Rotational and Translational Mobility of Small Molecules in Sucrose plus Polysaccharide Solutions

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The effect of different polysaccharides on the rotational (D_{rot}) and translational diffusion (D_{trans}) coefficients of small molecules in concentrated systems (sucrose solutions) was investigated. Dextran (1 or 10% w/w) with different molecular masses (from 10^4 to 2×10^6 Da), gum arabic, or pullulan was added to solutions of sucrose (57.5% w/w). Viscosity measurements of the diffusion medium studied (sucrose and sucrose plus polysaccharide) were made using a Rheometric Scientific viscometer in a temperature range from 20 to −10 °C. The rotational mobility of nitroxide radicals (Tempol) dispersed in the concentrated systems was measured by electron spin resonance. The translational diffusion coefficient of fluorescein was determined by the fluorescence recovery after photobleaching method. The studied temperature range for the latter two techniques was from 20 to −16 °C. For these conditions of concentration and temperature, there was no ice formation in the samples. No effect of the molecular mass of dextran on D_{rot} and D_{trans} was observed when solutions with the same dry matter content were compared. Only pullulan and gum arabic, at 10%, had a significant effect on D_{trans} of fluorescein. Temperature and total dry matter content were observed to be the most important factors controlling D_{rot} and D_{trans} in these concentrated systems.

Keywords: *Polysaccharide; sucrose; rotational and translational mobility; diffusion coefficient; subzero temperatures*

INTRODUCTION

Freezing is not a guarantee of an indefinite shelf life for food products; it is only a process to reduce or limit temporarily the rate of different phenomena (physical, chemical, and enzymatic reactions) occurring in the unfrozen phase of a frozen product. The presence of certain polysaccharides was suggested to be an important factor in increasing shelf life (Levine and Slade, 1988). Their presence in frozen products, especially ice creams, contributes on the one hand to improving sensory properties, producing smoothness in body and texture and providing uniformity to the product, and on the other hand to limiting physical changes (by increasing viscosity), reducing ice and lactose crystal growth and increasing resistance to melting (Goff and Sahagian, 1996).

Indeed, the mechanism of action of stabilizers in enhancing frozen stability is related to the control of the amorphous matrix surrounding the ice crystals (Levine and Slade, 1988). In the viscoelastic liquid surrounding ice crystals, the polysaccharide concentration can be relatively high, exceeding its critical concentration, and consequently, polysaccharide chains can become entangled. It has been suggested that they may restrict diffusion of water molecules to existing crystals during fluctuations in temperature and thus limit ice crystal growth (Goff et al., 1993).

Differences in stabilizer action have been related to the specific structure and molecular mass of each polysaccharide (Slade and Levine, 1991). Network formation can depend on the ability of flexible chains to entangle. At intermediate chain lengths, greater concentrations of chains are required for entanglement and network formation. For dilute solutions, high molecular mass polymers are necessary to produce gels or networks (characterized by very high macroscopic viscosity) (Slade and Levine, 1991).

The effect of polysaccharides on the mobility of water or small solutes is not clearly known (Parker and Ring, 1995) but is still widely studied (Martin et al., 1999; Basaran et al., 1999). Recently, Belton (1997) studied the motions of water in a polysaccharide gel by nuclear magnetic resonance (NMR). He showed that water molecules diffuse between and inside the meshes created in the network and that water also interacts with the polysaccharide. The possible orientations and the diffusion paths of water and solute molecules in polysaccharide networks seem to be varied and complex. The determination of diffusivities (rotational and translational) of small solutes would be useful for predicting reaction rates in complex systems and in understanding the mechanisms underlying the stability of foods.

The objective of this study was to understand the effect of the addition of polysaccharides on the mobility of small solutes in sucrose solutions. Polysaccharides with different structures (linear and branched), concentrations, and molecular masses were compared. The concentrations of the sucrose solutions were chosen, according to the sucrose state diagram (Blond et al.,

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Table 1. Model Systems Used To Study the Effect of the Addition of Polysaccharides on Rotational and Translational Mobility of Probes

model system	reference solution
sucrose 57.5% + polysaccharide 1%	sucrose 58.5%
sucrose 57.5% + polysaccharide 10%	sucrose 67.5%

1997), to avoid ice formation at temperatures down to −16 °C. Both rotational and translational diffusion coefficients of small probes were measured, using the techniques of electron spin resonance (ESR) and fluorescence recovery after photobleaching (FRAP), respectively. The diffusing probes were Tempol for ESR and fluorescein for FRAP. The measurements were performed from 20 to −16 °C.

