Optical investigation of diffusion of levofloxacin mesylate in agarose hydrogel

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Abstract. Real-time electronic speckle pattern interferometry method has been applied to study the diffusion behavior of levofloxacin mesylate (MSALVFX) in agarose hydrogel. The results show that the diffusivity of solute decreases with the increase of concentration of agarose and adapts to Kohlrausch's law. Furthermore, Amsden's model, based on the retardance effect associated with polymer chain flexibility, was employed to simulate the diffusion behavior. The consistent results suggest that the retardance effect dominates the diffusion process of MSALFVX in hydrogel; moreover, polymer chain flexibility greatly affects drug transport within the polymer matrix.

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The study of drug molecule transport in hydrogels, especially in biological hydrogels, is significant for gaining a better understanding of drug-gel interactions, and also for exploring some potential applications in diagnostic, therapeutic and bioseparation, etc.[1] Recently, investigation of diffusion transport has been reported using various optical techniques.[2–4] In particular, with the advent of an inexpensive laser, digital recording, computing facilities, and strong functional image-processing software, electronic speckle pattern interferometry (ESPI) has become a powerful tool for medical application.[4,5] In this work, the horizontal fringe mode of ESPI system was applied to study the diffusion of levofloxacin mesylate (MSALVFX) in agarose hydrogel. The interaction of MSALVFX and hydrogel was analyzed based on the experimental results and theoretical retardance models. MSALVFX is a mesylate of levofloxacin and a kind of new fluoroquinolone antibacterial agent.[6] In order to apply MSALVFX in a safer way or even guide some more excellent generation of fluoroquinolones, it is necessary and meaningful to understand the interaction mechanism between drug molecular and biological hydrogels.

The ESPI optical setup used in this study is as stated in Zhang et al.'s work.[4] It is similar to Mach–Zehnder interferometer and is a typical out-plane ESPI configuration. The object beam traverses the sample cell, and the reference beam is transmitted in a symmetric way. Then the two beams meet on the CCD detector. The main difference for ESPI system is that the ground glass plates as diffusors are required and located at the focus of the CCD lens. Because the speckle pattern is originated from the random scattered lights, ESPI method is beneficial to avoid the environment disturbance and compare the diffusion behavior between any pair of times. When the refractive index of gel varies with the medicine molecules diffusing, the change of concentration corresponding to subtracted ESPI fringe orders yields as follows:[4]

$$\frac{\varphi}{2\pi}\lambda = L[n(x,t_R) - n(x,t_L)] = L\frac{dn}{dc}[C(x,t_R) - C(x,t_L)], \tag{1}$$

$$C(x,t) = C_0 + \frac{m\lambda}{L(dn/dc)}, \tag{2}$$

where n, L, λ, and t_R and t_L are the refractive index value of gel, optical path of diffusion cell, wavelength of laser, time for recording at subtracted instance and reference instance; dn/dc denotes the refractive index increment; $C(x,t)$, C_0 are the concentration at the position x and the initial concentration.

By using the ESPI method, we investigated the diffusion of MSALVFX (molecular weight $M=475$, received as donation), in agarose hydrogel. The preparation of agarose solution has been described previously.[2] Briefly, the desired weight of dry agarose powder (Donghai Pharmacy Co., Ltd. Shanghai, China) was added in degassed distilled water. The mixture was boiled and then transferred to a glass spectrophotometric cuvette with a volume of $4.5\times1.0\times1.0$ cm^3 and a wall thickness of 1 mm. The injected height in the cuvette was $\sim$2.2 cm. A piece of rectangular flakelet was inserted into the hot solution to get a flat surface. Therefore, agarose hydrogels were prepared after the solution was left in ambient atmosphere for 1 h to complete gelation. When the MSALVFX solutions of different concentrations were injected into the upper part of the cuvettes (the down part occupied by as-prepared agarose hydrogels), drug diffusion began. The CCD recorded and the fringe images were displayed on computer in near real time.

