Self-Diffusion in Chitosan Networks: From a Gel−Gel Method to Fluorescence Recovery after Photobleaching by Fringe Pattern

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ABSTRACT: The diffusion properties of caffeine and dextran within chitosan networks with controlled structure were investigated using a gel−gel method and fluorescence recovery after photobleaching (FRAP) by fringe pattern. The center of mass diffusion was studied by varying the molecular weight of dextran macromolecules labeled with fluorescein isothiocyanate (FITC). It was shown that the diffusion was drastically slowed down above a critical molecular weight around 10^5 g/mol.

Introduction

Chitosan is a natural linear cationic polysaccharide obtained by N-deacetylation of chitin. The numerous physicochemical and biological properties of chitosan such as biocompatibility, non toxicity, mucoadhesion, absorption enhancement and pH-dependent swelling properties are particularly interesting for many applications in the biomedical and the pharmaceutical fields.[1−6] This is why chitosan networks are already used as hydrophilic matrix formers in controlled drug delivery systems.[7,8]

Chemical cross-linking of chitosan with dialdehydes such as glyoxal is the most commonly used method to obtain water insoluble polymeric gels whatever the pH and to improve the mechanical properties of the system.[9,10] This type of network offers the possibility to accurately control the release kinetics of incorporated drugs. They are particularly interesting for the delivery of large molecules such as proteins as the latter can relatively easily diffuse through the hydrogel.[5,11] The drug is usually dispersed or solubilized in a chitosan gel, and its release rate is mainly governed by the diffusion of the drug molecules within the gel network. It is therefore important to characterize the mechanism of this diffusion.

The diffusion of particles in a polymer network depends on many parameters:[12] the size of the particles relative to the mesh size of the network, the chemical interactions and the friction between particles and components of the network, the geometry of the particles (hard spheres, coils,...), and the dynamics of the polymer network mesh, e.g., the relative rate of polymer mesh rearranging while the particles move in it.

The methods to study the transport properties of molecules within gels can roughly be divided in two categories: indirect methods and direct methods. In the former, a receptor phase is adjoined to the sample (donor) initially containing the diffusing molecules. The diffusion properties of the donor are then extrapolated from the time evolution of the concentration measured in the receptor. This receptor medium is often a liquid phase,[13−15] partly for practical reasons, partly because this design mimics actual pharmaceutical applications. However, this method can induce errors due to a possible interaction between the donor and the receptor during the experiment or the diffusion through the interface. Convection in the liquid media may also affect the results. A way of avoiding these problems is to use the same gel as a receptor medium (gel−gel diffusion). The concentration profile in the matrix polymer of the receptor can be determined by interferometry,[16] using radiotracers,[17,18] opalescent[19] or fluorescent molecules.

Using direct techniques such as pulsed field-gradient-spin−echo NMR spectroscopy,[20] dynamic light scattering[21,22] or fluorescence recovery after photobleaching (FRAP),[23−25] it is possible to monitor the motion of particles in a matrix in real time and *in situ*, *i.e.*, without the use of a receptor phase. The main advantage of these techniques—when applied to the study of diffusion—is their microscopic scale. When the diffusion is slow, as can be expected for molecules inserted inside a gel, measurements can be performed while keeping the experiments reasonably brief (less than 24 h). We have chosen FRAP by fringe pattern, among the possible fluorescent profiles applied to the medium, as this technique allows one to precisely specify the distance over which the diffusion is followed (the interfringe pattern) independently of the photobleaching reaction and has successfully been used to study transport phenomena in complex matter such as biological[26] cells.

The diffusion properties of model molecules within chitosan gels have previously been studied using the gel-liquid techniques explained above.[27,28] To our knowledge, the results presented here are the first ones obtained on this subject by a direct method.

