Diffusion of Levofloxacin Mesylate in Agarose Hydrogels Monitored by a Refractive-Index Method

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ABSTRACT: The diffusion of levofloxacin mesylate (MSALVFX) within agarose hydrogels was investigated by an improved refractive-index method. The diffusion coefficient of MSALVFX in infinite dilution (D_0) was obtained as 4.01×10^{-6} cm^2/s (25°C) through extrapolation according to Kohlrausch's law. The diffusion behavior of MSALVFX under conditions of different solute concentrations, polymer volume fractions, and temperatures was studied in detail. In the considered range of agarose concentrations (0.5–2.5%, w/w), the ratio of D_g to D_0 (where D_g is the effective diffusion coefficient of MSALVFX in the agarose hydrogel) decreased from 0.938 to 0.835 because of the retardance effect of agarose fiber. Furthermore, the experimental data were analyzed with the Amsden, Clague and Philips, and Ogston models, and the data fit the Amsden model best. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 3000–3006, 2011

Key words: diffusion; hydrogels; refractive index

INTRODUCTION

Drug diffusion in porous hydrogels is significant for understanding the transport process in organisms[1–3] and the release from specific sustained-release dosage forms, such as hydrophilic matrix tablets[4,5] and sustained delivery implants.[6–8] Agarose, a polysaccharide consisting of 1,3-linked D-galactopyranose and 1,4-linked 3,6-anhydro-R-L-galactopyranose,[9] can form a physically crosslinked hydrogel by cooling a hot agarose aqueous solution. The pore size of the resultant hydrogel ranges from 1 to 900 nm with changing agarose concentration.[10] Because its typical characteristics resemble the living tissues in composition, rheological properties, and water content, the agarose hydrogel is widely used to study the molecular diffusion *in vitro*.[11–13]

Levofloxacin, the S-(−) isomer of ofloxacin, is one of the most excellent quinolone antibiotics with a broad spectrum of antibacterial activity. The antibacterial activity of levofloxacin is 8–128 times than that of the R-(−) isomer.[14] Levofloxacin mesylate (MSALVFX), which is more water soluble than levofloxacin, has a similar antibacterial activity to levofloxacin both *in vitro* and *in vivo*[15,16] and has been used to treat various microbial infectious diseases in clinics. To apply MSALVFX in a safe and efficient way and also to further understand the mechanism of molecular interaction *in vivo*, it is important to obtain additional information of the molecular transport of MSALVFX.

Over the past decades, several approaches have been developed to investigate the molecular diffusion in hydrogels; these include light scattering,[17,18] NMR microscopy,[1,19,20] fluorescence spectroscopy,[21–23] Fourier transform infrared microscopy,[24,25] and electrochemical techniques.[26,27] Most of these methods are usually carried out in an intermittent manner by detection of the change of the solute concentration (C) outside of the polymer hydrogels. Optical methods, such as electronic speckle pattern interferometry[28] and holographic interferometry,[29] are more accurate than intermittent methods because of *in situ* nondestructive monitoring. However, the instruments used for these two methods are expensive and complex. In our previous work,[30] we established a refractive-index method to investigate the diffusion of a solute in hydrogels. This optical method is simple, low cost, and proven to be promising for the study of the molecular diffusion in hydrogels.[30–32]

Herein, we investigated the diffusion behavior of MSALVFX in agarose hydrogels by using an improved refractive-index method coupled with a temperature-controlled device,[31] and we studied the effects of

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various agarose concentrations (C's) and temperatures on the drug diffusion. The results may provide a better understanding of the diffusion behavior of MSALVFX in a gel system, which is important for the *in vivo* investigation and design of advanced dosage forms.

EXPERIMENTAL

Reagents

Agarose was purchased from Donghai Pharmacy Co., Ltd. (Shanghai, China) and was used without further purification. MSALVFX (weight-average molecular weight = 475 g/mol) was received as a donation. Deionized water was used as a solvent.

Preparation of the agarose hydrogels

Agarose hydrogels with agarose concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5% (w/w) were prepared as described previously.[31] The initial refractive indices of the gels (n_0's) were taken from our previous work.[30] MSALVFX solutions were prepared by dissolution of the MSALVFX powder in deionized water at desired concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5% (w/v).