The relationships between rotational and translational diffusion coefficients and viscosity are represented by the Debye−Stokes−Einstein and Stokes−Einstein equations (eqs 1 and 2), respectively, where k_B is the

$$D_{rot} = k_B T / 8\pi\eta r^3 \qquad (1)$$

$$D_{trans} = k_B T / 6\pi\eta r \qquad (2)$$

Boltzmann constant, T is temperature (K), r is the hydrodynamic radius of the probe molecule (m), and η is the viscosity (Pa·s). Although these equations do not hold exactly for small molecules (Kovarskii et al., 1972), they are currently used to describe the behavior of solutes (Le Meste and Voilley, 1988).

According to these relationships, the diffusion of small molecules is controlled by properties of the diffusing molecule (its hydrodynamic size) and by the surrounding conditions. Earlier experiments have shown that the Stokes−Einstein equation can yield a good prediction for the translational diffusion of small solutes in sucrose solutions at concentrations up to 65% (w/w) in the temperature range from 20 to −10 °C (Champion et al., 1995; Girlich and Lüdemann, 1993, 1994). Viscosity measurements on the different sucrose and sucrose plus polysaccharide solutions were performed within the same temperature range to study the above relationships between viscosity and diffusion.

MATERIALS AND METHODS

Diffusion Media. Different sucrose and sucrose plus polysaccharide solutions were used as diffusion media (Table 1). The sucrose solutions were heated and agitated using a magnetic stirrer. Then, after cooling, water was added to compensate for the amount evaporated during heating. The polysaccharide, dextran (of different molecular masses), pullulan, or gum arabic was dissolved in cold water to avoid heat-damaging the polymers. Finally, the polysaccharide solution was added to the sucrose solution and mixed using a magnetic stirrer. Polysaccharide structures are presented in Table 2. Sucrose solutions with the same dry matter content as the sucrose plus polysaccharide solutions (58.5 and 67.5%) were used as references (Table 1). The studied temperature range was from 20 to −16 °C for both rotational and translational measurements. Under these conditions of temperature and concentration, there was no ice formation in the samples.

Diffusing Probes. The diffusing probes used for the D_{rot} and D_{trans} determinations were, respectively, Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) (Sigma Chemical Co., St. Louis, MO) (Table 3) at a concentration of 114 μM and fluorescein (disodium salt, Kuhlmann) at 3 μM.

Rotational Diffusion. The rotational diffusion of Tempol was measured using the ESR technique. The ESR spectra of Tempol were obtained with a Bruker EMX 10 spectrometer (Wissembourg, France) and analyzed with WINEPR software.

Table 2. Some Characteristics of the Polysaccharides Used in This Study

Polysaccharide	MW Daltons (x10³)	Nature	Structure
[a]Dextran	10 40 500 2000	Almost linear (the degree of branching is 4.8-5.5%) Main chain: (1→6)-α-glucan	
[b]Pullulan	270	Linear Main chain: (1→6)-α-D-maltotriose	
[c]Gum arabic	384	Branched Main chain: (1→3)-β-D-galactopyranosyl Side chains: (1→3)-β-D-galactopyranosyl joined to the main chain by (1→6)-linkages, both chains have attached units of: α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, 4-O-methyl-β-D-glucuronopyranosyl, and uronic acid as terminal ends of the chain branches.	

[a] Structure reproduced from Bovey (1979). Copyright 1979. Reprinted with permission from Academic Press. [b] Structure reproduced from Tsujisaka and Mitsuhashi (1993). Copyright 1993. Reprinted with permission from Academic Press. [c] Structure reproduced from Glicksman (1979). Copyright 1979. Reprinted with permission from Butterworth.
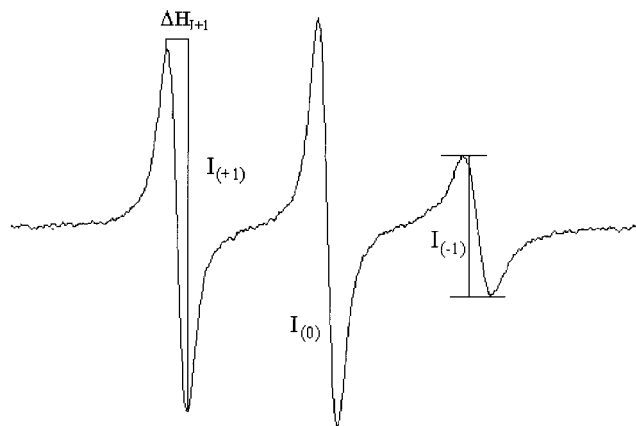
Table 3. Physical Characteristics of the Probes, Fluorescein and Tempol, Used, Respectively, for the Determination of Rotational and Translational Diffusion Coefficients