Figure 1(a) shows the sequential interference images of 5 mg/mL MSALVFX diffusing in 1.5% (w/w) gels at $t_R=0$ min, and t_L with an increment of 50 min. As the diffusion processes, the fringes increase and spread along the diffusion direction. On the basis of Fick's second law, the concentration can be solved with the initial and boundary conditions of $t=0$, $0<x<l_g$, $C=0$; $x=0$, $C=C_0$; $t>0$, $x=0$, $\partial C/\partial x=0$ as,[7]

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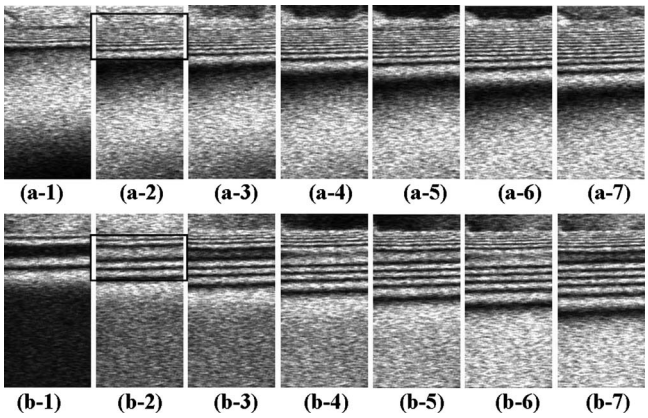

Fig. 1 Interference fringes evolution of 5 mg/mL MSALVFX diffusing in 1.5% (w/w) gels at sequential mode (a) $t_R=0$ min, t_L with an increment of 50 min; (b) $t_R=50$ min, t_L with an increment of 100 min.

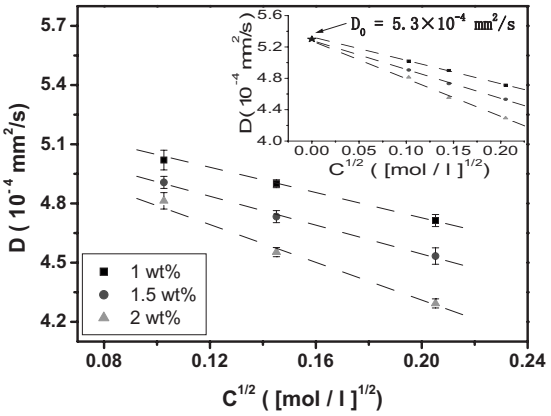

Fig. 2 Dependence of MSALVFX diffusion coefficient on the square root of the concentration of agarose hydrogels with a concentration of 0.5% (w/w) (□), 1% (w/w) (○), and 2% (w/w) (△), respectively. The inset shows the linear extrapolation for prediction of diffusion coefficient at the infinite concentration.

$$C(x,t) = C_0\left\{1 - \frac{4}{\pi}\sum_{k=0}^{\infty}\frac{1}{2k+1}\cdot\exp[-D(2k+1)^2\pi^2 t/(2l_g)^2]\sin\frac{(2k+1)\pi x}{2l_g}\right\}, \quad (3)$$

where l_g is the length of the gel, $\partial C/\partial x$ denotes the concentration gradient from the interface along the diffusion direction, and k is an integer. Equation (3), accompanied with Eq. (2), implies that the MSALVFX concentration at the interface of $x=0$ equals to that of the initial solution. The resultant fringes are too crowded as in Fig. 1(a). However, when we take the image at 50 min instead of 0 min as the reference, the more legible sequential images appeared, as shown in Fig. 1(b). The living images display that a heartland occurs and new fringes grow out incessantly with the drug diffusion. Obviously, the fringes distinguish better. Because of the same concentration at the boundary $x=0$, $C=C_0$, and $x=l_g$, $C\approx 0$, thus subtracting the speckled image corresponding to different pairs of times must give a maximum at a certain position from the interface due to the subtraction. The fringes with equal orders will locate at both sides of maximum. Moreover, the diffusion coefficient can be fitted from the fringe orders of speckle fringe images according to Eqs. (2) and (3), and the least-squares method. Here, the refractive index increment in Eq. (3) was measured to be 0.21 mL/g by a simple refractometer.

Figure 2 shows the fitted MSALVFX diffusion coefficients in agarose hydrogels of various concentrations. To ensure the results, all diffusion coefficient data were averaged from the subtracted images at $t_L=200$, 400, and 600 min and $t_R=50$ min, and the corresponding t_L was taken four times from the neighbored fringe images. As shown in Fig. 2, solute diffusion coefficients decrease as the concentration of hydrogel increases. It is believed that the increase of polymer concentration will lead to a decrease in the mesh size of the gel network and shrinkage of the available space for the diffusing solute,[8] thereby retarding the solute diffusivity. Meanwhile, the diffusion coefficient reduces as solute concentration increases. This may be because the hydrodynamic drag enhances with the amount of solute and thereby obstructs the diffusivity. For a different molecule concentration, Zhang et al. demonstrated that Kohlrausch's law was still holding at a high concentration of polymer-solvent electrolyte system.[4] The molar conductivity of an electrolyte solution linearly decreases with the increase in the square root of the electrolyte concentration, and the diffusion coefficient approximately yields as follows:[4] $D\approx D_0-SC^{1/2}$, where S is a constant independent of the concentration and D_0 is the extrapolated value of the diffusion coefficient at the infinite concentration. In present drug delivery system, all the experimental data showed good linearities, implying that the MSALFVX diffusion also adapts to Kohlrausch's law. Moreover, the diffusion coefficient D_0 can be obtained by linear extrapolation as about 5.3×10^{-4} mm^2/s (inset of Fig. 3).

