NMR Self-Diffusion of Molecular and Macromolecular Species in Dextran Solutions and Gels

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ABSTRACT: To provide insights into the diffusion properties of micelles in hydrogels and, in general, of solutes in dextran gels, self-diffusion coefficients of small organic solutes and large micelles were measured in dextran solutions and gels, using pulsed-field gradient NMR. The self-diffusion of the solutes was shown to be slower in dextran solutions than in D_2O and even slower in dextran gels. The extent of the diffusion reduction was more pronounced for higher dextran concentrations. For a series of molecules with a molecular weight between 46 and 78, the self-diffusion coefficient in dextran gels (20% w/w) corresponded, on average, to about 0.39 of the values measured in bulk water. This hindered diffusion was mainly associated with the obstacles created by the polysaccharide segments. There was no evidence in the diffusion measurements of interaction between dextran and solutes capable of hydrogen bonding. The reduction of diffusion in dextran solutions and gels was very drastic in the case of micelles. For Triton X-100, the self-diffusion coefficient in dextran gel was about 7% of that observed in water. The values were, in fact, on the same order of the dextran chain diffusion coefficient. This finding suggests that these large macroassemblies can hardly move in dextran gels.

Introduction

Because of many applications in different fields such as controlled release of drugs and cell immobilization in gels, there have been several studies on diffusion in polymer gels, and the conclusions are summarized in several review articles.[1−3] It is established that the diffusion of a solute is slowed down by polymers in solutions or gels. This reduction has been shown to depend on several factors including the size of the solutes, the concentration of the polymer, and the structure of the polymer.[2] In general, diffusion coefficients decrease as a function of the solute size and the polymer concentration. The diffusion in polysaccharide gels is no exception. For example, a systematic decrease of the diffusion coefficients of various solutes has been reported with increasing polysaccharide concentration.[4−6] In addition, the influence of the polysaccharide concentration on the diffusion appears to be dependent on the size of the solutes.[1,2]

Our research activities related to bacterial biofilms have led to a considerable interest in diffusion in polysaccharide gels. When bacteria attach to surfaces, they produce a hydrated polymeric matrix formed mainly of exopolysaccharides, in which bacteria microcolonies are embedded. These complex ultrastructures are referred to as biofilms.[7−9] Biofilms have shown an increased resistance to bacteriophage, chemical surfactants, and biocides, and it has been proposed that reduced penetrability of these agents into biofilms is partly the origin of their reduced antibacterial power.[7,10,11] The heterogeneous and complex structure of biofilms suggests that multiple transport mechanisms exist within a single biofilm.[12,13] While convective mass transport through void volumes and water channels should not be disregarded, diffusion within the exopolysaccharide matrix is considered as an important transport mechanism.[14]

Dextran is a homopolysaccharide with α-1,6-linked D-glucose units. Side chains are formed with α-1,3 and α-1,4 interconnections. Dextran is often found in bacterial biofilms produced by *Leuconostoc mesenteroides* and *Lactobacteriaceae* and by oral biofilms.[8,15] Thermoreversible gels are formed upon the addition of potassium ions to aqueous dextran solutions.[16] The dextran sol−gel transition is considered as a structural change from random coils to an infinite network of the polymers. Pockets formed by the flexible dextran chains fit well with the size of potassium ions, and the cross-linking ions would be trapped selectively in these pockets. The absence of ionic groups on the polymer chains indicates that the nature of the interactions between dextran polymers and potassium ions is likely of hydrogen-bonding type. The degree of cross-linking increases as the dextran and potassium ion concentrations increase.[17] Diffusion coefficients of various solutes in dextran-based hydrogels, formed by the polymerization of methacrylated dextran, have been determined. These gels show a potential use in controlled-release materials.[18,19] Diffusion coefficients are useful parameters for the prediction of the drug release rate. The gelification of dextran by potassium ions has been found relatively recently, and consequently, there are only few studies of the diffusion in these dextran gels.[17]

In present study, we have examined the diffusion properties of a series of solutes in dextran gels and solutions to investigate whether some distinctive features can be identified with this polysaccharide and to extend our knowledge relative to the solute diffusion in polysaccharide hydrogels. Diffusion coefficients of different solutes in water as well as dextran solutions and gels were measured and compared with each other to define the influence of the gels. It was reported that interactions between solutes and polymers such as hydrogen bonding may affect the diffusion properties

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of solutes.[20,21] For example, poly(vinyl acetate) and poly(methyl methacrylate) slow down the solute diffusion through hydrogen bonding in toluene. Polysaccharides have several hydroxyl groups, and they are expected to interact with solutes containing hydrogen-bonding groups. Therefore, the self-diffusion could be retarded, and the degree of retardation would depend on the strength of the interaction. The experiments reported here were originally designed to observe these specific interactions, mainly hydrogen bonding between solutes and dextran polymer chains by introducing solutes having different hydrogen-bonding capabilities. In fact, we found that the diffusion in dextran solutions and gels shows almost same trends as those in water regardless of the solute functionality. This implies the absence of specific interaction between solutes and dextran polymer chains.

