at $\varphi/2 = \pi/2$, $3\pi/2$, ..., and those of I_{add} are at $\varphi/2 = 0$, π, 2π, ...

The fringes in the interferograms used to calculate the diffusion constant are located far from the interface between gel and solution. It avoids the influence of "mirage" effects at phase boundaries.[12,17] The partition coefficient k was set to 1 in the calculation for simplicity.

Surfactant Diffusion. Figure 3 shows the diffusion coefficient of sodium sulfate of various concentrations in agarose gels obtained by ESPI. To confirm that the diffusion coefficient of the simple molecules is not sensitively affected by the agarose concentration, the experiment was carried out for two samples of agarose with concentration of 2 wt % and 4 wt %.

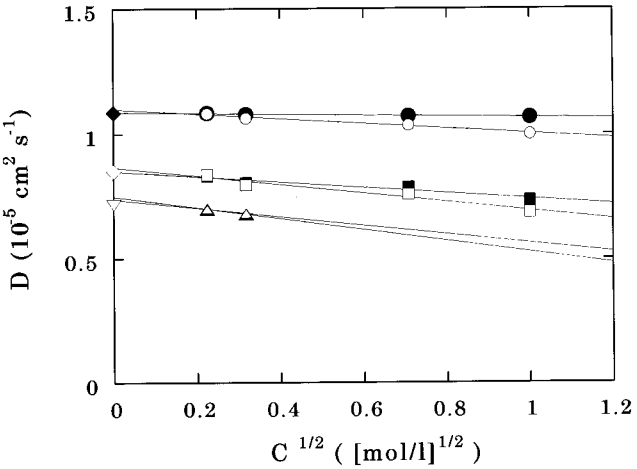

Figure 3. Diffusion coefficients of sodium sulfate in 2 wt % (closed marks) and 4 wt % (open marks) agarose gel: (●,○), SMS; (■,□), SBS; (▲,△), SOS. The data on the vertical axis are the values obtained in water by electrical conductance measurement: (◆), SMS; (◇), SBS; (▽): SOS.

For the ionic surfactants, the diffusion coefficient obtained is the harmonic average of the cation D_c and the anion D_a[18]

$$D = 2D_cD_a/(D_c + D_a) \tag{17}$$

When $D_c \approx D_a$, we approximately have

$$D \approx (D_c + D_a)/2 \tag{18}$$

On the other hand, the diffusion coefficients of ions can be related to their molar conductivity Λ_i by the relation of[18]

$$D_i = RT\Lambda_i/F^2 \qquad (i = \text{c,a}) \tag{19}$$

where R, T, F are the gas constant, the absolute temperature, and the Faraday constant, respectively. The molar conductivity of an electrolyte solution $\Lambda = \Lambda_c + \Lambda_a$ linearly decreases with the increase in the square root of the electrolyte concentration according to Kohlrausch's law[18]

$$\Lambda = \Lambda_0 - SC^{1/2} \tag{20}$$

where Λ_0 is the molar conductivity at infinite concentration and S is a constant which does not dependent on the concentration. Combining eqs 17, 18, and 19 we get

$$D \approx D_0 - \frac{RTS}{F^2}C^{1/2} \tag{21}$$

where

$$D_0 = \frac{RT}{2F^2}\Lambda_0 \tag{22}$$

All of the experimental data showed a good linearity between D and the square root of concentration $C^{1/2}$, demonstrating that the Kohlrausch's law is still holding at such high concentrations (Figure 3). The extrapolated values of the diffusion coefficient D_0 at the infinite concentration are listed in Table 1. The diffusion coefficients at infinite concentration obtained from the electrical conductance measurement, using the relation of eq 22, are also listed in Table 1. As shown in the table, the discrepancies between the results obtained from ESPI and from the electrical conductance measurement were less than 5%. This

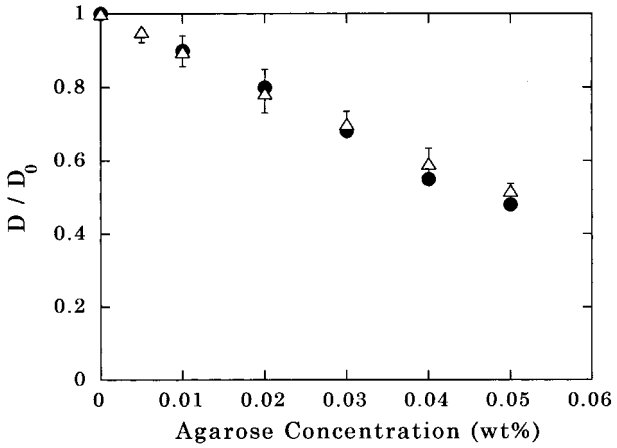

Figure 4. Diffusion coefficients of myoglobin in agarose gel: (△), measured by ESPI; (●), reported by ref 1.