Measurements

The diffusion coefficient of MSALVFX in the agarose hydrogel (D_g) was measured by the improved temperature-controlled refractive-index method.[31] A triangular cell with one acute angle of 30° was made accurately. The bottom of the cell with the gel inside was immersed in a container of the diffusion solution, which was magnetically stirred to keep the diffusion solution homogeneous. The temperature of the diffusion cell was controlled by a special temperature-controlled device (Fig. 1). The principle of the experiment was described previously.[32] A continuous-wave semiconductor laser ($\lambda = 632.8$ nm) was used as the light source. The laser beam transmitted first through a set of lenses and a linear aperture to obtain a linear parallel light strip with a length of 14.3 mm. The object light strip then passed through the triangular gel cell and formed a linear light strip on the screen. The interface between the gel and diffusion solution was defined as $x = 0$ (where x is the diffusion distance). To avoid a mirage effect,[33] the bottom of the light strip was set at $x = 2.5$ mm. During the diffusion process, the bottom of the linear light strip became bending because of the refractive-index change (Δn) of the gel system, which resulted from the diffusion of drug in the gel. The light strip was immediately monitored with a charged coupling device (CCD) camera (JT-2172) Shenzhen Dijiete Electronics Co., Ltd. (Shenzhen, China). Images of the light strip were recorded at 5-min intervals and treated with specific software.

Theoretical analysis of the experimental data

When the solute diffused upward into the hydrogels contained in the triangular cell, the refractive index of the hydrogels changed; this led to a shift of the light strip (Fig. 1). The change of the refractive index [$\Delta n(t)$] can be represented as

$$\Delta n(t) = \frac{\Delta l \cos \alpha}{L \sin \theta} = \frac{\sqrt{1 - n_0^2 \sin^2 \theta}}{L \sin \theta} \Delta l(t) \quad (1)$$

where L is the distance between the emergence point and the screen, $\Delta l(t)$ is the shift distance of the light strip from its initial point at time t, θ is the incidence angle, and α is the refracted angle. The diffusion of solute in the hydrogels is a dynamic process. The change of C is a function of time. A transform between the profiles of Δn values and the profiles of C in the gel is expressed as[34]

$$n(x, t_1) - n(x, t_2) = k[C(x, t_1) - C(x, t_2)] \quad (2)$$

where t_1 and t_2 are two optional time in the whole diffusion process, n is the refractive-index value of the hydrogel. $C(x, t)$ is the concentration of the diffusing solute in the hydrogel in x at time t and k is a constant. Combined with eq. (1), the distribution of concentration in the hydrogel at any time t could be obtained. Moreover, the maximum value of the refractive-index change (Δn_{max}) from Δl_{max}, the maximum shift distance of the light strip from its initial point, according to eq. (2), is considered to correspond to the initial concentration of the diffusion solution (C_0).

Because the diffusion process is one dimensional and controlled by Fick's second law, the diffusion coefficient (D) could be simulated by the least-squares procedure in terms of the analysis solution of Fick's second law [eq. (3)] with the initial and boundary conditions. (1) Initially ($t = 0$), C is zero in the whole diffusion field ($0 < x < l$),. (2) At the interface of the solution and hydrogel ($x = 0$), C is C_0. (3) During the diffusion process ($t > 0$), C is steady ($\partial C/\partial x = 0$) at the interface of the solution and hydrogel ($x = 0$):[35]

$$\frac{C(x,t)}{C_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \cdot \exp[-D(2n+1)^2 \pi^2 t/4l^2] \cdot \cos \frac{(2n+1)\pi x}{2l} \quad (3)$$

where l is the length of the gel and $\partial C/\partial x$ is the concentration gradient along the diffusion direction

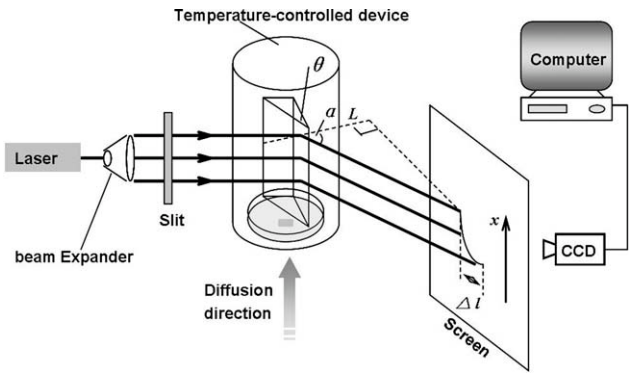

Figure 1 Schematic image of the refractive-index method with a temperature-controlled device for monitoring the diffusion process.

(x axis; Fig. 1). During the data processing, we only considered x to range from 2.5 to 16.8 mm. The coarseness of the light strip was the main source of error in the digital image processing. D, therefore, was the average value estimated from three images recorded at different diffusion times.