The characterization of the macromolecular diffusion in gels is necessary for several aspects. Surfactants are a class of antibacterial agents currently used against biofilm-type infections. For example, cetylpyridinium chloride (CPC) is used in mouth rinse to fight dental plaque, a well-known biofilm. Surfactants likely exist under a micellar form, and it is the diffusion of this species that should be relevant. In parallel, novel therapeutic approaches proposed to improve the antibacterial power of bactericides by their trapping in liposomes of hundreds of nanometers of diameter.[22] The diffusion of these macroassemblies in slimy biofilms needs to be characterized. Previous studies, using infrared spectroscopy, have shown that the diffusion of sodium dodecyl sulfate (SDS) in bacterial biofilms can be hindered in some conditions.[10,23] In the present paper, we have characterized the diffusion of macroassemblies, namely micelles, in dextran gels and solutions to provide information relative to their interactions with dextran molecules and their diffusion behavior in hydrogels.

Experimental Section

Sample Preparation. Chemicals were obtained from EM Science (Cincinnati, OH) for methanol, Aldrich (Milwaukee, WI) for ethanol, ethylene glycol, and D_2O, Anachemia (Montreal, QC) for 1-propanol, American Chemicals (Montreal, QC) for acetone, and Sigma (St. Louis, MO) for 1,2-propanediol, 2-butanol, DMSO, SDS, CPC, and Triton X-100. Dextran powder (average molecular weight of approximately 40 000) was obtained from Sigma (St. Louis, MO) and used as received.

1% (w/w) of the diffusing species in D_2O samples and dextran solutions containing a diffusing species were directly prepared in 5 mm NMR tubes. The dextran concentrations are expressed by weight relative to the dextran−D_2O weight whereas diffusing species concentrations are relative to the total weight. The NMR tubes were then sealed and heated to about 90 °C in an oven for at least 5 h in order to get a homogeneous solution. Subsequent heating and cooling did not affect significantly the measurements. The same protocol was applied for the diffusion measurements of the micellar species in dextran solution. The surfactant concentrations were 38, 33, and 18 mM for SDS, CPC, and Triton X-100, respectively. These values are considerably higher than their cmc measured in aqueous solutions (8.3, 0.84, and 0.205 mM for SDS, CPC, and Triton X-100, respectively).[24,25] Triton X-100 micelles formed a stable colloidal suspension in water, but they led to phase separation in dextran solutions. The Triton X-100/water phase separated from the dextran/water phase, preventing us from measuring its diffusion properties. In dextran gels, Triton X-100 led to clear and macroscopically homogeneous samples.

For dextran gel samples, dextran powder was dissolved in a KCl aqueous solution (2.5 M) made up with D_2O, at about 90 °C, and stirred vigorously until the mixture became homogeneous. The sample holders then were sealed carefully to prevent the loss of deuterium oxide. The solution was transformed into a gel by cooling the samples down to room temperature. The heated gel was transferred into 5 mm NMR tubes while in the solution phase. Subsequently, the diffusing species was added, and the tubes were sealed, reheated to 90 ° C, and cooled to get a homogeneous gel phase. The NMR experiments were done at least 2 days after the sample preparation. Ionic micelles (SDS and CPC) precipitated in these conditions because of the high ion concentration in dextran gels (2.5 M KCl). Therefore, the diffusion measurement of these micelles in dextran gels could not be carried out.

NMR Measurements. Self-diffusion coefficient (D_s) measurements were carried out on a Bruker Avance AMX-300 NMR spectrometer operating at 300.13 MHz for protons, and the Stejskal−Tanner pulse sequence was used.[26] A Bruker magnetic resonance imaging probe, Micro 2.5, was used in conjunction with a gradient amplifier (BAFPA-40). Gradient pulses were applied only in the z direction. The effect of the Eddy currents that were induced by strong gradient pulses were eliminated by modifying the pulse shape with a preemphasis technique.[27] The gradient strength was calibrated with one-dimensional image profile along the z-axis of a well-defined solid object in water solution containing traces of $CuSO_4$ to shorten the relaxation time. The daily checkup of the gradient strength and temperature was accomplished with 2% H_2O in D_2O solution by measuring the self-diffusion coefficient of HDO at 37 °C (2.5×10^{-9} m^2/s). The temperature was set at 37 °C. The temperature calibration was performed using ethylene glycol peaks.[28]

