Molecular dynamics of confined glucose solutions

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Silica gels containing solutions of glucose in heavy water at different concentrations have been prepared by a sol-gel method. Dynamical studies with quasielastic neutron scattering, compared with previous results on bulk solutions, show that the dynamics of the glucose molecules are not appreciably affected by the confinement, even though the gels behave macroscopically as solid materials. Small-angle neutron-scattering spectra on the same systems, fitted with a fractal model, yield a correlation length that decreases from 20 to 2.5 nm with increasing glucose concentration, suggesting a clustering of glucose molecules in concentrated solutions that is consistent with the dynamical measurements. These two sets of results imply that 20 nm is an upper limit for the scale at which the dynamics of glucose molecules in solution are affected by confinement. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1884989]

INTRODUCTION

Freezing and drying are the major problems for living cells because the resulting damage to their membranes usually kills the cells. A key factor is the composition of the liquids present inside and outside the cell, containing a variety of aqueous solutions that protect the membranes against extreme cold or drought.[1–4] Among the solutes present are salts, sugars, and macromolecules. Sugars are especially interesting because they are produced in response to desiccation or freezing in numerous animal and plant species living in hostile environments.[1] Recent studies have demonstrated the role played by sugars such as the disaccharides sucrose and trehalose in structural membranes.[1] Wolfe *et al.* have shown that sugar solutions can vitrify in the spaces inside the protein structure of the membrane and maintain that structure against further dehydration.[1,5] These carbohydrates exhibit unusually high glass transition temperatures T_g, and the dynamics of both water and sugar molecules in the solutions depend strongly on sugar concentration.[2,6] From one sugar to another, the variations in T_g are huge, the disaccharide trehalose, for example, exhibiting a glassy state at room temperature. D-glucose is interesting for two reasons: as a basic monomeric component of more complex sugars such as trehalose, and as the monosaccharide with one of the highest T_g.

Smith *et al.*[7] and Talón *et al.*[8] have studied the response of the dynamics of glucose and water molecules to changes in sugar concentration and temperature with quasielastic neutron scattering (QENS). The presence of glucose slows down dramatically the dynamics of the water molecules, while the dynamics of the glucose molecules in turn depends strongly on sugar concentration. These bulk solution studies were a necessary first step towards understanding the phenomena underneath the dynamics of glucose solutions. In a biological context, however, the effect of the nature and size of the environment on the dynamics has to be taken into consideration, and the dynamics of glucose solutions for environments that mimic those of the living cell is of considerable interest in the understanding of the glassy state in membranes. To our knowledge, there has been little, if any, work on the dynamics of sugar solutions confined in matrices with different degrees of porosity.

A first step towards this goal is presented in this paper—an investigation of the dynamics of glucose molecules confined within an aqueous silica gel. Silica gel obtained by a sol-gel process provides an original and interesting way to understand how the size and the geometry of the confinement affect the dynamics of the solutes. QENS is a technique well adapted to this kind of study because neutrons are able to selectively probe the dynamics of a complex organic system by means of H/D contrast. With this method it

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is possible to distinguish the dynamics of solute and solvent and to access the time scales appropriate to the dynamics of each. We report here the results of QENS experiments on solutions of glucose confined in deuterated silica gel, in order to determine the time and length scales of the dynamics of the glucose molecules. We have also used small-angle neutron scattering (SANS) to characterize the structure of the pores and the distribution of the sugar molecules inside them.