RESULTS AND DISCUSSION

Diffusion of MSALVFX in the agarose hydrogels

According to the MSALVFX distribution within the agarose hydrogels, the diffusion process was evaluated successfully. Shown in Figure 2, in 1.5 wt % agarose hydrogels, are the Δn values with x in the presence of a 30.0 mg/mL MSALVFX aqueous solution at 25°C. Δn denotes the transport of MSALVFX in the hydrogel. There was an exponential decrease of the refractive index with an increase of x; this indicated a large concentration gradient of MSALVFX formed in the agarose hydrogel during the diffusion process. Furthermore, Δn_{max} at $x = 2.5$ mm increased from 1.73×10^{-3} to 2.72×10^{-3} as the diffusion time was prolonged from 155 to 300 min; this demonstrated that the refractive-index method could trace the whole diffusion process under nondestructive circumstances.

On the basis of eq. (2), $\Delta n(t)$ could be converted to the change of MSALVFX concentration $C(x,t)$ in the agarose hydrogels. As a function of x, the concentration of MSALVFX in the 1.5 wt % agarose hydrogels at different diffusion times is illustrated in Figure 3. At $x = 2.5$ mm, the concentration of MSALVFX increased from 9.15 to 13.42 mg/mL as the diffusion time increased from 155 to 300 min. On the basis of Fick's second law, the experimental results of the MSALVFX concentration profiles were fitted with eq. (3). D_g was obtained as 3.44×10^{-6} cm^2/s in 1.5 wt % agarose hydrogels at 25°C.

Although there was some data scattering, the experimental results agreed with the theoretical curves. The data scattering may have arisen from the interaction between the MSALVFX and the surrounding polymer network. Previous study has indicated that the agarose exhibited a weakly ionic nature in the gel state because of the presence of sulfate, ketal pyruvate, and carboxyl groups located at the 2 and 6 positions of β-D-galactopyranose in the agarobiose backbone.[36] Under different ionic strength and pH conditions, the electrostatic interaction between agarose hydrogels and various ions (organic and inorganic ions) has been reported.[9] Similar to those charged ions, MSALVFX is partly ionized in neutral environments. Therefore, a weak electrostatic interaction between MSALVFX and the agarose hydrogel networks may have existed and affected the diffusion process thereof. Other possible factors, such as hydrogen-bonding interaction, hydrophobic interaction, and trapping effects of the rigid agarose matrix, also influenced the diffusion of MSALVFX in different ways. Systematic deviations also contributed to the data scattering.

Influence of the temperature on the MSALVFX diffusion

In general, the diffusivity of solutes in porous mediums increased with the surrounding temperature. To determine how temperature influenced the diffusion of MSALVFX in the agarose hydrogels, additional diffusion experiments for MSALVFX in

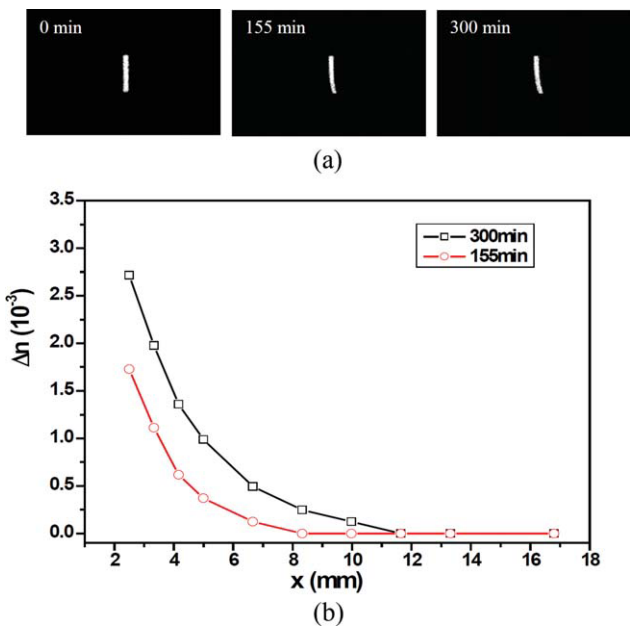

Figure 2 Diffusion of MSALVFX in agarose hydrogels. (a) The typical images of the light strip at different diffusion times for 30.0 mg/mL MSALVFX in 1.5 wt % agarose hydrogels ($T = 25$°C). (b) Δn as a function of x deduced from the typical images. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