Diffusion coefficient measurements were performed with increasing gradient field strength, G, from 5 to 80 G/cm for all the experiments. The other parameters, optimized for each sample, were kept constant. The 90° pulse lengths varied between 18.2 and 20.4 μs, the gradient pulse length, δ, was between 0.7 and 1.5 ms, and the delay between the two gradient pulses, Δ, was between 30 and 500 ms. Typically, each spectrum was obtained with single scan, and the relaxation delay between the scans was 40 s. After the Fourier transform of the FID, absolute value calculations were done to eliminate the phase disturbance of multiplets caused by homonuclear spin−spin coupling.[27,29] The integrated areas of characteristic bands were used for the diffusion coefficient calculations. The echo attenuation can be expressed by

$$I_{(2\tau)} = I_{(0)} \exp(-2\tau/T_2) \exp[-\gamma^2\delta^2G^2D_s(\Delta - \delta/3)] \quad (1)$$

where $I_{(2\tau)}$ and $I_{(0)} \exp(-2\tau/T_2)$ represents the echo intensities with and without the gradient pulses, τ is the delay between 90° and 180° pulses, T_2 is the spin−spin relaxation time, and γ is the gyromagnetic ratio of ^{1}H (2.675×10^{11} rad G^{-1} s^{-1}). For the different G values, the logarithm of the echo intensities was plotted vs b values ($b = -\gamma^2\delta^2G^2(\Delta - \delta/3)$), and D_s was obtained from the slope of the graph. The experimental uncertainty on D_s was estimated to be 2% or less. Dextran resonance peaks appeared at around 4 ppm (Figure 1a). The peaks associated with the diffusing species that were selected for the measurements did not overlap with the dextran peaks in most cases. Methanol and ethylene glycol spectra exhibited only one peak overlapping with those of dextran. In these cases, integrals including dextran resonances were directly used under the hypothesis that dextran resonance intensities were constant. This is well supported by the fact that diffusion coefficients calculated from two different peaks of ethanol, one overlapping with the dextran signal (3.7 ppm) and another one in a region free of dextran contribution (1.2 ppm), were the same within the experimental error. Moreover, peak areas obtained by band fitting of the overlapping ethylene glycol and dextran peaks led to no significant change of the self-diffusion coefficient in dextran solution samples. This spectral interference existed only for the dextran solutions since dextran peaks were not observed in the gel samples, probably due to their short T_2.

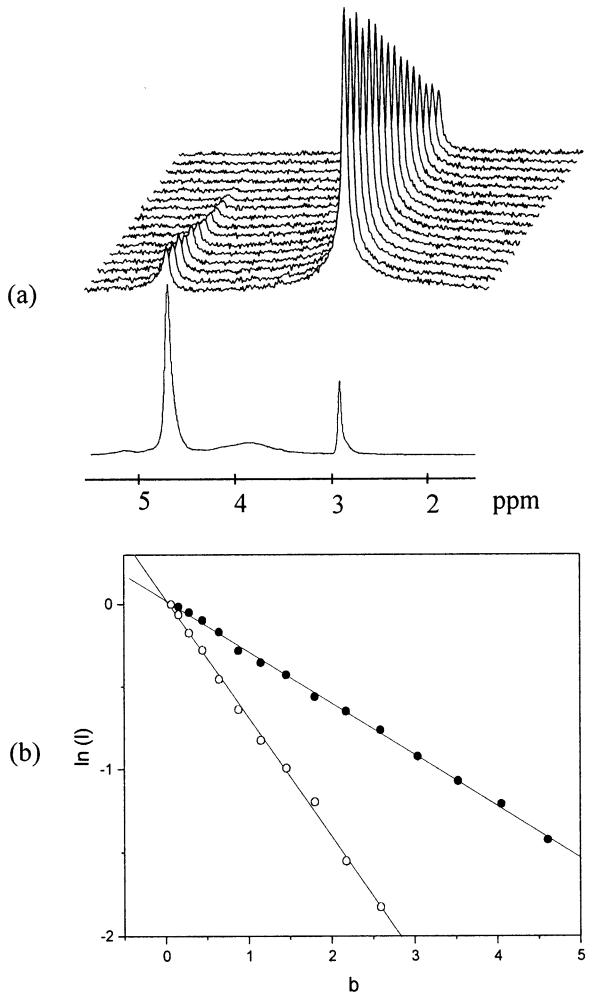

Figure 1. (a) Echo attenuation of DMSO and HDO in a 20% dextran gel as a function of the increasing gradient strength. G = 5−80 G/cm, δ = 1.3 ms, Δ = 60 ms. (b) Normalized intensity of the HDO (○) and DMSO (●) echoes in (a) as a function of the b values ($b = -\gamma^2\delta^2G^2(\Delta - \delta/3)$). The slopes of the least-squares fits represent the diffusion coefficients.