The major aims of this study are to evaluate the diffusion properties of different molecules within chitosan gels and to assess the physicochemical conditions that influence them. Two types of diffusing molecules are used: a model drug molecule of low molecular weight, caffeine, and dextran, a macromolecule which is available under various molecular weights and labeled with fluorescent dyes making them very suitable for diffusion studies. These two types of tracers are chosen in order to cover

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Table 1. Average Molecular Weight, Ratio of FITC per Mole of Glucose, and Hydrodynamic Radius for the Different Dextran−FITC Samples (Supplier Data)[a]

dextran−FITC	FD4	FD20	FD40	FD70	FD150S	FD250S
M_w (kg/mol)	4.4	19.5	43.2	77	150	282
FITC/glucose	0.004	0.006	0.01	0.013	unknown	0.009
R_h (Å)	7.04 ± 0.02	15.5 ± 0.1	21.5 ± 0.2	29.3 ± 0.8	48 ± 5	60 ± 5

[a] R_h are calculated from the diffusion coefficients measured by FRAP (see the Results and Discussion data).

the range of molar mass classically encountered in pharmaceutical applications. FRAP by fringe pattern allowed to measure and to compare the diffusion of dextran macromolecules in solvent, chitosan solutions and gels for different dextran molecular weights. The diffusion of caffeine was studied by a gel−gel method since the FRAP experimental set up used in this study was not adapted to measure diffusion coefficient lower than 3×10^{-6} cm^2•s^{-1}. The obtained results are interpreted in terms of hindered diffusion when the size of the diffusing object becomes comparable to the correlation length (e.g., the mesh size) deduced from small angle neutron scattering experiments (SANS) in chitosan solutions and gels.

Materials and Methods

Chitosan. Chitosan was purchased from Aldrich. A deacetylation degree of 84% was measured according to Brugnerotto's method, using FTIR spectroscopy.[29] The average viscosimetric molecular weight ($M_v = 1.0 \times 10^6$ g/mol) was determined by capillary viscosimetric measurements using the experimental conditions of Roberts and Domszy.[30]

Caffeine. Caffeine ($M = 194$ g/mol) was used as a model drug to study the diffusion of low molecular weight compounds within the cross-linked chitosan network using the gel−gel diffusion method. Indeed caffeine is well-known in the pharmaceutical field to exert an analgesic effect.[31] Moreover, its concentration can easily be assessed by UV spectroscopy (272 nm).

FITC−Dextran (Fluorescent Probes). Dextrans have been chosen as probes because they are available under a wide range of molecular weights, which is an important parameter in a diffusion process. Six dextran samples labeled with fluorescein isothiocyanate (FITC-dextran) were purchased from Sigma and named FD4, FD20, FD40, FD70, FD150S, FD250S according to their nominal weight average molecular weights. The weight average molecular weights and ratios of FITC per mole of glucose are listed in Table 1. The molecular weights were determined by the vendor using gel permeation and small angle light scattering (SALS). Doucet and co-workers[32] have recently characterized FITC−dextran purchased from Sigma. These authors found that the molecular weights measured using gel permeation chromatography combined with small angle light scattering (GPC/MALS) either agree with or slightly exceed the vendor-supplied values.

FITC is a widely used dye, for example in cellular biology,[33] but there are very few studies on the effect of the different physicochemical parameters on its fluorescence properties. To establish the optimal experimental conditions for diffusion measurements, a fluorimetry study was performed on the solution of FD250S with different concentrations and at different acid pH ranging from 2 to 6. It was shown that the higher the pH is, the stronger the emission intensity is, whatever the wavelengths of excitation and emission are. However chitosan is only soluble in acid aqueous solutions (pH < 6.5). As a compromise, the pH was fixed at 5 for the FRAP experiments. The optimal values of the excitation wavelength and of the dextran concentration were found to be 488 nm and 0.1 wt % respectively. It is worth noting that for all used weight fractions, this concentration is lower than the overlap concentration of dextran. Using the radius of gyration of FITC-dextrans in buffer determined by Andrieux and co-workers[34] with small-angle X-ray scattering in the same range of weight average molecular mass as in this study, the overlap concentration, here defined as $C^* = 3M/N_A 4\pi R_g^3$, is expected[35] to be between 21.8 wt % (Dextran-FITC FD4) and 13.0 wt % (dextran−FITC FD150S).