SYNTHESIS

Aqueous silica gels containing the monosaccharide D-glucose were obtained by the sol-gel process.[9] The SiO_2-glucose sol was prepared using tetraethyl orthosilicate (TEOS), D_2O, HNO_3, urea, and simple glucose as precursors. First, TEOS and D_2O were mixed together, and HNO_3 was added to adjust the pH to approximately 2. In acidic media, the hydrolysis of TEOS $[Si-(OC_2H_5)_4]$ leads to the formation of silicic acid $[Si(OH)_4]$. When the hydrolysis of the alkoxide was complete, urea and a specific amount of D-glucose (Sigma-Aldrich) were added to the solution under vigorous stirring. The thermohydrolysis of the urea generates ammonia, increasing the pH of the sol and polycondensing the silicic acid into silica gel. After stirring, the sol was aged for periods of several hours to several days, depending on the temperature. We prepared gels with a molar ratio of glucose: water=0, 1:55, and 1:20 respectively, in order to correspond to the solutions studied previously.[7,8] The total molar compositions were thus TEOS: D_2O: OCN_2H_4: HNO_3: glucose =7.2: 1110: 1.6: 0.025: x, where $x=0$, 20.2, and 55.5, respectively. These concentrations would correspond to 0, 13.4, and 30 wt % of glucose in fully protonated solutions, or 0, 14.2, and 31.6 wt % taking only the glucose and water into account. We will denote these samples as A1 (pure gel), A2 (gel+1:55 solution), and A3 (gel+1:20 solution), respectively.

For the samples A2 and A3, D-glucose was first mixed with heavy water to replace the five exchangeable hydrogen atoms per molecule by deuterium. The solution was then dried in a vacuum oven and used as described above.

EXPERIMENT

For the QENS experiments, two spectrometers at the NIST Center for Neutron Research (CNR) were used: the disk chopper time-of-flight spectrometer[10] (DCS) and high-flux backscattering spectrometer[11] (HFBS). In both cases, the samples were loaded into annular aluminum cans with an annular spacing of 0.4 mm, sealed, and placed within the radiation shield of a closed-cycle refrigerator in which the temperature could be controlled to ±1 K.

The DCS experiments were carried out at an incident wavelength of 6 Å. The wave-vector transfer Q (for elastic scattering) covered the range of 0.25–1.93 $Å^{-1}$. All samples were measured at 300 K with run times of approximately 12 h. The energy resolution and intensity normalization were determined from a measurement with a vanadium hollow cylinder in the same conditions as those used for the sample measurements; the resolution was found to be 57 μeV [full width at half maximum (FWHM)]. The HFBS experiments were carried out at a wavelength of 6.271 Å. The resolution was measured from vanadium to be 1.2 μeV with an energy-transfer window of ±36 μeV and the Q values covered the range of 0.36–1.52 $Å^{-1}$. All samples were measured at 300 K with runs of approximately 42 h.

The QENS spectra were corrected for container scattering and background and the scattering functions $S(Q,E)$ were obtained, where E is the energy transfer. Data sets were reduced and analyzed with the DAVE package developed at NIST.[12]

For the SANS experiments, the samples 2 mm thick were sandwiched between two quartz windows. The measurements were conducted on the NG-3 30-m SANS instrument[13] at the CNR at an incident wavelength of 6 Å. Detector distances of 1.3, 4, and 13 m were used sequentially to cover a large range of Q (0.0035–0.47 $Å^{-1}$).

RESULTS: DYNAMICS

Various combinations of theoretical functions could fit the measured $S(Q,E)$. For the DCS results, the best fit was obtained with a combination of one delta function, two Lorentzians, and a sloping background to allow for a slowly varying inelastic scattering component:

$$S(Q,E) = c_1(Q)\mathcal{L}(W_1,E) + c_2(Q)\mathcal{L}(W_2,E) + a + bE, \quad (1)$$

convoluted with the resolution function of the instrument. In Eq. (1) $\mathcal{L}(W,E)$ denotes a Lorentzian function with FWHM W. For the HFBS results, only a single Lorentzian could be fitted, due to the limited dynamical range. To obtain information on the dynamics of the glucose molecules, the data from the pure gel (A1) were subtracted from those for the gels containing glucose (A2 and A3). We denote these results as (A2–A1) and (A3–A1), respectively. To take into account the different attenuations by the pure gel and the gel containing sugar, the scattering for the pure gel was multiplied by the attenuation factor for the glucose prior to the subtraction. The validity of this procedure rests, of course, on the assumption that the effect of the glucose on the dynamics of the gel host is negligible. This is supported by the fact that the gel is deuterated and contributed relatively little to the total scattering, as shown below.