D_{trans} (FRAP)	Diffusion coefficient	D_{rot} (ESR)
$C_{20}H_{12}O_5$	Formula	$C_9H_{18}O_2N$
332.3	Molecular Weight (gmol⁻¹)	172
5.02[a]	Hydrodynamic radius (Å)	2.5[b]
Fluorescein	Structure	Tempol

[a] Hydrodynamic radius of probe molecules was estimated by Mustafa et al. (1993). [b] Hydrodynamic radius of probe molecules was estimated by Le Meste et al. (1991).

Three-line spectra (Figure 1) were obtained for the different studied solutions. The rotational correlation times (τc) of Tempol were determined from eq 3, deduced from the Freed and Fraenkel theory (1963), where I_{+1} and I_{-1} are the heights

$$\tau c = 6.65 \times 10^{-10}(\Delta H_{I+1}) \left[(I_{+1}/I_{-1})^{1/2} + 1 \right] \qquad (3)$$

Influence of Polysaccharides on Mobility

J. Agric. Food Chem., Vol. 48, No. 4, 2000 **1011**

Figure 1. ESR spectra of Tempol in a solution of sucrose 57.5% plus pullulan 10% at 20 °C.

of the lines I_{+1} and I_{-1} (Figure 1) and ΔH_{I+1} (G) represents the width (peak to peak) of the I_{+1} line.

Rotational diffusion coefficients (D_{rot}) were calculated from the relationship

$$D_{rot} = 1/6\tau c \tag{4}$$

The accuracy of the system for the control of temperature was ± 0.3 °C. The measurements were made every 2 °C, from 20 to −16 °C. Prior to measurement, the samples were equilibrated at the required temperature for at least 1 min.

Translational Diffusion. The FRAP method has been previously described in detail by Hervet et al. (1978), Yguera-bide et al. (1982), and Champion et al. (1995). The principle is based on the incidence of an attenuated beam on a small area of the sample (where the fluorescent molecules are uniformly dispersed). The fluorescence intensity, excited by the beam, is monitored as a function of time. At a predetermined time, the optical attenuator is momentarily removed for a fraction of a second to bleach some of the probe molecules in the illuminated area. The fluorescence intensity is reduced by the bleaching pulse, but the intensity subsequently recovers through diffusion of unbleached molecules from the surrounding unilluminated area. From a graph representing the evolution of the recovery of fluorescence as a function of time, we evaluate τ (relaxation time of diffusion, corresponding to the interference fringe period), and then from eq 5, the translational diffusion coefficient (D_{trans}), expressed in m^2 s^{-1}, is determined. d represents the interference fringe period.

$$D_{trans} = d^2/4\pi^2\tau \tag{5}$$

Preliminary measurements in sucrose and sucrose−polysaccharide solutions were made to verify that there was no effect of the different interference fringes used (from 5 to 23 μm) on the determination of D_{trans}. A Peltier system was used for the control of temperature, with an accuracy of ±0.2 °C.

Viscosity. The viscosities of the different diffusion media, sucrose and sucrose plus polysaccharide, were determined in a Rheometric Scientific viscometer (Piscataway, NJ) with a plate−plate (50 mm) geometry, in a temperature range from 20 to −10 °C. This viscometer works in conjunction with RSI Orchestrator software. Stress was measured as a function of shear rate. The viscosity, for solutions that have a Newtonian behavior, was calculated from the relationship

$$\eta = \tau/\dot{\gamma} \tag{6}$$

where η is the viscosity (Pa·s), τ is the stress (Pa), and $\dot{\gamma}$ is the shear rate (s^{-1}).

When the stress versus shear rate line is constant, the liquid is thought to be Newtonian; that is, the viscosity of the solution does not depend on the shear rate. For the sucrose plus polysaccharides solutions that did not exhibit Newtonian

behavior, an apparent viscosity could be calculated from

$$\tau = K\dot{\gamma}^n \tag{7}$$

In the case of non-Newtonian solutions, the coefficient K was approximately equal to the viscosity.

When $n < 1$, the material is said to be pseudoplastic (it undergoes shear thinning), and the viscosity falls as shear rate is increased. When $n > 1$, the material is said to undergo dilatancy (shear thickening), and the viscosity increases as the shear rate increases.

A suitable shear rate was chosen in the range from 100 to 360 s^{-1}, with a gap between the two plates of 1 mm for all experiments. The sample was always loaded at room temperature, and the plate was slowly lowered. The samples were then equilibrated at the required temperature for at least 5 min before the evaluation of viscosity. The measurements were repeated at least three times.