As a typical example of a data set, Figure 1a shows the DMSO echo attenuation in 30% dextran gel as a function of the increasing gradient strength at 37 °C. The NMR lines are broadened near the baseline in comparison to Lorentzian bands due to the absolute value calculations. Dextran gel signals, which appear as a broad band near 4 ppm in a normal spectrum (Figure 1a, lower panel), are absent in the spin echo spectrum (upper panel). The intensity of the HDO signal at 4.7 ppm decreases faster than that of DMSO signal at 2.9 ppm, illustrating the faster Brownian motions of HDO molecules than those of DMSO molecules. Area integrals are plotted vs b values in Figure 1b, and the self-diffusion coefficients are derived from the slopes of the plots.

Results and Discussion

Self-Diffusion of Small Solutes in Water. Despite the fact that the self-diffusion coefficients of molecular species in water were measured only for comparative purposes, few comments should be made regarding these measurements. The diffusion coefficient of a solute in solution depends on its size. Aspects of this dependence have been described by the well-known Stokes−Einstein equation,[30] a relation that has been developed for solutes much larger than the solvent molecules. Indeed, the diffusion of solutes in liquids and polymer systems has been widely examined in the literature (for reviews, see refs 1, 2, 31, and 32). Recently, an approach, based on free volume theory, has been successfully applied to the diffusion of molecular solutes.[33] It was found that the ratio of the solute tracer-diffusion and the solvent self-diffusion could be related to the size and molecular weight of the solute and the solvent, and consequently, these measurements provide an excellent means to estimate molecular size. Equation 2 describes the relation, where D_s is the diffusion coefficient of the solute, D_1 is the self-diffusion coefficient of the solvent, R_{w1} and R_w are the van der Waals radii of the solvent and the solute, and m_1 and m are the molecular weight of the solvent and the solute.

$$D_s/D_1 = [2/(1 + R_w/R_{w1})]^2[(1 + m_1/m)/2]^{1/2} \quad (2)$$

In the present paper, R_w of the investigated molecules was calculated from the van der Waals volume (V_w) following the method suggested by Bondi and Edward.[30,34] V_w of the molecule is the sum of the volume increment values corresponding to the structural elements such as CH_3, CO, OH, etc., and R_w is defined as $R_w = (3V_w/4\pi)^{1/3}$. R_w are considered as a reasonable choice for the small molecules examined in this paper.[30] In addition to describing successfully the diffusion of small solutes in solvent where interactions like hydrogen bonding is negligible, the approach was shown to be also satisfactory in aqueous milieu when hydration effects are taken into account. We followed the method that Edward suggested for the calculation of the degree of hydration.[30] This method assumes that each hydroxyl group of a tracer molecule forms, on average, one hydrogen bond with one water molecule, which results in an increase of V_w by 18.6 Å^3. It was found that the extent of the hydration does not appears to be proportional to the number of hydroxyl groups in the solutes, and the hydration by (or addition of) one water molecule to solutes having two hydroxyl groups, such as ethylene glycol, provided the best fit.[33] Along this line, one hydration water molecule is assumed for both alcohols and diols in the present paper. The corrected R_w values are listed in Table 1. Adopting the approach described above, D_s of small solutes in D_2O at 37 °C determined in the present study is plotted against $1/R_w^2$ in Figure 2. Since eq 2 has been developed for binary solution systems, it cannot be directly applied to analyze the diffusion in dextran solutions or gels. Therefore, a simplified relation (D_s vs $1/R_w^2$) was adopted for the analysis of the diffusion results in the present paper. Interestingly, D_s increases almost linearly as $1/R_w^2$ increases, and the resulting least-squares fitted line is drawn in Figure 2. It is clearly seen from the figure that, for a given solvent and temperature, the diffusion of solutes is properly described by their size.

Self-Diffusion of Micelles in Water. We have examined the self-diffusion of SDS, CPC, and Triton X-100 micelles in water as a guideline for our analysis of the diffusion in dextran solutions and gels (Table 1). For the three investigated micellar systems, the measured D_s in water were smaller than those of the investigated molecular solutes by about 1 order of magnitude. This observation can be easily rationalized qualitatively, considering the large size of the micelles. To display the influence of the size on the diffusion, we have considered the hydrodynamic radii (R_h) of the micelles that are obtained with the Stokes−Einstein equation, using the self-diffusion coefficients measured by the PFG−NMR experiments. The R_w values as