Typical spectra are shown in Figs. 1 and 2 for the DCS and HFBS results, respectively. The $S(Q,E)$ functions exhibit considerable differences, due to the very different resolutions and dynamic ranges (by nearly two orders of magnitude) of the two instruments. It is clear that the solvent/matrix contribution is weak compared to that of the glucose. The fitted values of the energy widths W_1 and W_2 are plotted against Q^2 in Figs. 3 and 4. In Fig. 3 the HFBS results are also plotted for the lower Q values, where the quasielastic peaks still fall mostly within the energy window. The Q^2 dependence of the W_n was least-squares fitted by the following expressions used in the previous studies of bulk glucose solutions:[7,8]

$$W_1 = \alpha_1 + \frac{\beta_1 Q^2}{(1+\gamma_1 Q^2)}, \quad (2a)$$

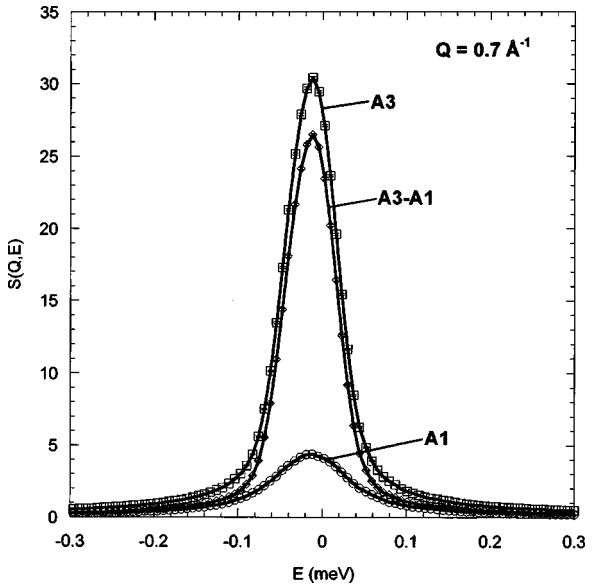

FIG. 1. DCS spectra for the samples A1 (circles), A3 (diamonds), and A3–A1 (squares) at 300 K at $Q=0.70$ Å^{-1}. The lines are a guide for the eye.

$$W_2 = \alpha_2 + \beta_2 Q^2. \tag{2b}$$

These fits are shown as continuous lines in Figs. 3 and 4, and the values of the parameters for the DCS results are given in Table I. With increasing glucose concentration, the widths decrease, indicating a slowing down of the translational and rotational dynamics of glucose molecules.