Statistical Treatment of Results. The measurements of rotational and translational diffusion were repeated at least three times, each with an accumulation of six curves. The statistical analysis of data was done with the Statistical Analysis System (SAS) program. A Student−Newman−Keuls (SNK) test was done to test for significant influence of the added polysaccharide. Comparisons between measurements were made with $\alpha = 0.01$.

RESULTS

Viscosity of Diffusion Media. Table 4 shows the viscosity values of the sucrose plus 10% polysaccharide solutions at 20 °C. The sucrose solutions containing 1 or 10% dextran, gum arabic, or pullulan exhibited Newtonian behavior at 20 °C, as did the reference sucrose solutions (58.5 or 67.5%). Dextrans of higher molecular mass (5 × 10^5 and 2 × 10^6 Da), gum arabic, and pullulan at 10% in the sucrose solution exhibited non-Newtonian behavior at the lower temperatures studied; they exhibited a pseudoplastic character. The viscosity values were compared only at 20 °C, at which all the solutions exhibited Newtonian behavior. The addition of 1% polysaccharide to a 57.5% sucrose solution did not increase the viscosity (data not shown) more than did the addition of 1% sucrose (reference sucrose solution at 58.5%). On the other hand, the addition of 10% polysaccharide significantly increased the viscosity, compared to the viscosity of the reference solution (sucrose solution at 67.5%). For 10% dextran, the higher the molecular mass of the dextran, the greater was the increase in viscosity. Ten percent pullulan increased the viscosity remarkably, compared to the other polysaccharides.

Rotational Diffusion of Tempol. The rotational diffusion coefficients of Tempol, measured from 20 to −16 °C in sucrose and sucrose plus polysaccharide solutions, are presented in Figure 2. D_{rot} of Tempol decreased when the dry matter content of the diffusion medium increased from 58.5 to 67.5% w/w, whichever the polysaccharide added. No significant effect ($\alpha = 0.01$) of the nature or structure (linear or branched) of the polysaccharide on D_{rot} of Tempol was observed.

Translational Diffusion of Fluorescein. The translational diffusion coefficients of fluorescein were measured, as a function of temperature from 20 to −10 °C, in solutions containing sucrose and sucrose plus (1 or 10%) polysaccharide; results are shown in Figure 3. The lateral mobility of fluorescein was not affected by the addition of 1% polysaccharide to the sucrose solution (57.5%). However, in the presence of 10% polysaccha-

Table 4. Viscosities of the Different Sucrose and Sucrose (57.5%) plus Polysaccharides (10%) Solutions Measured at 20 °C[a]

| | | η (Pa s) | | | | | |
| | | sucrose 57.5% + polysaccharide 10% | | | | | |
T (°C)	sucrose 67.5%	dextran 10^4	dextran 4×10^4	dextran 5×10^5	dextran 2×10^6	gum arabic	pullulan
20	0.28 ± 0.02	1.12 ± 0.01	2.28 ± 0.58	5.32 ± 0.14	6.54 ± 0.86	2.06 ± 0.14	12.23 ± 1.09