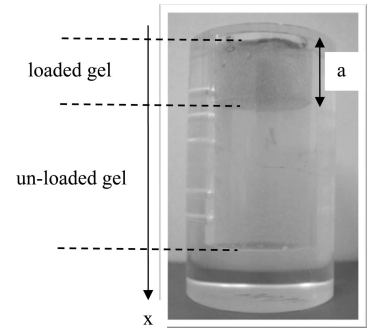

Figure 1. Cell for gel−gel diffusion method.

Preparation of Chitosan Solutions and Gels. Chitosan was dissolved in a 0.1 mol/L acetic acid aqueous solution to a concentration of 2 wt %. This stock solution was diluted with distilled water to obtain solutions with different chitosan weight fractions ranging from 0.05 to 2 wt % for preliminary rheological measurements in order to evaluate the entanglement concentration of chitosan. It was not possible to obtain a good dissolution for chitosan concentration higher than 2% with the studied molecular weight (1.0×10^6 g/mol). Then all diffusion measurements were performed at a chitosan concentration of 1.5 wt % because this concentration was already used in a previous work on the preparation of micro network of chitosan by a dropping process in view to obtain a new drug carrier excipient for the oral route.[36]

Caffeine preliminarily solubilized in 0.1 mol/L acetic acid aqueous solution was added to the chitosan solution (concentration of 2 wt %) to obtain a solution of 1.5 wt % of chitosan at pH = 4 with a final caffeine concentration of 0.7 wt %. FITC-dextran preliminarily solubilized in water was added to the 2 wt % chitosan solution to obtain a solution of 1.5 wt % of chitosan at pH = 5 with a dextran concentration of 0.1 wt %. Cross-linking of chitosan chains was achieved by adding 20 μL of an aqueous solution of glyoxal (40 wt %, purchased from Riedel-De-Haen) to 5 g of polymer solution.

Characterization of Chitosan Network. The viscosity of chitosan solutions for concentration between 0.05 and 2 wt % was measured with MCR 500 Physica rheometer fitted by a double cylinder geometry.

Small-angle neutron scattering data were obtained at Laboratoire Leon Brillouin (CEA, Saclay, France) on the PAXE spectrometer. The XY detector contained 64 × 64 cells of 1 × 1 cm^2 mounted on a moveable trolley placed within a cylindrical tube kept under vacuum. The scattering data were collected with an incident wavelength $\lambda = 14$ Å and a sample−detector distance of 5 m. The wave vector range was then 4.45×10^{-4} to 4.87×10^{-2} Å^{-1}. Deuterated water was used as solvent for the studied 1.5 wt % chitosan solution and gels.

Gel−Gel Diffusion Cell−Diffusion of Caffeine. The homemade diffusion cell was a plexiglas hollow cylinder, bored at 6 different heights (see Figure 1). The holes (0.4 cm in diameter) were blocked up for the filling. First, the cell was filled up to 5.6 cm with a gelling chitosan solution after addition of glyoxal (1.5 wt %, pH = 4), without diffusing molecules. The gel formed in approximately 1400 s. The same chitosan solution containing the diffusing molecules (caffeine) was then further added with an additional height of 1.3 cm. The time at which we added this additional solution was considered as the beginning of the diffusion process.

The cell was then stored at 25 °C in humid environment to avoid evaporation.

The gel was sampled after 24 and 48 h by coring through the holes using hollow glass tubes. The samples (0.2 g) were left in water (20 to 50 mL) for approximately 30 h to release the probes. After this time, it could be safely assumed that the concentration of caffeine within the gel and in water was the same, considering the relative volumes and the release rate of caffeine in water.[27] Indeed, Vachoud and co-workers determined that the diffusion coefficient of caffeine from chitosan gel to water is 3.8×10^{-6} cm^2/s. Finally, the caffeine concentration was measured by UV spectroscopy at 272 nm, using a calibration curve of absorbance previously obtained from gel samples with known caffeine concentrations.