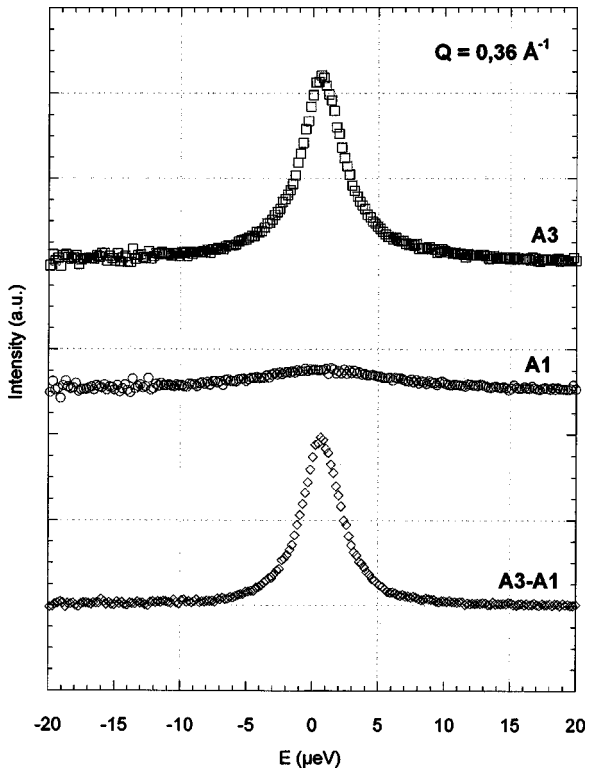

FIG. 2. HFBS spectra for the samples A1 (circles), A3 (squares), and A3–A1 (diamonds) at 300 K at $Q=0.36$ Å^{-1}.

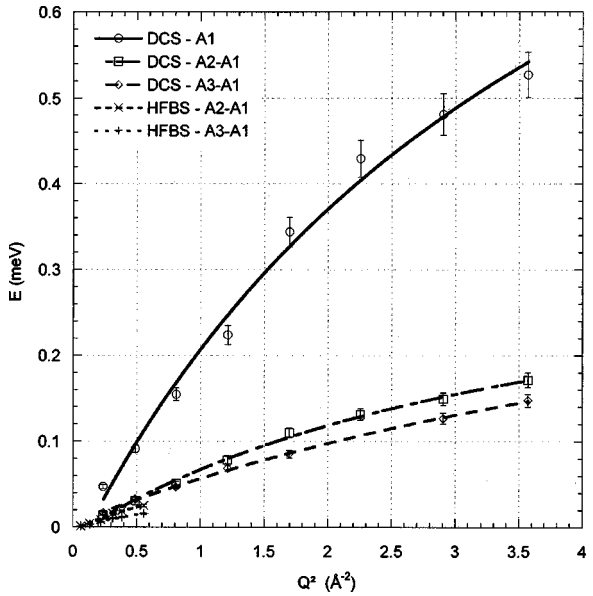

FIG. 3. Full width at half maximum, W_1, of the narrower Lorentzian fitted to the QENS spectra. The lines represent fits for the samples A1 (circles for DCS), A2–A1 (squares for DCS and × for HFBS) and A3–A1 (diamonds for DCS and + for HFBS).

Translational dynamics

According to the model of Teixeira *et al.* (TBCD),[14] first proposed for water and used in the previous studies of bulk solutions,[7,8] β_1 and γ_1 can be associated with the parameters for rapid jump diffusion:

$$\beta_1 = 2\hbar D, \tag{3a}$$

$$\gamma_1 = \frac{l^2}{6}, \tag{3b}$$

where D is the translational diffusion constant and l an effective jump distance. Fitted values of D and l are shown in

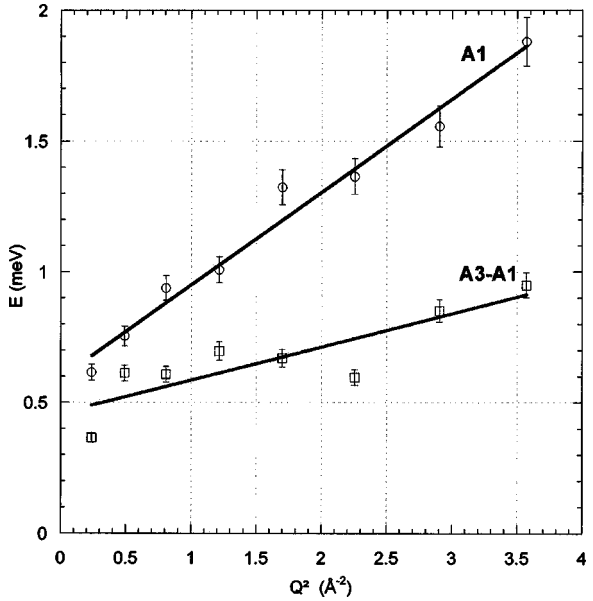

FIG. 4. Full width at half maximum, W_2, of the broader Lorentzian fitted to the QENS spectra measured on the DCS spectrometer. The lines represent fits for the samples A1 (circles) and A3–A1 (squares).