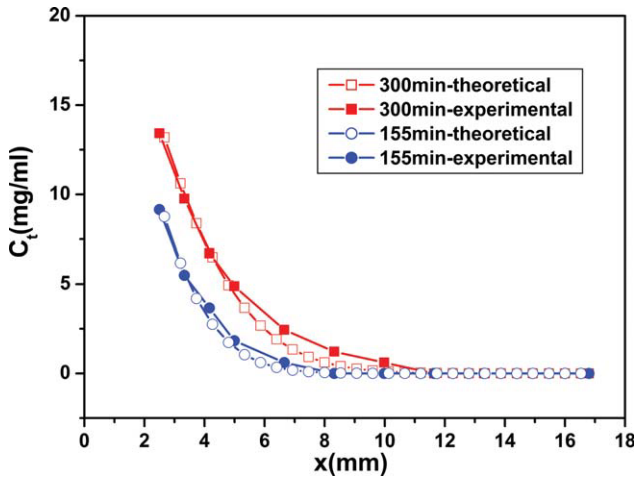

Figure 3 Distribution of the solute concentration (C_t) as a function of x at two diffusion times for 30.0 mg/mL MSALVFX in 1.5 wt % agarose hydrogels at 25°C: (○, □) Theoretical and (●, ■) experimental curves. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

1.5 wt % agarose hydrogel were performed at temperatures of 20, 25, 30, 37, and 40°C, respectively. Figure 4 presents a plot of ln D_g as a function of $1/T$. As expected, D_g increased with the increase of temperature. This was due to the fact that the low temperature increased the microscopic viscosity of the solvent entrapped in the agarose hydrogel network, which had a retardation effect on the solute diffusion.[27,37] Here, the dependence of ln D_g on $1/T$ was linear; this agreed with the theoretical result predicted by an Arrhenius-like equation. Consequently, when the temperature increased, there was a tendency for the polymer network to dissociate because of the weakening of hydrogen bonding and hydrophobic interactions between polymer chains. This dissociation in the microstructure of the agarose hydrogels caused a decrease of hindrance effects on the diffusion of MSALVFX and, thus, accelerated the diffusion of MSALVFX at higher temperatures.

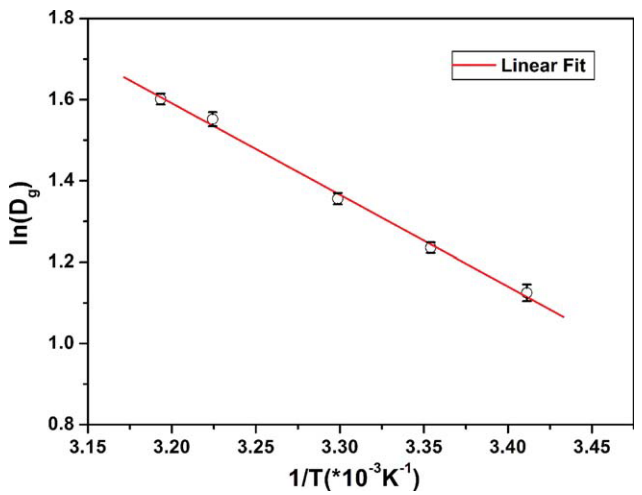

Figure 4 Influence of the temperature on the diffusivity of MSALVFX in 1.5 wt % agarose hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Influence of the MSALVFX concentration on the diffusion

According to Kohlrausch's law, D of an electrolyte can be approximately written as[34]

$$D \approx D_0 - SC^{1/2} \tag{4}$$

where D_0 is the diffusion coefficient of a solute in a liquid at infinite dilution, S is a constant independent of the concentration at a given temperature, and $C^{1/2}$ is the square root of concentration. Also, Tan et al.[38] demonstrated that eq. (4) was applicable for the MSALVFX–agarose diffusion system by means of electronic speckle pattern interferometry. The change of D_g as a function of the square root of the MSALVFX concentration is plotted in Figure 5. The results show that D decreased slightly with the increase of MSALVFX concentration in the initial solution. The extrapolated value of D_0 was 4.01 × 10^{-6} cm^2/s. The hydrodynamic radius of MSALVFX could be obtained according to the following Stokes–Einstein equation:

$$D_0 = k_B T/6\pi\eta r_s \tag{5}$$

where k_B is Boltzmann's constant (1.38 × 10^{-23} J/K), T is the temperature in Kelvin (298.15 K), η is the solvent viscosity (0.8949 × 10^{-3} Pa·s), and r_s is the hydrodynamic radius of the diffusing solute. r_s was thus obtained as 6.09 Å.