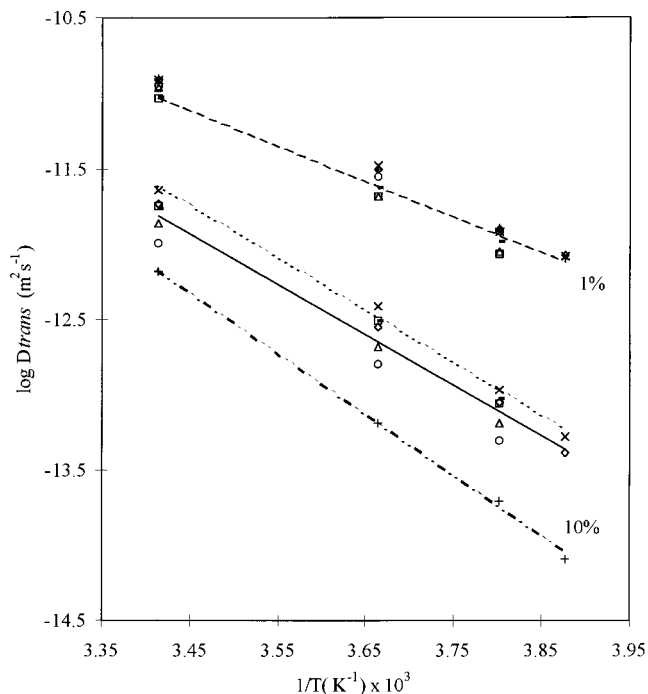[a] Newtonian behavior was equated to $N > 0.91$.
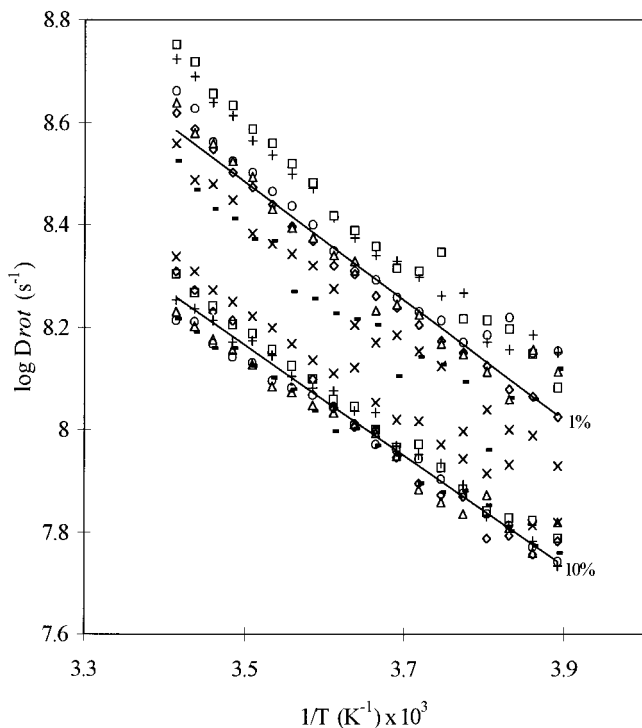
Figure 2. Rotational diffusion of Tempol, as a function of temperature, in solutions with sucrose 57.5% w/w plus 1 (or 10% w/w) polysaccharide (dextran: □, 10^4; ○, 4×10^4; −, 5×10^5; △, 2×10^6; ×, gum arabic; and +, pullulan) and in reference solutions [◇, sucrose 58.5% (or 67.5% w/w)]. Lines are drawn for samples that are not significantly different ($\alpha = 0.01$).

Figure 3. Translational diffusion of fluorescein, as a function of temperature, in solutions with 57.5% sucrose plus 1 (or 10%) polysaccharide (dextran: □, 10^4; ○, 4×10^4; −, 5×10^5; △, 2×10^6; ×, gum arabic; and +, pullulan) and in reference solutions [◇, sucrose 58.5% (or 67.5%)]. Comparisons were significantly different at $\alpha = 0.01$.

ride, the translational diffusion was observed to depend on the polysaccharide added. On the one hand, the addition of 10% dextran, whatever its molecular mass, induced the same effect on D_{trans} of fluorescein as did the addition of 10% sucrose (reference solution at 67.5% w/w). On the other hand, the addition of 10% pullulan induced a decrease in fluorescein D_{trans}, and the addition of 10% gum arabic significantly increased the values of D_{trans}, compared to that for the sucrose solution (67.5% w/w) used as a reference.

Temperature Influence on Mobility. Arrhenius-like representations, log $D = f(1/T)$, for D_{rot} and D_{trans}, for the different sucrose plus polysaccharide and reference solutions were used to show the effect of temperature on the diffusion of Tempol and fluorescein (Figures 2 and 3). A decrease in rotational and translational coefficients of probes with a decrease in temperature was observed for both concentrations of polysaccharide (1 and 10%) studied in the sucrose matrix.

Concerning activation energy (E_a), there was no significant difference in the activation energy of Tempol D_{rot} in the reference and sucrose plus (1 or 10%) polysaccharide solutions: 23.6 ± 3.2 kJ mol^{-1} for the solutions at 58.5% dry matter (with or without polysaccharide) and 20.4 ± 1.5 kJ mol^{-1} for all of the solutions

at 67.5%. In contrast, an increase in the activation energy of D_{trans} was observed when the total dry matter was increased. Indeed, an E_a value of 49.4 ± 2.0 kJ mol^{-1} was obtained for all of the solutions at 58.5% dry matter (with or without the addition of 1% of polysaccharide); it increased to 64.4 ± 1.9 kJ mol^{-1} for the solutions at 67.5% (with or without the addition of 10% dextran). The addition of 10% gum arabic or pullulan resulted in higher activation energies (67.2 ± 0.5 and 76.9 ± 1.2 kJ mol^{-1}, respectively) than for the addition of 10% dextran.