Table 1. Size and Diffusion Properties of the Solutes in Various Media at 37 °C

			self-diffusion coefficients, D_s ($\times 10^9$ m^2/s)			
	R_w (Å)[a]	R_h (Å)[b]	D_2O	20% dextran solution	20% dextran gel	30% dextran gel
HDO	1.7	1.1 (1.9)	2.5	1.7	1.4	1.0
methanol	2.1 (2.4)	1.7 (2.3)	1.6	1.1[c]	0.82	0.51
ethanol	2.3 (2.6)	2.1 (2.6)	1.3	0.73, 0.72[c]	0.52	0.36
2-propanol	2.7 (2.8)	2.6 (3.0)	1.1	0.56	0.40	0.22
ethylene glycol	2.5 (2.7)	2.3 (2.8)	1.2	0.64[c]		0.31
1,2-propanediol	2.4 (2.9)	2.8 (3.2)	1.0	0.52	0.37	0.25
acetone	2.6	1.9 (2.5)	1.4	0.82	0.59	0.39
2-butanone	2.7	2.3 (2.8)	1.2	0.70	0.46	
DMSO	2.6	2.1 (2.6)	1.3	0.71		0.31
SDS		17 [18]	0.16	0.024		
CPC		29 [38]	0.094	0.0086		
Triton X-100		53 [54]	0.052		0.0039	0.0038

[a] van der Waals radii of the small solutes. R_w values corrected for their hydrated forms as described in the text are indicated in parentheses. [b] Hydrodynamic radius of the solutes. R_h calculated with corrected Stokes−Einstein relation are indicated in parentheses for small solutes. Literature values for micelles are shown in brackets.[35,39,40] [c] Calculated from a peak overlapping with a dextran peak.

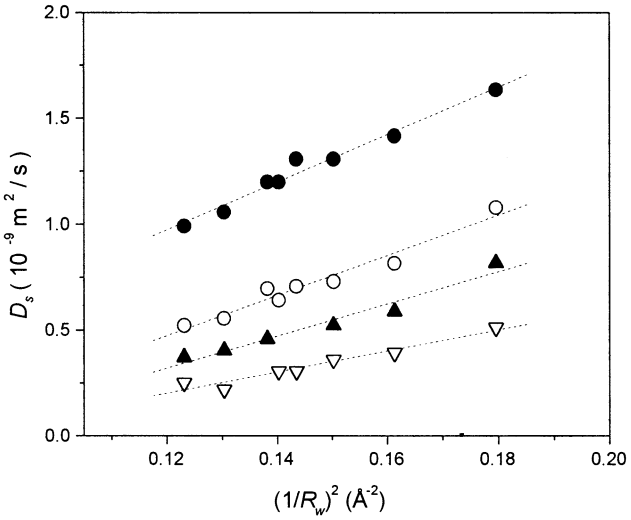

Figure 2. Self-diffusion coefficients of the small solutes (●, in D_2O; ○, in 20% dextran solution; ▲, in 20% dextran gel; ▽, in 30% dextran gel) at 37 °C.

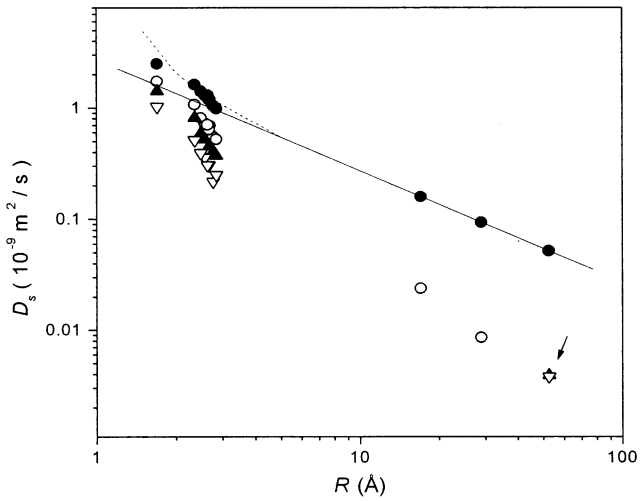

Figure 3. A log−log plot of the self-diffusion coefficients of the small and micellar solutes as a function of the solute radii at 37 °C. The symbols indicate the same media as in Figure 2.