TABLE 1: Comparison of the Diffusion Coefficients D_0^{ESPI} Measured by the ESPI Method and D_0^{el} Obtained from the Electrical Conductance Measurement

surfactant	D_0^{el} (10^{-5} cm^2/s)	agarose gel concn	D_0^{ESPI} (10^{-5} cm^2/s)	$\Delta D_0/D_0^{el}$ (%)
SMS	1.086	2 wt%	1.088	0.12
		4 wt%	1.100	1.28
SBS	0.852	2 wt%	0.851	0.10
		4 wt%	0.868	1.83
SOS	0.718	2 wt%	0.749	4.37
		4 wt%	0.738	2.81

good agreement demonstrates the validity of the ESPI method for the measurement of diffusion constants of simple molecules.

Protein Diffusion. To investigate the possibility of applying the ESPI to the diffusion of macromolecules, we have further carried out the experiment on the diffusion of myoglobin in agarose gel. Figure 4 shows the agarose concentration dependence of the diffusion coefficient of myoglobin obtained by ESPI. To make a comparison, the results obtained from the literature[1] are also shown in Figure 4. The results obtained from the ESPI showed a good agreement with those reported, demonstrating the validity of the ESPI method for the measurement of diffusion coefficients of macromolecules.

The main source of errors of this method comes from the coarseness of the fringe pattern, which limits the accuracy of location of the fringe peak in the digital image processing.

Spatial Distribution of Concentration. Figure 5 shows the refractive index change distribution in 2 wt % agarose gel with 0.1 M SBS as the diffusion solution at diffusion times of 5, 20, 50, and 120 min, respectively. To get the concentration values we investigate the relation of surfactant concentration to refractive index. By best linear fitting, we get the relation of $dn/dc = 0.01912$ M^{-1}. Therefore, the refractive index data shown in Figure 5 can be transferred into the concentration values, which are also shown in Figure 5. Because the surfactant concentration is low, the refractive index gradient is small. The "mirage" effect could be ignored from the calculation on the basis of reference 18.

Supposing that D_g and D_s are constants, using eq 7 to fit the experimental values, we can get the theoretical predictions for the diffusion as shown in Figure 5 with solid lines. The diffusion coefficients of SBS in water, $D_s = 8.05 \times 10^{-6}$ cm^2/s, is used in the calculation. The result shows that the theoretical prediction is in conformity with the experimental data only in the primary diffusion time with $D_g = 8.05 \times 10^{-6}$ cm^2/s. It is clearly seen from Figure 5 that the refractive index variation near the surface

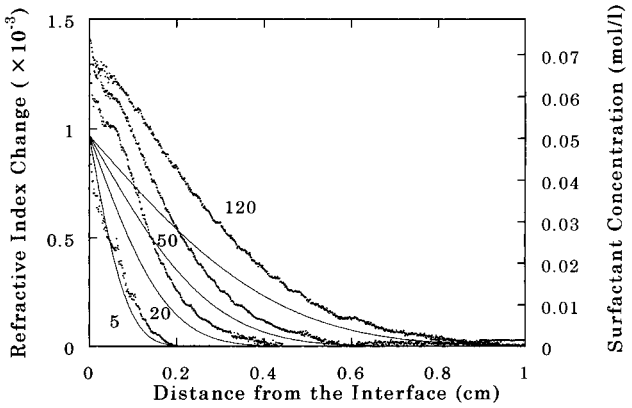

Figure 5. The refractive index change distributions and SBS concentration distributions in 2 wt % agarose gel. The numbers on curves are values of diffusion time (minutes). The interface of gel and solution is at $x = 0$, the diffusion solution is 0.1 M SBS. Points, experimetal data; solid lines, theroetical curves with $D_g = 8.05 \times 10^{-6}$ cm^2/s predicted by eq 6 and 7.

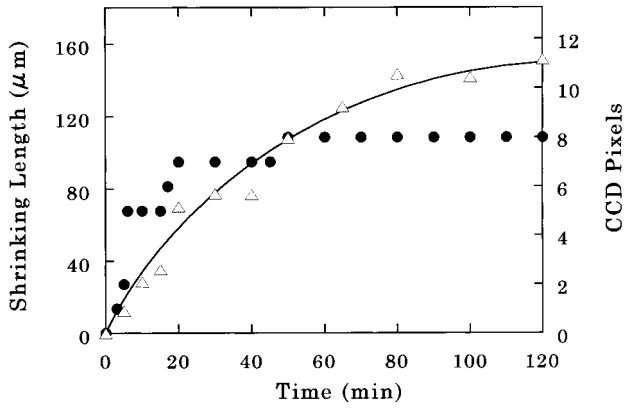

Figure 6. The shrinking length of 2 wt % agarose gel in 0.1 M SBS solution as a function of diffusion time (minutes) measured by CCD camera (●) and calculated by eq 23 and Figure 7 (△).

of the gel increases with time, which is much different than with theoretical values.