DISCUSSION

Influence of Polysaccharides on the Viscosity of Sucrose Solutions. The influence of polysaccharides on sucrose solution viscosity was observed only at 20 °C, at which solutions with dextran (of different molecular masses), gum arabic, and pullulan at 10% exhibited Newtonian behavior (Table 4). The large viscosity increase observed in the presence of pullulan cannot be explained only by its molecular mass (270×10^3 Da). Indeed, dextran, at the highest molecular mass studied (2×10^6 Da), showed a lower viscosity than pullulan. Moreover, gum arabic (384×10^3 Da) caused an increase in viscosity similar to that of dextran at 40×10^3 Da. If we compare polysaccharides of different structures, a discrepancy is thus observed in the relationship between

Influence of Polysaccharides on Mobility

J. Agric. Food Chem., Vol. 48, No. 4, 2000   **1013**

viscosity and molecular mass. However, we can observe that polysaccharides with the same structure and composition (dextran for instance), but different molecular masses, produce an increase in viscosity that is a function of molecular mass.

The intrinsic viscosity of polysaccharides provides an indication of the hydrodynamic volume occupied by the macromolecules in diluted solution. This volume depends on the macromolecule's molecular mass and flexibility and also on its interactions with the solvent. Dextran, at 5×10^5 Da, shows a lower intrinsic viscosity (0.63 mL/g) (Belder, 1993) than does gum arabic (25.4 mL/g at 1.2×10^6 Da) (Mitchell, 1979) or pullulan (209 mL/g at 10^6 Da) (Launay et al., 1986). It is generally observed that the higher the intrinsic viscosity, the lower the critical concentration, C^*, at which viscosity increases very strongly with a limited increase in concentration, as a result of chain overlapping and entanglement (Morris, 1989). Our results suggest that this critical concentration was reached for pullulan, whereas it was not for the other polysaccharides. Thus, neither the molecular mass nor the structure, branched or linear, can be used alone as a good indicator to predict the effect of polysaccharides on the viscosity of solutions.

Effect of Dextran Molecular Mass on D_{rot} of Tempol and D_{trans} of Fluorescein. The effect of the addition of dextran (1 or 10%), with molecular masses from 10^4 to 2×10^6 Da, to sucrose solutions (57.5%), on D_{rot} of Tempol and D_{trans} of fluorescein is shown, respectively, in Figures 2 and 3. The lack of sensitivity of Tempol D_{rot} and fluorescein D_{trans} to dextran molecular mass could be attributed to the relatively low polysaccharide concentration (1 or 10%) added to the sucrose solutions. Indeed, Häglund et al. (1988) showed that fluorescein translational diffusion was sensitive to the molecular mass of dextran, when the concentration of polysaccharide (in distilled water) was raised to 20%. Voilley and Le Meste (1985) showed that the values of Tempol D_{rot} were not affected by the molecular mass of polyethylene glycol (from 600 to 35×10^3 Da) at water contents >10%. The influence of polysaccharide molecular mass on rotational and translational mobility might depend on the nature and concentration of the polysaccharide. However, whatever its molecular mass up to 2×10^6 Da, dextran was observed not to reduce significantly the mobility (translational and rotational) of small molecules at low concentrations commonly used in foods (<10%), compared to the mobility of the sucrose solution with the same dry matter content.

Effect of the Structure of Polysaccharides on D_{rot} of Tempol and D_{trans} of Fluorescein. Gum arabic, dextran, and pullulan, at 1 or 10% in a 57.5% sucrose solution, induced similar effects on Tempol D_{rot} as for the reference solution at the same dry matter content. Indeed, the structure and nature of the polysaccharides did not appear to be parameters that determined the rotational mobility of Tempol in the sucrose matrix. Several authors (Voilley and Le Meste, 1985; Le Meste et al., 1991; Roozen et al., 1991) have suggested that small molecules can rotate freely in "cavities" or "holes" created in the network of biopolymers. The local environment of the rotating probe does not seem to be modified by the presence of polysaccharides, even if the polysaccharide induces changes on a larger scale, as reflected by an increase in viscosity.

Gum arabic, dextran, and pullulan, at 1% in the sucrose solution (57.5%), had the same influence on translational mobility of fluorescein, and this 1% addition did not reduce the probe's mobility more than did the addition of 1% sucrose. A similar effect was observed in the NMR studies by Martin et al. (1999) and Basaran et al. (1999), in which the presence of locust bean gum (0.5 and 1%) or xanthan (1%), in sugar solutions at concentrations up to 62%, did not modify the diffusion properties of fructose, sucrose, and water.