Diffusion Coefficient Measurement by FRAP. Fluorescence recovery after photobleaching by a fringe pattern[23] was used to study the diffusity of FITC-dextran molecules within the chitosan hydrogels. This experiment consisted in forming a fringe pattern with interfringe distance i at the crossing point of two coherent laser beams (argon laser at 488 nm). First, a fluorescence concentration profile was created in the sample by photobleaching locally a small fraction of the fluorescent probes with a short pulse of high intensity in the sample. Then, the beams were attenuated and the fluorescence recovery was monitored while the spatial position of the fringes was modulated at a chosen relatively high frequency $\omega/2\pi$ (few 10^3 Hz). This modulation of the spatial position of the fringes with respect to the fixed position used for the photobleaching leads to a modulation of the fluorescence intensity (at twice $\omega/2\pi$), allowing one to use a lock in amplifier to extract the fluorescence recovery signal from noise. This method of modulating the position of the fringes thus allows one to work with low photobleaching dose (less than 10% of the total concentration of fluorescent probes). This is of particular interest to minimize possible perturbations of the system by the photobleached species. The use of fringe pattern is an easy way of specifying with no ambiguity the diffusion distance, whatever the degree of bleaching used, contrary to the now more commonly used spot photobleaching technique, often available with most of the biological microscopes. From the fluorescence recovery signal due to the progressive blurring out of the concentration profile through diffusion of the photobleached probes, it was possible to extract the diffusion coefficient. The fluorescence signal can be decomposed into a harmonic series with respect to the fundamental modulation frequency of the position of the fringes. In a case of a simple diffusion process, the second harmonic component decays exponentially with time, with a characteristic time τ related to the lateral diffusion coefficient D according to the following equation:

$$D = \frac{i^2}{4\pi^2\tau} \tag{1}$$

The experiments were performed at 25 °C.

The experimental set up used in this study was designed to form interfringe spacing not higher than 30 μm and consequently the range of lateral diffusion coefficients was lower than 3.10^{-6} cm^2/s.

A typical graph of the second harmonic modulated fluorescence recovery signal for FITC-dextran in a chitosan gel is shown in Figure 2. The characteristic relaxation time ($\tau = 0.39 \pm 0.01$ s) is deduced from the best fit to a monoexponential decay.

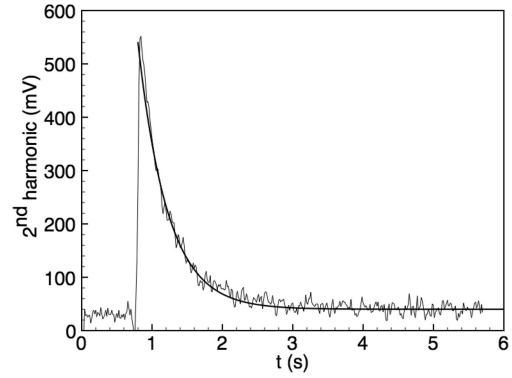

Figure 2. Recording of the second harmonic modulated fluorescence recovery signal for a chitosan hydrogel (1.5 wt %) loaded with FITC-dextran 0.1 wt %, $M_w = 19500$ g/mol). The interfringe spacing i is 21.51 μm. The solid line is the best adjustment to an exponential decay (exp $-t/\tau$): $\tau = 0.39 \pm 0.01$ s.

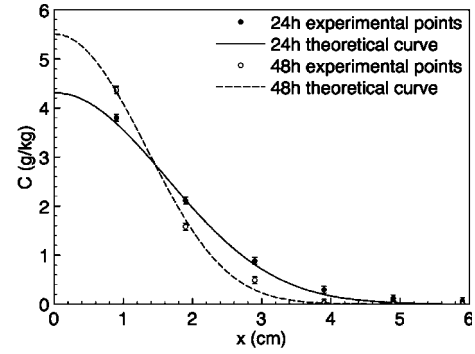

Figure 3. Concentration of caffeine versus sampling height in the chitosan gel at $t = 24$ h (●) and $t = 48$ h (○). The theoretical curves are obtained from the fit of the experimental data according to eq 3.