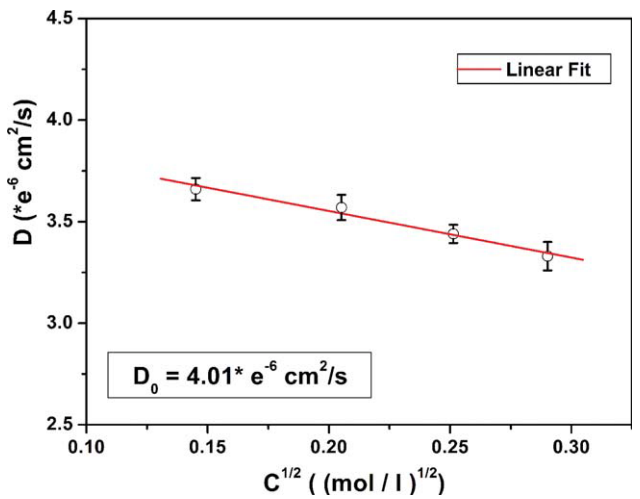

Figure 5 Dependence of MSALVFX D on $C^{1/2}$ in the agarose hydrogels (25°C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

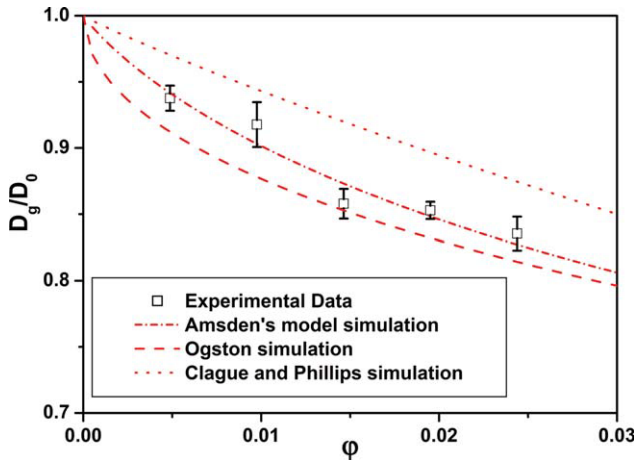

Figure 6 Dependence of the diffusivity (D_g/D_0) on ϕ in the agarose hydrogels (25°C). The scattered symbols are the experimental data for MSALVFX (30 mg/mL). The dashed lines are simulated results by the Amsden, Ogston, and Clague and Philips models. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Influence of the agarose concentration on the MSALVFX diffusion

The available volume between the fibers of the gel network was the most important factor affecting the diffusion process. An increase of gel concentration would decrease the mesh size of the gel network and lead to a reduction of available space for the diffusing solute and an increase in the path length for diffusive transport. Figure 6 shows the influence of the polymer volume fraction (ϕ) on D_g of 30.0 mg/mL MSALVFX in agarose hydrogels at 25°C. The volume fraction of agarose in the gel was calculated by Pluen's[39] method as follows:

$$\varphi = C_{\text{agarose}}/(\rho_{\text{agarose}}\omega_{\text{agarose}}) \tag{6}$$

where ρ_{agarose} is the density of dry agarose powder (1.64 g/mL),[39] ω_{agarose} is the mass fraction of agarose in the agarose hydrogel fiber (0.625),[40] and C_{agarose} is the weight fraction of agarose in the agarose hydrogel. With the increase of agarose concentration from 0.5 to 2.5 wt %, the ratio of D_g/D_0 decreased from 0.938 to 0.835 for MSALVFX. A slightly systematic decrease in the D values with increasing agarose concentration in the gels was observed; this was similar to the relationship between poly(ethylene glycol)s and the agarose hydrogel concentration revealed by our earlier studies.[30] Decreased D values suggested a relatively low mobility. This could be attributed to the fact that the increase in the volume fraction of agarose in the gels decreased the space for the diffusion of MSALVFX and resulted in the increase of obstruction effects.

Analysis of the experimental data with theoretical models

The diffusion of MSALVFX in the hydrogels may have taken place primarily within the space delineated by crosslinked polymer chains.[41] Therefore, several factors, including the mobility and concentration of polymer chains, the crosslinking density of polymer networks, the size of the solute in relation to the size of the space between polymer chains, and the presence of active groups on polymer networks, affect the diffusivity of solutes in a crosslinked polymer network. Commonly, the diffusivity of a solute through a physically crosslinked hydrogel decreases with increasing size of the solute and the crosslinking density. Polymer chains could also delay the movement of MSALVFX in hydrogels by increasing the hydrodynamic block and reducing the available average free volume per molecule or acting as physical resistance to increase the path length of diffusion.[41] To get more information of the diffusion behavior of MSALVFX in the agarose hydrogels, a comparison between the experimental data and theoretical prediction was carried out by fitting the experimental data with the Amsden, Clague and Phillips, and Ogston models.