McCurdy et al. (1994) have demonstrated that dextran (molecular mass = 5×10^5 Da) at a low concentration (4.7% w/v) shows typical properties of a "random coil", but with an increase in concentration (19% w/v), the polymer chain adopts a more compact coil geometry. Those authors suggested that this conformational change could modify the diffusion rate of small molecules. Our statistical test showed that the addition, in 57.5% sucrose solution, of 10% gum arabic or 10% pullulan modified D_{trans} of fluorescein, whereas the addition of 10% dextran did not affect D_{trans} more than did the addition of 10% sucrose. Indeed, pullulan at 10% was the only polysaccharide that was observed to induce a significant decrease in D_{trans}. Similar behavior was observed with gelatin gels (Gillies et al., 1996); the translational motions of a spin probe were much more sensitive to changes in water content than were rotational motions. When the concentration of gelatin reached 55%, the measurable translational diffusion was virtually stopped, whereas the rotational diffusion was decreased only by a factor of ~10. Those results were interpreted in terms of a model in which increasing density of biopolymer strands restricted translational diffusion, but relatively free rotation still took place in cavities created by the lattice of the gel. The influence of 10% pullulan on D_{trans} could be due to its ability to form physical entanglements in water.

The high sensitivity of the FRAP method and the statistical analysis allowed us also to distinguish a different effect of gum arabic on D_{trans}, but its effect was not so pronounced as that for pullulan. The values of D_{trans} were higher in the presence of gum arabic than the values obtained for the reference solution (at the same dry matter content). The presence of gum arabic might induce some heterogeneities in the diffusion medium; probe molecules might move more freely in the regions where the network was less dense. More investigations are needed to understand the effect of polysaccharides on translational diffusion.

Temperature Influence on Viscosity and Mobility. All of the E_a values calculated for D_{trans} were higher than the values found for D_{rot} in the sucrose and sucrose plus polysaccharide solutions. The higher values might be attributed to differences in the mechanisms of rotational and translational motions of the solutes. It might be assumed that the cooperative character is greater when the displacement of the probe through the diffusion medium is considered. Indeed, D_{trans} appears to be more sensitive than D_{rot} to changes in the composition and viscosity of the medium.

Predicted diffusion coefficients were calculated with the Stokes–Einstein equation (eqs 1 and 2) to compare measured data with theory. The measured and predicted D_{trans} values were in relatively close agreement when the diffusion media were only sucrose solutions (Figure 4). However, the predicted D_{trans} values were much higher than the measured ones when polysaccharides were added to the solutions (results not shown). The viscosity increase caused by the addition of polysac-

1014 *J. Agric. Food Chem.,* Vol. 48, No. 4, 2000

Contreras-Lopez et al.
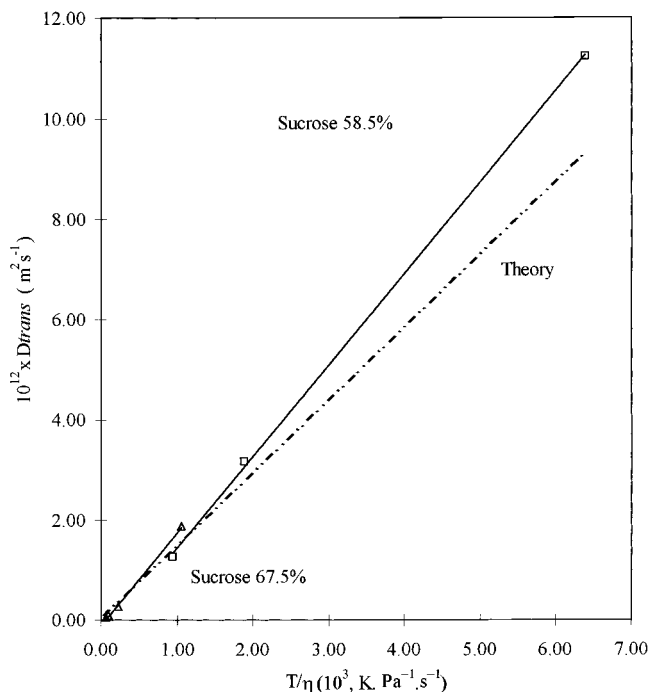
Figure 4. D_{trans} as a function of T/η for the sucrose solutions of ($\square$) 58.5 and ($\triangle$) 67.5% w/w. Theoretical values of D_{trans} were calculated from the Stokes–Einstein equation, using measured viscosity data, and are represented by the dotted line.