Results and Discussion

Characterization of Chitosan Solutions and Gels. A shear thinning behavior was observed in the shear rate dependence of the viscosity after a shear independent plateau at low shear rates. The entanglement concentration (0.6 wt %) was evaluated by the crossover of the unentangled and entangled regimes as classically observed in the polymer concentration dependence of the zero shear viscosity.[37]

The dynamic component of the SANS scattering intensity I as a function of the magnitude q of the wave vector was analyzed with the Ornstein−Zernike equation, $I \propto 1/(1 + \xi^2 q^2)$ as expected for uncross-linked or cross-linked flexible polymers networks.[38−40] The dynamic correlation length ξ between adjacent chemical or physical junctions is a characteristic of the mesh size of the chitosan networks. We found $\xi = 512 \pm 30$ Å for a 1.5 wt % chitosan solution. The values for 1.5 wt % chitosan gel are $\xi = 549 \pm 44$ Å (pH = 5) and $\xi = 616 \pm 84$ Å (pH = 4). These values are in reasonable agreement with those obtained by G. Mangiapia and co-workers[41] who have investigated the structure of chemically cross-linked chitosan by means of the combined use of small-angle neutron scattering (SANS), electron paramagnetic resonance spectroscopy (EPR), intradiffusion, and swelling degree measurements. These authors have demonstrated that these hydrogels may be described in terms of an inhomogeneous structure composed by polymer-rich and polymer-poor regions. The correlation distance of polymer-rich regions ranged between 600 and 850 Å, in good agreement with our results.

Gel−Gel Diffusion. Diffusion was assumed to take place in a homogeneous semi-infinite medium from an initially C_0 concentrated region to an unloaded region and was considered monodimensional along the x-axis of the cylinder with the following initial boundary condition:

$$C(x,t) = C_0 \quad \text{for } t = 0, \quad 0 < x < a \tag{2}$$

The concentration profile of caffeine molecules in the cell derives from Fick's equations and reads as follows:

$$C(x,t) = \frac{1}{2}C_0\left[\mathrm{erf}\frac{x+a}{2\sqrt{Dt}} + \mathrm{erf}\frac{x-a}{2\sqrt{Dt}}\right] \quad (3)$$

where erf is the error function, D the diffusion coefficient of caffeine, and a the initial height of the loaded gel. The samples were analyzed after 24 and 48 h of diffusion in two different cells. The variations of concentration as a function of the height of sampling in the diffusion cell are reported in Figure 3 for two 24 h and 48 h aged samples.

The assumption that the medium is semi-infinite is reasonable, since the concentration of caffeine at the edge of the gel is around 0.06 g/kg, *i.e.*, is negligible for the two measurements (the accuracy is 0.07 g/kg). Fitting the data according to eq 3 allows the determination of the diffusion coefficient of caffeine: we obtained respectively $D = 5.1 \times 10^{-6} \pm 0.3 \times 10^{-6}$ cm^2/s and $D = 5.5 \times 10^{-6} \pm 0.3 \times 10^{-6}$ cm^2/s after 24 h and 48 h of diffusion. These values are almost identical which means that the system was stable over this time period. They are also very close to (but slightly below) the value $D = 5.9 \times 10^{-6} \pm 0.1 \times 10^{-6}$ cm^2/s for the diffusion of caffeine in water at 25 °C deduced from the Stokes−Einstein equation:

$$D_0 = \frac{k_B T}{6\pi\eta R_H} \quad (4)$$

where $\eta = 1$ mPa·s is the viscosity of water and R_H is the hydrodynamic radius of the probes ($R_H = 3.4$ Å).[42,43]

This result must be related to the fact that the mesh size of the gel is much larger than the probes so that the gel has little effect on the diffusion. Indeed, the size of caffeine molecule is less than 4 Å while the mesh size of 1.5 wt % chitosan gel at pH =4 is $\xi = 616 \pm 84$ Å. We can thus conclude that there is no specific hindrance of the diffusion due to the gel, i.e., no chemical interaction between chitosan and caffeine. It appears clear that this kind of hydrogel will not be able to promote the sustained release of noninteracting low molecular drugs by a diffusion mechanism.