TABLE I. Parameters fitted to W_1 and W_2 from DCS results.

	W_1			W_2	
	α_1 (meV)	β_1 (meV Å^2)	γ_1 (Å^2)	α_2 (meV)	β_2 (meV Å^2)
A1	−0.04±0.03	0.30±0.06	0.24±0.09	0.59±0.04	0.35±0.02
A2	0.019±0.005	0.088±0.007	0.03±0.02	0.51±0.03	0.22±0.01
A3	0.018±0.004	0.059±0.007	0.11±0.04	0.51±0.04	0.14±0.02
A2–A1	−0.01±0.005	0.097±0.011	0.26±0.05	⋯	⋯
A3–A1	0.004±0.002	0.061±0.004	0.15±0.02	0.05±0.03	0.11±0.01

Table II for the fits to the DCS and HFBS data. In spite of the discrepancy between the magnitudes of the DCS and HFBS results, due to the very different resolutions and dynamic ranges (by nearly two orders of magnitude) of the two instruments, in both cases there is a reduction in the value of D by about a factor of 2 in going from the 14 wt % glucose (A2–A1) to the 30-wt % glucose (A3–A1) solution. A similar reduction by a factor of 2 was observed in the bulk solutions measured on HFBS (Ref. 7) (denoted there 1:55 and 1:20).

We now consider the effect of confinement, i.e., the change in the values of D in the present glucose–gel solutions from the values obtained in previous work on bulk solutions. Unfortunately the previous measurements on bulk solutions of natural glucose in D_2O were carried out only at 280 K as opposed to the 300 K of the present work. We can estimate the effect of the 20-K change in three ways:

(1) In their NMR measurements on 40 wt % glucose solutions Moran and Jeffrey[15] find an activation energy for the diffusion of glucose molecules of 27.8±0.6 kJ/mol, giving an increase by a factor of 2.2 on going from 280 to 300 K.
(2) They also find a nearly identical activation energy for diffusion of glucose and water molecules, suggesting that we can apply the activation energy for diffusion of *water* molecules found in our previous work[8] to be 15.5±0.8 kJ/mol; for the 15 and 33 wt % solutions, giving an increase of 1.6 on going from 280 to 300 K.
(3) Our recent measurements at ISIS (Ref. 16) on solutions of natural *fructose* in D_2O gave an increase in the diffusion rate of 1.65 on going from 280 to 300 K.

These results taken together provide an estimate of approximately a factor of 2 for an increase in the diffusion rate of glucose molecules on going from 280 to 300 K. Applying this to the values found for the 1:20 and 1:55 bulk solutions of 0.25 and 0.12×10^{-5} cm^2 s^{-1} on HFBS,[7] we obtain values for D at 300 K of about 0.5 and 0.25×10^{-5} cm^2 s^{-1} for the two solutions. Comparing the results in Table II with these values, we find essentially no change in the diffusion rate of glucose molecules between the bulk solutions and those confined in silica gel at the same temperature.

There is no marked dependence of the effective jump distance l on concentration, and the values in the range of 1–2 Å obtained here on both HFBS and DCS are similar to that obtained in the earlier work for the 33 wt % bulk solution. This implies that the Brownian-type motions of glucose molecules within a cage of neighboring sugar molecules, as proposed by Roberts and Debenedetti[17] on the basis of numerical simulations, are also not affected by either confinement or concentration. It appears that the rapid dependence of diffusion rate on both temperature and concentration results from changes in the jump frequency rather than jump distance.