To further understand the diffusion behavior of MSALVFX in agarose hydrogels, we compared the experimental results to some empirical physical mechanism to elucidate the solute transport. Concerning small molecule transport in a hydrogel, the polymer chains may retard the movement of the molecules in the polymer matrix, Amsden's model based on the retar-

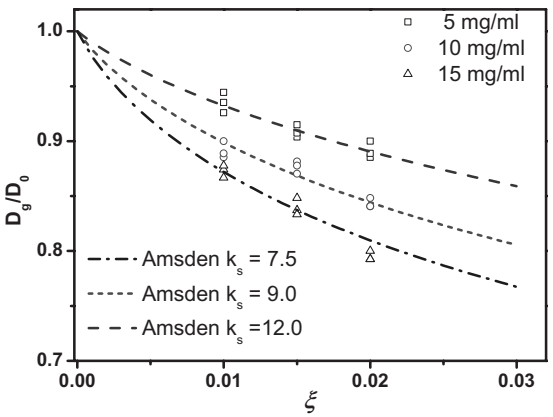

Fig. 3 Application of the Amsden (dash line) models to the experimental data showing the effect of polymer volume fraction on solute diffusivity. The scattered symbols are the experimental data for different MSALVFX concentration of 5 mg/mL (□), 10 mg/mL (○), and 20 mg/mL (△), respectively.

dance effect was chosen to simulate the diffusion behavior of MSALVFX in heterogeneous agarose gels.[8] This model describes that the polymer fibers in the polymer gel are stiff and the solute transport is determined by the probability of the solute finding enough space between polymer fibers. Combining the free volume theory with the retardance theory and a scaling law, a mathematical description of the restricted movement of solutes yields[8]

$$\frac{D}{D_0} = \exp\left[-\pi\left(\frac{r_s + r_f}{k_s \xi^{-1/2} + 2r_f}\right)^2\right], \tag{4}$$

where k_s is the scaling parameter and depends on the flexibility of the polymer chains, which decrease as polymer concentration increase.[8] Because agarose hydrogel fibers are composed of bimodal bundles of α-helix chains with 87% having a radius of 15 Å and 13% of 45 Å, r_f is taken as 19 Å in the application,[8] whereas, r_s can be estimated to be 4.6 Å from the Stokes–Einstein equation $r_s = k_b T / 6\pi\eta D_0$ ($k_b = 1.38 \times 10^{-23}$ J/K; $T = 298.15$ K; $\eta = 0.8949 \times 10^{-3}$ Pa s). By employing Eq. (4), the normalized diffusion coefficients of MSAVFX as a function of the polymer volume fraction are plotted in Fig. 3. Obviously, Amsden's model shows a good agreement with most of the experimental data by adopting the scaling parameters of 7.5, 9.0, and 12.0 Å for MSALVFX concentration of 5, 10, and 20 mg/mL, respectively. The difference in scaling parameters is originated from the flexibility of polymer chains in different concentration of polymer-solute system. It suggests the diffusion process of MSALFVX within hydrogel not only is dominated by retardance effect, but also is greatly affected by polymer chain flexibility.

In summary, real-time ESPL is employed to analyze the diffusion of medicine molecule MSALVFX in agarose hydrogels. To get more distinguishable fringes, reference images recorded at different instances are chosen. The diffusion coefficient decreases with the increase of concentration of agarose hydrogels and MSALVFX solution as well. Meanwhile, Amsden's model is employed to study the interaction between drug molecule and agarose hydrogel matrix. The results suggest that the retardance effect dominates the diffusion process of MSALFVX in hydrogel, and polymer chain flexibility also affects the drug transport in polymer matrix. The present investigation of drug transport in biological hydrogels provides a meaningful means to understand the control release of drugs in living tissues and design viable drug delivery device.

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