FRAP Measurements. In this section, we describe the diffusion of FITC-dextran molecules of various molecular weights within water, chitosan solutions and chitosan gels. The choice of these three different diffusion media enabled us to compare the effect of the free polymer chains (in solution) and the effect of cross-linking (in hydrogel).

In view of the gel−gel diffusion results presented above (little effect of the chitosan network on the diffusion of small molecules such as caffeine), it appeared interesting to determine the minimum size of a particle for which the diffusion is effectively slowed down by the chitosan network. For that, a precise and convenient technique (FRAP) was used and the size of the object diffusing through the gels was varied in a systematic manner, keeping constant the possible chemical interactions with the diffusing media. This could be achieved by using various molecular weights of fluorescent FITC−dextran.

The variations of the relaxation time τ of the fluorescence recovery signal as a function of the square of the interfringe spacing i^2 for the different molecular weights of FITC−dextran investigated are plotted for the different chitosan systems. An example of these variations is given in Figure 4. The relaxation times were found to vary linearly with respect to i^2 and the linear regression lines cross the origin. This is typical of a single diffusion of irreversibly bleached fluorophores. This was observed in all media (water, polymer solution or gel). Thus, the diffusion coefficient D of FITC-dextran in a given medium could easily be extracted by linear regression (eq 1).

The values of D for the different molecular weights of dextran obtained in water (D_0), in the chitosan solution (D_{sol}) and in the chitosan gel (D_{gel}) are listed in Table 2.

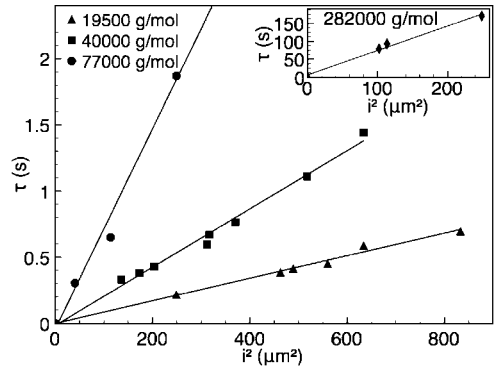

Figure 4. Relaxation time τ as a function of the square of the interfringe i^2 for three molecular weights of FITC−dextran in chitosan hydrogel (1.5 wt %). The results for the highest molecular weight of FITC−dextran (282000 g/mol) are given in the inset.

Table 2. Diffusion Coefficients for Different Molecular Weights of Dextran in Water (D_0), in 1.5 wt % Chitosan Solution (D_{sol}) and in 1.5 wt % Chitosan Gel (D_{gel})

dextran	$D_0 \times 10^6$ (cm^2/s)	$D_{sol} \times 10^6$ (cm^2/s)	$D_{gel} \times 10^6$ (cm^2/s)
FD4	3.10 ± 0.01	nondetermined	0.99 ± 0.01
FD20	1.41 ± 0.01	0.37 ± 0.01	0.28 ± 0.02
FD40	1.00 ± 0.01	0.111 ± 0.002	0.12 ± 0.01
FD70	0.75 ± 0.02	nondetermined	0.033 ± 0.02
FD150S	0.45 ± 0.05	0.031 ± 0.002	0.00045 ± 0.00003
FD250S	0.36 ± 0.03	0.022 ± 0.001	0.00033 ± 0.00004