calculated for small solutes were shown to be incorrect for large solutes, and the Stokes−Einstein equation is ideal for spherical solute molecules that are much larger than solvent molecules, like large micelles.[30] In fact, the description of micelles and macromolecules diffusion in aqueous solutions using this approach has been extensively described in the literature.[6,35−37] The calculated R_h values, considering the viscosity of D_2O equals 0.8329 cP,[38] are listed in Table 1. They show a good agreement with those previously reported in the literature.[35,39−40] In Figure 3, D_s values are plotted against R_h with a log−log scale, and the straight solid line for the diffusion data in D_2O indicates the Stokes−Einstein relation. In addition, R_h of small solutes was also derived from the PFG−NMR experiments, and the resulting values are included in Table 1 and Figure 3. As mentioned before, the Stokes−Einstein relation is developed for solutes with larger size than the size of the solvent, and indeed, Figure 3 indicates that the solid line displaying the Stokes−Einstein relation does not describe the diffusion of small solutes very well. Some corrections to the relation were proposed on the basis of the diffusion data and the size of solutes calculated with their van der Waals radius.[30] The dashed line indicates the expected behavior with the Stokes−Einstein model, including these corrections. It can be seen that this approach describes well the diffusion coefficients that we have measured for the complete size range.

Self-Diffusion in Dextran Solutions and Gels. D_s values of small solutes in 20% dextran solutions and gels are included in Table 1 and displayed in Figure 2. A similar dependence of D_s on $1/R_w^2$ observed in aqueous solution is also reported for the dextran solutions and gels. Figures 2 and 3 clearly show that the solute diffusion is hindered by the addition of dextran polymers. The self-diffusion coefficients of all the investigated solutes are reduced in dextran solution relative to water. When the solution is transformed into a gel by the cross-linking of the dextran polymers with potassium ions, an additionnal decrease in the self-diffusion is observed. This observation is valid for all the investigated molecules including micelles, and the magnitude of the decrease appears to be independent of the solute functionality.

Dextran molecules have lots of hydroxyl groups that can participate in hydrogen bonds. The association of a diffusing species with the polymer chains through hydrogen bonding should lead to a limited diffusion because the dextran polymer diffusion is considerably slow, on the order of 10^{-12} m^2/s.[17] However, we did not observe any specific behavior of the solutes that bear functional groups that can be involved in hydrogen

bonding. For example, diols such as ethylene glycol have two hydroxyl groups. Despite their larger number of hydroxyl groups than alcohols and ketones, the diffusion of diols did not show any further decrease of D_s compared to alcohols and ketones when the diffusion medium changed from water to dextran solutions and dextran gels. Instead, the overall diffusion behavior in all the media remained unchanged. The prevailing role of nonspecific interactions between solutes and polysaccharide gels was previously reported for 1% alginate and 2.4% agarose gel.[41] These results suggested that the polysaccharide chains work simply as a steric barrier to the solute diffusion rather than an interactive medium. The system investigated here has relatively high polysaccharide concentrations (20−30% dextran). Even at these concentrations, the interactions between solutes and water molecules were considered to be prominent in the dextran solutions and gels. One must note that water molecules are by far the most abundant species that can participate in hydrogen bonding. The present results do not exclude completely interactions between solutes and dextran, but these putative interactions do not significantly slow down the diffusion of specific solutes.

The self-diffusion of SDS and CPC micelles was characterized in dextran solutions (Table 1). The presence of the polysaccharide has a large impact on the micelle diffusion. The D_s values are decreased by about 1 order of magnitude compared to the values obtained in water. This reduction is much more pronounced than the decrease observed for the molecular species, which is considered to be a phenomenon likely due to the large size of micelles.

The diffusion coefficient of Triton X-100 micelles was measured in dextran gels. Similar to the observations for dextran solutions, the diffusion of Triton X-100 micelles is severely hindered by the presence of polysaccharide gels, the reduction of D_s being by more than a factor 13 compared to the measurements in water. Interestingly, the D_s of Triton in 20 and 30% dextran gels remains almost same (marked with arrow in Figure 3). This behavior contrasts with that observed for small molecular solutes for which the D_s is systematically slower in 30% dextran gel than in 20% gel. In fact, the values of D_s of Triton in these dextran gels are in the same order of the diffusion coefficient of the dextran polymer chains themselves. It is likely that the micelles are actually embedded in the cross-linked dextran gels, and the motions of micelles are somehow severely limited and concerted with those involving the polymer chains. In this case, the restricted self-diffusion of these micelles in dextran gels is proposed. The structural description of dextran gels is fairly limited. In the case of 30% dextran gels (with 0.5−2 M K^+), the pore size was estimated to be 8−10 μm from the water self-diffusion coefficients.[17] The pore size of dextran gels was also shown to vary as a function of the dextran and the K^+ concentration. Considering our conditions (20−30% dextran with 2.5 M K^+), the pore size of the gels we prepared should be similar to or slightly larger than those reported, and consequently is much larger than the Triton micelle size, on the basis of the measurements made in aqueous solution (see Table 1). To examine whether restricted diffusion could be observed in our systems, the self-diffusion coefficient of Triton micelles in 20% dextran gels has been measured for diffusion time (the interpulse delay Δ) varying between