Rotational dynamics

According to the TBCD model,[4] α_2 can be associated with the relaxation time for free orientational diffusion,

$$\alpha_2 = \frac{2\hbar}{3\tau_R}. \tag{4}$$

For the sample A2–A1 on DCS, the only case in which a second Lorentzian could be satisfactorily fitted, we obtain $\tau_R = 8.2$ ps. Comparing the result on HFBS for the 33 wt % bulk solution (there also it was not possible to fit a second Lorentzian at 15 wt %) of ~9 ps, we find that the rotational dynamics also do not appear to be affected by the confinement in the gel.

TABLE II. Physical constants calculated from the fits to W_1 and W_2 as a function of Q realized to DCS and HFBS data: D (diffusion constant), l (jump length), and τ_R (orientational relaxation time).

	DCS			HFBS	
	D (10^{-5} cm^2 s^{-1})	/(Å)	τ_R (ps)	D (10^{-5} cm^2 s^{-1})	/(Å)
A1	2.3±0.5	1.2±0.2	0.74±0.06	0.59±0.06	⋯
A2	0.66±0.05	0.4±0.2	0.86±0.05	0.8±0.2	3.2±0.6
A3	0.45±0.05	0.8±0.2	0.86±0.06	0.29±0.04	1.9±0.2
A2–A1	0.74±0.08	1.3±0.1	⋯	0.6±0.1	2.2±0.4
A3–A1	0.46±0.03	0.93±0.08	8±4	0.31±0.05	2.0±0.3