The dependences of D upon the molecular weight of the dextrans, M_w, is shown in Figure 5. As could be expected, the diffusion was slowed down when the molecular weight of the probe increased (in all media). The data obtained in water are well fitted by a power law with an exponent value of −0.53 in the investigated range of dextran molecular weights. This value is in good agreement with the experimental results obtained by Smit and co-workers[44] and Bu and co-workers[45] for the diffusion of dextran in water studied respectively by quasi-elastic light scattering and fluorescence recovery after photobleaching. Moreover this value is in between the expected results for a linear chain either in a good solvent ($R_g \sim M^{0.588}$) or in a Θ solvent ($R_g \sim M^{0.5}$). The difference between the experimental exponent and the theoretical prediction can be an indication of a situation of moderately good solvent for the range of molecular weights of dextran used. It could also be indicative of a random branching in the dextran chains. Indeed, dextrans are considered to belong to the class of branched polysaccharides and long-chain branching is observed in dextran molecules with molecular weight exceeds 150000 g/mol.[32,46]

Concerning the chitosan solutions, the diffusion coefficient of dextran also follows a power law with molecular weight in

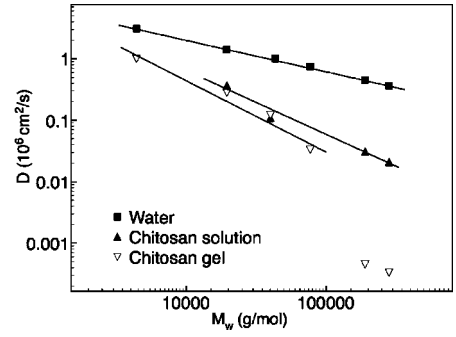

Figure 5. FITC−dextran diffusion coefficients versus FITC−dextran molecular weights in water, in 1.5 wt % chitosan solution and in 1.5 wt % chitosan gel. The solid lines correspond to the best fit to a power law.

the investigated range of molecular weights. The exponent is −1.02, much larger than in water. In the framework of polymer diffusion, a diffusion coefficient inversely proportional to the molecular weight is characteristic of Rouse dynamics. A similar exponent was reported by Doucet and co-workers[32] for the diffusion of labeled dextrans or pullulans in a solution of dextran at a concentration of 15 wt %%. With such a high concentration, these authors were expecting a value of −2 (reptation mechanism) if the solution of matrix dextran was in the entangled domain. They attributed the observed Rouse-like value to the branching of dextran molecules delaying and preventing the formation of entanglements. As chitosan may be considered as a linear binary copolymer[47] and as chemical modifications are necessary to induce branching[48] such an argument is not valid in our systems.

We rather think that our result indicates that the chitosan solution acts on the dextran molecules to screen the hydrodynamic interactions[49] but that the chitosan solution is not concentrated enough to induce a reptation-like behavior for the dextran molecules. Indeed, if the number of entanglements per chain is not larger than 10, tube renewal and fluctuations of the chain inside their tube are expected to accelerate the dynamics compared to reptation, and to widen the cross over from Rouse to reptation-like dynamics.[50]

Conversely to the decreases observed in water and in chitosan solutions, the variations of the diffusion coefficient with molecular weights in chitosan hydrogel was not monotonic. Two different domains appeared. The first, at low molecular weights (from FD4 to FD70), was well described by a power law, with an exponent close to −1, very similar to what was observed in chitosan solutions showing that the effect of cross-linking of the chitosan coils is not important in the diffusion mechanism of small dextran.

Quite surprisingly, for the two larger dextran probes (FD150S and FD250S), there is slowing down by a factor 100 of the diffusion in the gel compared to the solution. The two larger molecules appear extremely sensitive to the presence of cross-linking points in the chitosan matrix even though their hydrodynamic radius (see table 1) remains rather small compared to the mesh size of the chitosan matrix ($\xi = 549 \pm 44$ Å, pH=5). As these results have been obtained in a situation where the gel was not really confining the dextran chains, one may reasonably think that the drastic slowing down of the diffusion of the dextran molecules observed in the gel arises from specific interactions between the dextran and the gel, which only appear effective when the dextran molecules are large enough so that they are forced to approach the chitosan gel.