Because of the stiffness of the polymer fibers in the polymer gel, the Amsden model assumes that the diffusion of solutes is determined by the probability of solute to find an enough space between polymer fibers for passing through. Combined with the free-volume theory with the obstruction and scaling concepts, the relationship between the diffusivity of the solute and the volume fraction of the polymer in the gel is written as[42]

$$\frac{D_g}{D_0} = \exp\left[-\pi\left(\frac{r_s + r_f}{k_s\varphi^{-1/2} + 2r_f}\right)^2\right] \tag{7}$$

where r_f denotes the radius of the polymer fibers and k_s is the scaling parameter, which is constant for a given polymer system.

As both the steric or tortuosity effects and hydrodynamic effects were rigorously taken into account, Clague and Phillips developed a model based on the fact that the diffusivity in a gel can be written as a product of a hydrodynamic factor and a steric factor.[43] This model is well viable for the hindered diffusion of spherical solutes in porous media composed of straight and randomly placed cylindrical fibers and allows for the prediction of the rates of protein diffusion in agarose accurately with no adjustable parameters. In general, this model can be expressed as follows:[44]

$$\frac{D_g}{D_0} = \left(1 + \frac{2}{3}\alpha\right)^{-1}\exp(-\pi\varphi^{0.174\ln(59.6r_f/r_s)}) \tag{8}$$

where

$$\alpha = \varphi\left(\frac{r_s}{r_f} + 1\right)^2 \quad (9)$$

The Ogston model focuses on the probability that a hard spherelike molecule of radius r_s can complete a step through a randomly oriented array of long and straight fibers of negligible thickness but neglects hydrodynamic interactions between the mobile solute and the polymer matrix. When applied to a dimensionless ratio of r_s/r_f and ϕ, the predicted relative diffusivity is given by[45]

$$\frac{D_g}{D_0} = \exp\left[-\frac{r_s + r_f}{r_f}\sqrt{\varphi}\right] \quad (10)$$

As agarose hydrogel fibers contain bimodal bundles of α-helix chains with 87% having a radius of 15 Å and 13% having a radius of 45 Å, r_f is thus taken to be 19 Å during application.[41] Moreover, r_s was obtained to be 6.09 Å on the basis of the Stokes–Einstein equation.

As is shown in Figure 6, the experimental D values of MSALVFX obtained for different agarose concentrations at 25°C were fitted with eqs. (7), (8), and (10), and were plotted with the Amsden, Clague and Philips, and Ogston models. The Amsden model agreed the experimental data very well by adopting $k_s = 10.8$ Å. It was reported that the application of the Amsden model to the diffusion of myoglobin, bovine serum albumin (BSA), $C_{12}E_8$ micelles, and $C_{12}E_{10}$ micelles in agarose hydrogels produced consistent values of the regression k_s ranging from 10.65 to 13.75 Å.[41] On the other hand, the Clague and Philips model, containing no free parameters in the calculation, exhibited a large deviation to our results because this model is applicable only to the diffusion of large molecules, with r_s greater than 20 Å, in hydrogels of relatively high polymer fraction.[41] Coinciding with the report in the literature indicating that the Ogston model is often unsatisfactory for agarose hydrogels, it was shown that the Ogston model presented a large deviation to our data as well.

CONCLUSIONS

We have investigated the diffusion of MSALVFX, a typical quinolone antibiotic, in agarose hydrogels by an improved refractive-index method with a precise temperature-controlled device. The results indicated that D of the drug in the gel increased with the experimental temperature and decreased with increasing agarose and diffusing concentrations. D_0 of MSALVFX was 4.01×10^{-6} cm^2/s through extrapolation according to Kohlrausch's law. Among the three typical theoretical models applied, the Amsden model revealed a good fit with a scaling constant $k_s = 10.6$ Å for agarose gels, whereas the Clague and Philips and Ogston models showed a large deviation to our experimental data. This work will provide a better understanding of the transport property of MSALVFX in biological gel systems; this is necessary for progress in the investigation of the molecular transport of quinolone antibiotics *in vivo* and for the development of advanced dosage forms.

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