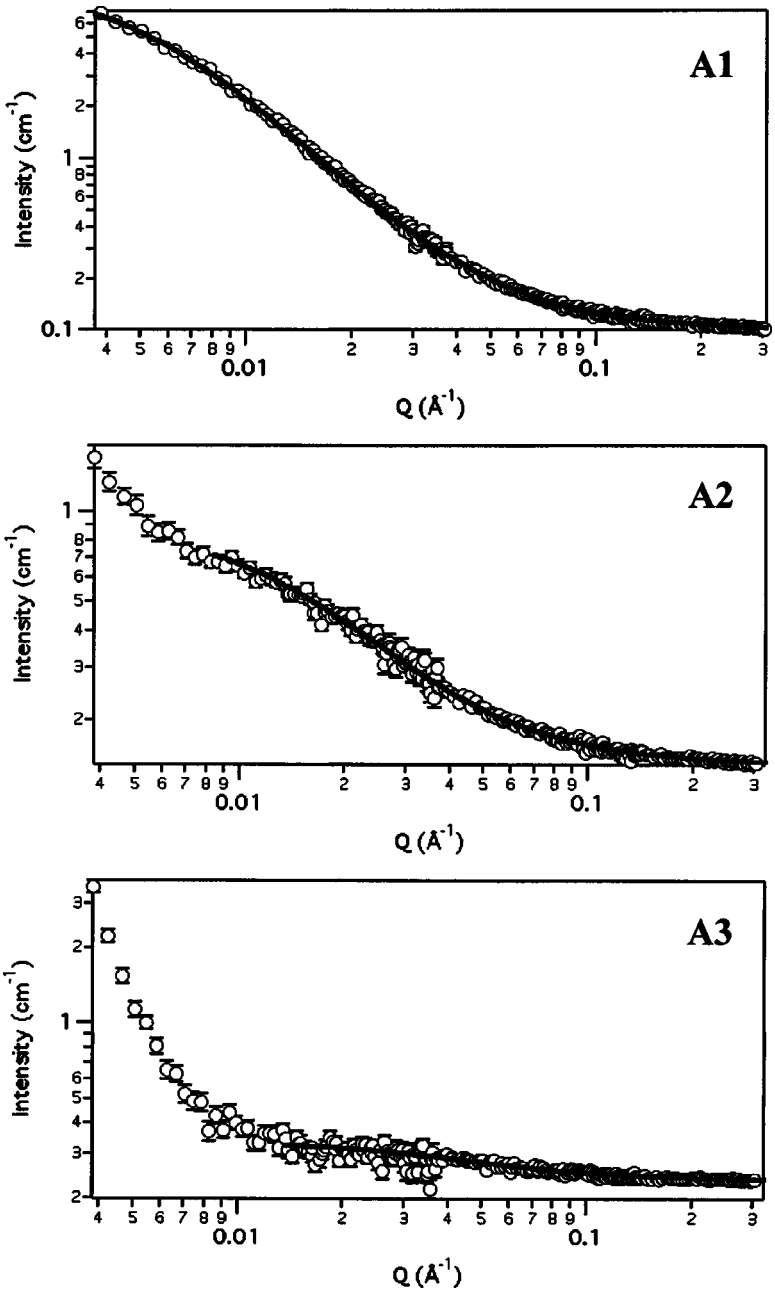

FIG. 5. SANS spectra for samples A1, A2, and A3. The lines represent the fits of the fractal model described in the text.

RESULTS: STRUCTURE

The SANS results of scattering intensity versus Q, shown in Fig. 5, give an indication of the arrangement of the glucose molecules within the pores of the silica gel and the distribution of pore sizes. We observe significant changes with increasing glucose concentration. A fractal model[18] is often used to fit spectra from silica gels using the form

$$I(Q) = P(Q)S(Q) + \text{bkgd},$$

where $P(Q)$ is the form factor for the spherical subunits of the fractal structure, and bkgd is the q-independent incoherent background. The fractal structure factor, $S(Q)$, is given by[18]

$$S(Q) = 1 + \frac{\sin[(D-1)\tan^{-1}(Q\xi)]}{(QR_o)^D} \frac{D\Gamma(D-1)}{[1+1/(Q^2\xi^2)]^{(D-1)/2}}.$$

Here D is the fractal dimension, R_o the radius of the primary silica particles, and ξ the correlation length, interpreted as the pore size of the silica gel.

This model[19] fits the pure gel very well. For the samples A2 and A3, the fit is also good except for an upturn in the intensity at small Q values, which could be due to large-scale inhomogeneities in the sample, "sugar-rich" domains, or phase separation. From the fractal model fits, we find that the fractal dimension $D=1.9\pm0.1$ and the primary particle size $R_o=6\pm1$ Å are nearly identical for all three gels, but the correlation length decreases from 188 ± 4 Å for the pure gel to 70 ± 3 and 25 ± 7 Å for the 1:55 and 1:20 solutions, respectively, which may indicate that the glucose is clustering, consistent with the interpretation of previous QENS results on

bulk solutions[8] and also of numerical simulations,[17] or the glucose may be forming a layer on the walls of the pores of the gel. Further SANS measurements with contrast matching of both gel and glucose are planned to distinguish these possibilities.

CONCLUSIONS

In this work, we have succeeded in synthesizing aqueous silica gels containing glucose solutions at varying concentrations. The dynamical studies with QENS show that the diffusion rate of glucose molecules slows down by a factor of 2 as the sugar concentration is doubled. A similar behavior was previously observed in bulk solutions and the rates for translational and rotational diffusion were similar, indicating that the confinement in silica gel does not substantially modify the dynamics of the sugar molecules. Both SANS and QENS results suggest a clustering of glucose molecules at higher concentrations, consistent with numerical simulation results in the literature and previous studies of bulk solutions. The estimate of the pore size diameter of 20 nm obtained from the SANS measurement on the pure silica gel by itself provides an upper limit of the length scale at which the dynamics of the glucose molecules are affected by confinement. The silica gels containing glucose solutions studied in this work present macroscopically solid behavior with the microscopic dynamics of the corresponding bulk liquid solutions. In considering the behavior of sugar solutions in biological environments, it is clearly necessary to go to pore size diameter around 5 nm, a characteristic distance between lipid bilayers.[20] Work is in progress to synthesize and measure glucose solutions confined in mesoporous materials such as MCM-41 where one can alter the confinement size and fabricate smaller pores.

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