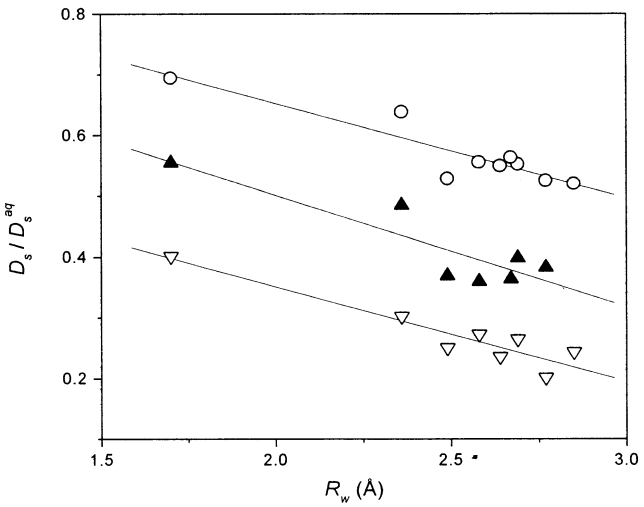

Figure 4. Relative diffusion coefficients, D_s/D_s^{aq}, of the small solutes in different media. The symbols indicate the same media as in Figure 2.

100 and 500 ms. This approach has been successfully applied on porous media and gels to probe limited diffusion associated with inhomogeneities such as pores and barriers.[42−44] In our case, no variation of D_s could be detected using different diffusion times, and the values remained small, $\sim 3.5 \times 10^{-12}$ m^2/s (data not shown). During the diffusion delay, it is calculated that the distance (root-mean-square distance) travelled by a Triton micelle is about 2 μm. This is considerably shorter than the estimated pore size of the dextran gels. Therefore, the limited effect of the increase of dextran concentration from 20 to 30% on the Triton macroassembly diffusion may not be simply associated with the formation of a pore network in which the micelles are tightly entrapped. It is possible that interactions between Triton and dextran polymer chains lead to the change of the geometry of the Triton micelles, to some sort of association of the micelles with the polysaccharide, etc. Additional work should be done to provide a detailed understanding on this phenomenon. However, our results firmly established that the diffusion coefficient of Triton micelles in dextran gels (20 and 30%) representes only 7% of the value obtained in water, indicating a dramatic constraint. A similar behavior has been reported for few other systems. A previous study reported a very limited diffusion of large solutes such as albumin and γ-globulins in alginate gel beads.[45] Conversely to small solutes such as water that did not experience any limited diffusion during their liberation from alginate gels, albumin and γ-globulin release was strongly restricted from these gels to the point that they could not diffuse out the gels. Similarly, it was observed that large proteins can be completely entrapped in methacrylated dextran gels when the cross-linking density is high, and their release was only possible by the degradation of the gel.[18,19]With other polysaccharides, it has been shown that the diffusion of small solutes are less dependent on the polysaccharide concentration than those of larger ones.[1] The results presented in this paper show that dextran gels follow this behavior. It is positively illustrated by the variations of the relative diffusion coefficients of the solutes, D_s/D_s^{aq} vs R_w and R_h (Figures 4 and 5). In these graphs, the influence of water on the solute diffusion is somehow eliminated by the normalization relative to D_s^{aq}. Figures 4 and 5 reveal the existence of the size dependence of

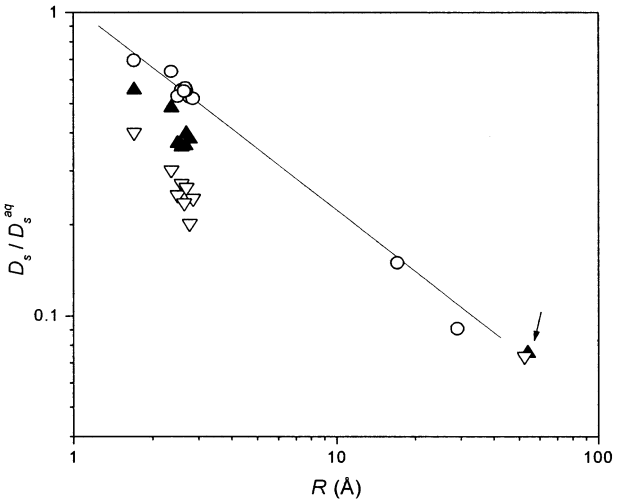

Figure 5. A log−log plot of relative diffusion coefficients, D_s/D_s^{aq}, of the small and micellar solutes as a function of the solute radius in different media. The symbols indicate the same media as in Figure 2.