A transition in the diffusion behavior of a polymer in a polymer network or entangled melts has already been observed. For example, in polydimethylsiloxane (PDMS) melts, Leger and co-workers have shown that the diffusion of PDMS was described by Rouse dynamics for small molecules, while it was decribed by reptation for larger ones.[51] The data obtained for dextran in chitosan gels thus agree qualitatively with Leger's ones, but the transition we observe in the chitosan gels cannot be attributed to reptation: first the mesh size of the gel is much larger than the size of the diffusing chains, and second the exponent we obtain (around −4 if the data are fitted for the three highest molecular weights) is much higher than the exponent −2 characteristic of the reptation behavior.

The chitosan gels slow down the diffusion of dextran molecules much more efficiently than what would be observed in the case of a linear polymer diffusing through the gel and only sensitive to steric hindrance and confinement, leading to a reptation-like motion. The dextran−chitosan system is however more complicated than the melts of polymer solutions investigated by Leger and co-workers[51] as two different polymers are involved, with eventually specific attractive or repulsive interactions. Such a mixture of two different polymers plus solvent has been investigated by Rotstein and Lodge.[52] They measured the diffusion of linear polystyrenes in swollen poly(vinyl methyl ether) (PVME) gels by dynamic light scattering. The results were compared to the values obtained in corresponding PVME solutions. They were the first to make a direct comparison of probe diffusion in entangled solutions and in chemical gels of the same composition. They reported that the diffusion in the gels was equal to or slighly less than in solutions showing that the presence of chemical cross-links has no particular effects on the probe mobility, as we do observe for Dextans FD4 to FD70. They also found that in the gels the diffusion coefficient scaled as $M^{-2.8}$ or $M^{-2.7}$ depending on the concentration which is indeed expected to have an important effect on the diffusion coefficient in the case of mutual diffusion. These authors suggested that a possible explanation for their observation of strong deviations of the reptation prediction might be the broad distribution of entanglements (i.e., mesh size) within the gels leading to the presence of "entropic traps": tracer chains are preferably localized in larger-than-average pores where their conformational entropy is higher. Such an hypothesis is supported by the work of Muthukumar and Baumgärtner[53] who investigated the diffusion of a flexible chain through an array of obstacles by Monte Carlo simulations. When the obstacles are randomly distributed regimes with apparent molecular weight exponents as strong as −3 are obtained. One can note that our exponent value is even stronger. This stronger dependence on molecular weight might be due to the long branching of the dextran molecules occurring for the two higher molecular weight samples (150 Da, 280 Da).[46] Indeed, Lodge and co-workers[54] have compared the diffusion of linear and star branched polysterenes in PVME solutions. They have showed that in entangled solutions the star polymers diffused more slowly than linear polymers of comparable size. They also noticed that the onset of this architecture-dependent diffusion requires entanglements in the viscoelastic sense and not merely coil overlap. This could explain why the effect of the tracer architecture is observed in our chitosan gels and not in chitosan solutions.

Conclusion

The aim of this study was to investigate the diffusion of molecules of various molecular weights in chitosan biogels with controlled structure covering the range of molecular sizes classically encountered in pharmaceutical applications. Caffeine was chosen as a low molecular weight tracer and its diffusion properties were investigated by a gel−gel method. It was shown that the chemical cross-linking did not slow down the diffusion of caffeine compared to its diffusion in water. The diffusion of higher molecular weight model compounds was then studied by the powerful method based on fringe pattern photobleaching recovery. This technique was used to study the diffusion of dextran molecules within the chitosan matrix (cross-linked or not). We were able to determine the Brownian diffusion coefficients of FITC-dextrans with various molecular weights in water, in chitosan solutions and in chitosan hydrogels.

The effect of the molecular weight of FITC−dextran on the center of mass diffusion was clearly demonstrated. For a large range of molecular weights of dextran, the cross-linking junctions in the chitosan gel were observed to have no effect on the dextran mobility. The highest molecular weights investigated (around 10^5 g/mol) exhibited a drastically slowed down diffusion due to the cross-linking points in the chitosan gel. This opens the way to more systematic experiments in which the mesh size of the gel could be externally addressed, in order to modulate the transport properties through the gel.

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