Figure 5. D_{rot} as a function of T/η for the sucrose solutions of ($\square$) 58.5 and ($\triangle$) 67.5% w/w. Theoretical values of D_{rot} were calculated from the Debye–Stokes–Einstein equation and are represented by the dotted line.

charides was not accompanied by a similar decrease in the mobility of small molecules, as expected from eq 2. The probes could, in fact, still move freely through the network created by the polymer; their motions were mainly under the influence of the frictional forces between the molecules of like size: sucrose and fluorescein.

The rotational diffusion coefficients of Tempol could not be predicted by the Debye–Stokes–Einstein equation; the measured D_{rot} values were always higher than the predicted ones, with or without polysaccharide in the diffusion medium (Figure 5). The viscosity of the sucrose solution, which reflects intermolecular interactions, could not be used as the only reference parameter for probe rotational motions, probably because the Tempol molecule did not show the same behavior as a sucrose molecule. Different interactions between probes and the diffusion medium have been commonly observed in ESR studies (Le Meste and Voilley, 1988; Roozen and Hemminga, 1990). Indeed, a coupling parameter was introduced in the Stokes–Einstein equation by Kowert and Kivelson (1976) to take into account the interactions between the diffusion medium and the spin probe. The relatively high D_{rot} values might demonstrate that the coupling of the probe molecule with the medium is poor, possibly due to relatively few hydrogen bonds being formed between the probe molecule and the medium. These H-bond interactions occur with water (due to the basic character of the probe molecule) rather than with sucrose. As the sucrose concentration increases, the system changes from a solution of hydrated sucrose molecules (<40%) to a sucrose–water phase (>40%), in which all of the water molecules are directly or indirectly involved in hydrogen bonds with sucrose (Roozen and Hemminga, 1990).

Conclusions. The addition of a low concentration of polysaccharide (1%) modifies neither the diffusivity properties of Tempol and fluorescein nor the viscosity
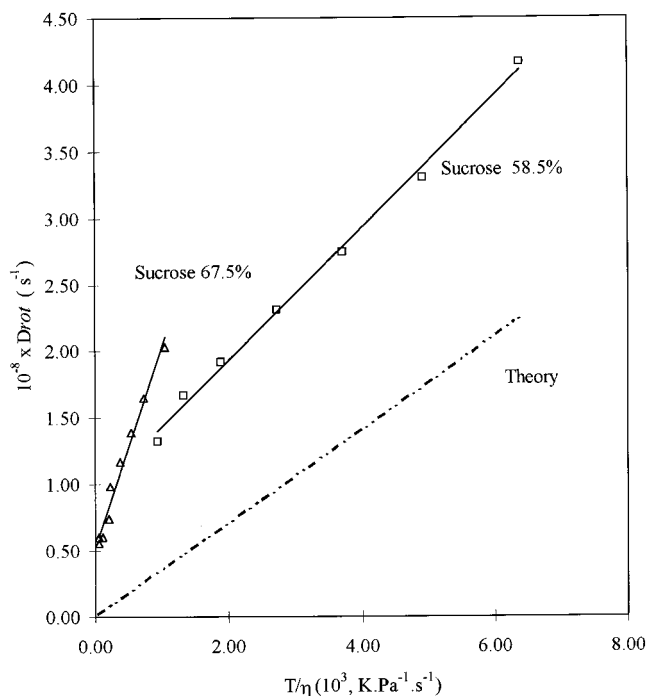
of sucrose solutions. However, a higher concentration of polysaccharide (10%) had an influence on viscosity.

In contrast to what is predicted by the Debye–Stokes–Einstein and Stokes–Einstein equations, viscosity is not the main factor affecting rotational and translational diffusivity of small molecules (Tempol and fluorescein) in concentrated solutions with polysaccharides. On the one hand, dry matter content and temperature are the main factors that affect the mobility of Tempol and fluorescein. On the other hand, the theoretical and experimental D_{rot} values for the sucrose solutions suggest an influence of interactions between the probe molecule and the diffusion medium. For fluorescein D_{trans}, a good agreement between theoretical and experimental values was observed, but only for the sucrose solutions. These results suggest that the mechanism of the motions shown by probe molecules (rotational or translational) could be the origin of the difference between theoretical and experimental values, rather than the probe molecule itself. Indeed, the large-scale translational mobility of a molecule depends on contributions from several other molecules in the medium, but the rotation of a molecule would be influenced only by its local neighborhood. Further investigations will be done to study the reasons for the differences between measured and predicted D_{rot} and between the D_{rot} and D_{trans} changes with temperature.

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Influence of Polysaccharides on Mobility

J. Agric. Food Chem., Vol. 48, No. 4, 2000 **1015**

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