the diffusion restriction induced by dextran. For all the investigated systems, D_s/D_s^{aq} of small solutes decreases linearly as the solute size increases (Figure 4). Small solutes seem to be less hindered by the dextran polymers than larger solutes (Figure 5). The fact that D_s/D_s^{aq} shows no relation with the functionality of the solutes, such as the number of hydrogen-bonding groups, leads to the conclusion that no specific interactions between solutes and dextran polymers have a significant effect on the solute diffusion, even at considerably high dextran content. For the polymer solutions as well as the gels, the large solutes experience a more pronounced reduction of their self-diffusion in the presence of dextran than the small ones (Figure 5). The restriction is more marked for dextran gels than dextran solutions. In addition, a higher dextran content in the gel leads to a greater reduction of the self-diffusion of the molecular species except for Triton-X 100 micelles. Triton X-100 shows almost the same D_s/D_s^{aq} values in 20 and 30% dextran gels (marked with an arrow in Figure 5) because of the similar diffusion coefficients in both gels.

The major differences between dextran gels and the most of the other polysaccharide gels are the high concentrations of the gelifying ion (K^+) and of polysaccharide that are needed to form gels. As a comparison, agarose and alginate easily form hydrogels with a polysaccharide content less than 1%. As a result, the diffusion coefficients of solutes in dextran gels are usually lower than the values reported for other polysaccharide hydrogels. For example, the diffusion of small solutes such as water and glucose is not altered very much by the presence of agarose and alginate (up to 2.4%) in water.[41] However, in the case of 20% dextran gels, the relative diffusion coefficient of HDO was only 0.82. A similar behavior seems to hold for large solutes. For example, albumin ($R_h \cong 41$ Å) and γ-globulin ($R_h \cong 63$ Å) display relative diffusion coefficients of 0.13 and 0.15 in 4% alginate gels.[45] These values are higher than 0.09 and 0.075 obtained for CPC in 20% dextran solutions and Triton X-100 in 20% dextran gels, respectively.

The present results reinforce the concept that diffusion in polysaccharide matrices is strongly affected by the size of the solute. The conclusion can be most likely be extended to bacterial biofilms. Previous works investigating biofilms have in fact reported similar size dependences.[12,46−48] A recent review[46] reported that the diffusion coefficient in biofilms relative to that measured in water, D_s/D_s^{aq}, was reported to be dependent on the molecular weight. The average value of this ratio for the solutes referred to as large (molecular weight of 45 or more) was 0.39. This value is in fact similar to that we observed since the average D_s/D_s^{aq} ratio for molecular solutes heavier than ethanol (molecular weight of 46) in 20% dextran gels was 0.39. We also report that the diffusion of micelles in dextran matrices is drastically limited, a finding analogous to the conclusions obtained from the diffusion of dextran with different molecular weight in biofilms.[47] For dextran with a molecular weight of 40 000, a polymer with a R_h similar to that of Triton X-100 micelles, the diffusion coefficient ratio between the value measured in bulk water and in biofilms is 0.031. This ratio is in the same order of magnitude of that obtained from the diffusion coefficient of Triton X-100 micelles in water and in dextran gels. These similarities between the diffusion properties in dextran gels and biofilms suggest that a large part of transport properties in biofilms can be understood on the basis of a proper description of diffusion in polysaccharide matrices, despite their limited complexity. Indeed, it should be added that biofilms have a much more complex architecture than dextrans gels, and these ultrastructural features lead to heterogeneous diffusion behavior within the biofilms themselves.[12,47] In addition, the solute diffusion behavior may become more complex when biofilm polysaccharides carry charges and electrostatic interactions have to be taken into account.[49] These levels of complexity have to be eventually included to provide a complete description of the diffusion in biofilms.

Conclusion

The determination of the self-diffusion coefficients of small and large solutes measured in dextran solutions and gels indicates that the size of the solutes and the dextran concentration are the major parameters controlling the diffusion in these systems. These features are general in the diffusion of solutes in polymer solutions and gels as previously described for other systems. We have investigated the behavior of solutes bearing a different number of hydrogen-bonding groups. The fact that they show a similar diffusion behavior in dextran solutions and gels indicates that the interactions between solutes and dextran are weak and have no particular effect on solute self-diffusion. In the case of micelles, the diffusion is severely hindered by dextran solutions and gels. In particular, Triton X-100 micelles seem to experience practically no free movement in dextran gels. This observation suggests that the invasion of bacterial biofilms by macromolecules or macroassemblies may be severely limited. Micellar antibacterials are included in mouth rinses to fight the bacteria in dental plaque. Similarly, more efficient antibiotics delivery has been attempted by using liposome.[22] The penetration of this types of supramolecular structures in biofilms must be examined in more details to determine their penetration ability, a parameter linked to the antibacterial efficiency.

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