The additional variation of the refractive index shown in Figure 5 might be caused by the shrinking of the gel in surfactant solution. We investigate the position of interface between gel and solution in the diffusion process and find that the gel has slightly shrunken in the 0.1 M SBS solution. The gel length measuring resolution is about 13.6 μm per pixel with the CCD camera. Figure 6 shows the relation of diffusion time and gel shrinking length.

The diffusion in fibrous media has been predicted in a variety of ways.[19−22] According to these results as well as the results in Figure 3, the diffusion of the surfactant, which is not of colloidal size, would not be distinctively affected by the change in the agarose concentration of gels at the experimental conditions. Thus, D_g could be treated as a constant upon gel contraction.

As shown in Figure 6, the shrinking length of gel is very small with respect to the diffusion length shown in Figure 5 and the displacement of solute−gel interface in diffusion also can be ignored. Therefore, the effect of gel shrinking to diffusion is negligible and the distribution of surfactant concentration in gel would obey eq 7.

For reasons given above, we think the additional refractive index change mainly results from the change of gel density due to shrinking. The value of dn/dc of agarose gel is measured about 1.41×10^{-3} (wt %)$^{-1}$. Subtracting the theoretical values

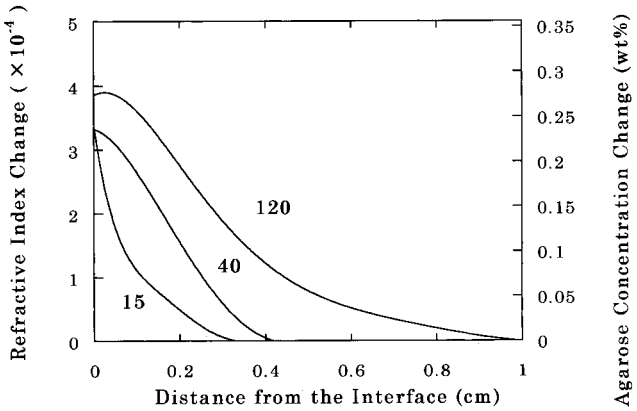

Figure 7. Distributions of refractive index change and agarose concentration variation in the diffusion process of surfactant. The numbers on the curves are values of diffusion time (minutes). The initial concentration of agarose gel was 2 wt %.

predicted by eq 7 from the experimental data shown in Figure 5, we get the distribution of additional refractive index change and gel density change, as shown in Figure 7. Assuming that the shrinking per unit of gel is uniform along the three spatial directions, the shrinking length ΔL can be obtained approximately from Figure 7 using the relation

$$\Delta L = \frac{1}{3}\int_0^{l_g} \frac{\Delta\phi}{\phi_0}\,\mathrm{d}x \qquad (23)$$

where ϕ_0 and $\Delta\phi$ are the initial concentration and concentration change of gel due to shrinking, respectively. The results are also shown in Figure 6 and in comparison with the values measured by the CCD camera.

The difference between the measurement data by CCD camera and the integration data by eq 23 is considered mainly to result from the assumption that the shrinking of gel is isotropic. In practice, the local shrinking of gel is dependent on the densities around it. In the diffusion cell in our experiment, the gel shrinking along the diffusion direction might be larger that other directions. It would introduce errors in calculating the shrinking length by eq 23. Further investigation on the contraction of gel is under progress.

Conclusion

The ESPI method is valid for measurement of diffusion coefficients in hydrogel. The method is simple and accurate. Furthermore, we need only know times t_1 and t_2 and measure the distances at which the interference fringes appear. Moreover, it is possible to obtain several diffusion coefficient values from each interferogram and an accurate mean value can be calculated, in contrast to other methods which obtain only one value. The accuracy is higher than when conventional techniques are used, and the diffusion process can almost be visualized with electronic speckle pattern interferometry, whereas with conventional techniques, the diffusion coefficient has to be measured by means of indirect methods.

The dynamic diffusion process of surfactant into gel can be followed by the ESPI technique, and the spatial distribution of refractive index change in gel with time can be determined accurately. The refractive index change reflects the variation of surfactant concentration and hydrogel density. Comparing the experimental results with the theoretical prediction based on diffusion theories, the local information of diffusion in gel can be obtained. This is beneficial to study the interactions between surfactants and hydrogels in a microscopic view.

The agarose gel shrinks slightly in surfactant solution and increases its density. The additional refractive index change due to gel shrinking is observed by ESPI. The theory of diffusion in fiber media shows that the diffusion coefficient varies very little when the radius of solute is much smaller than that of fiber. Therefore, the distribution of refractive index change attributed to the diffusion of surfactant could be obtained on the basis of Fick's law, and the quantitative information of shrinking behaviors of agarose was acquired successfully. The results presented in this paper show that ESPI is a promising technique for study the diffusion behaviors of molecules in hydrogels.

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