Proton, Deuterium, and Oxygen-17 Nuclear Magnetic Resonance Relaxation Studies of Lactose- and Sucrose–Water Systems

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Comparison of the ^1H, ^2H, and ^{17}O nuclear magnetic resonance (NMR) transverse relaxation rate (R_2) of water associated with lactose- and sucrose–H_2O (or D_2O) systems was studied as a function of pH and sugar concentration. The 3.6 g of lactose/mol of H_2O ^1H NMR R_2 measurements varied greatly as a function of pH, reaching a maximum near neutral pH, whereas the ^2H NMR R_2 measurements varied only slightly as a function of pH. The difference in the contribution of chemical exchange to the transverse relaxation experienced by the ^1H and ^2H nuclei was proposed to explain the observed variation between the two nuclei. For all three nuclei, ΔR_2 increased with increasing sugar concentration. However, the magnitude of these increases, as well as the trends in ΔR_2 vs concentration for each nucleus and sugar type, varied widely. Comparison of the ^1H, ^2H, and ^1H-decoupled ^{17}O NMR R_2 values, on a normalized basis ($\Delta R_2/R_{2F}$), suggested that only the ^{17}O monitors directly the water mobility in the two sugar–H_2O (or D_2O) systems studied here.

INTRODUCTION

When water mobility is studied by nuclear magnetic resonance (NMR) relaxation techniques, three water nuclei can be probed: proton (^1H), deuterium (^2H), and oxygen-17 (^{17}O). The majority of NMR hydration and molecular dynamic studies have probed the ^1H nucleus [spin (I) = $^1/_2$]; however, problems with the interpretation of ^1H NMR relaxation data have been found. In pure water, ^1H relaxation is dominated by intramolecular dipole–dipole interactions. However, in heterogeneous systems other mechanisms may contribute to water relaxation. Two relaxation mechanisms that have been identified as possible contributors to ^1H relaxation are (1) cross-relaxation, the transfer of magnetization between the solvent and macromolecule spin systems (Edzes and Samulski, 1978; Lankhorst and Leyte, 1984; Piculell and Halle, 1986); and (2) chemical exchange (also called proton exchange) between distinctly different types of protons or deuterions in the system (i.e., "bound" and "free" water or ionizable groups on the macromolecule) (Koenig et al., 1975; Kalk and Berendsen, 1976; Sykes et al., 1978; Bryant and Shirley, 1980).

Because of these problems, recent interest in measuring water dynamics has been directed to the quadrupolar water nuclei ($I > ^1/_2$), ^2H ($I = 1$), and ^{17}O ($I = -^5/_2$). These nuclei are relaxed efficiently through the intramolecular quadrupole mechanism and thus are not significantly affected by dipolar cross-relaxation. However, the ^2H relaxation is still affected by chemical exchange (Piculell, 1986; Piculell and Halle, 1986), whereas ^{17}O relaxation is unaffected by chemical exchange, except for a narrow pH range around neutrality (Halle and Piculell, 1982; Richardson, 1989). Proton decoupling can be used to eliminate this proton-exchange broadening (PEB) in the ^{17}O NMR transverse relaxation rate measurements (Earl and Niederberger, 1977; Richardson, 1989; Lai and Schmidt, 1990).

Comparison of the relaxation behavior of all three water nuclei has most often been carried out in protein–water systems (Koenig et al., 1975; Piculell, 1985; Piculell and Halle, 1986; Kakalis and Baianu, 1988; Kakalis et al., 1990) but has not been investigated in many other systems. To date, only two studies have compared the relaxation behavior of all three nuclei in a carbohydrate–water system (Mora-Gutierrez and Baianu, 1990; Hills, 1991; Belton et al., 1991). Other studies that have investigated carbohydrate–water systems using only one of the three water nuclei are listed here for the interested reader: ^1H (Harvey and Symons, 1976, 1978; Bociek and Franks, 1979; Hills et al., 1989b; Mora-Gutierrez and Baianu, 1989; Padua, 1989); ^2H (Suggett et al., 1976; Richardson et al., 1987); and ^{17}O (Tait et al., 1972; Suggett et al., 1976; Belton and Wright, 1986; Richardson et al., 1987; Lai and Schmidt, 1990).

The objective of this research was to determine the ^1H, ^2H, and ^{17}O NMR transverse relaxation rates (R_2) of water in lactose- and sucrose–H_2O (or D_2O) systems as a function of pH and concentration and to compare the resultant relaxation behavior of the three nuclei.

MATERIALS AND METHODS

Materials. Lactose (Sigma Chemical Co., St. Louis, MO), which contained 98% α-lactose monohydrate and 2% β-lactose (manufacturing specifications), and sucrose (J. T. Baker, Phillipsburg, NJ) were used. The moisture content of the sugars was determined by vacuum oven, using 60 °C and 29.8-in. Hg vacuum for 24 h. The moisture content of lactose was 0.025%, while sucrose had 0% moisture. The water was distilled, and deuterium oxide was 99.8% D_2O (Sigma). The DCl and NaOD were obtained from Sigma.

Sample Preparation. *pH Samples.* For ^1H and ^{17}O NMR transverse relaxation rate (R_2, s^{-1}) measurements of H_2O varying in pH between 0.8 and 12.5, samples were prepared by adjusting the pH of distilled H_2O (initial pH 5.6 ± 0.1) by adding the appropriate amount of 1 N HCl or 1 N NaOH. For ^2H NMR R_2 measurements of deuterium oxide (D_2O) varying in pD between 0.85 and 12.35, samples were prepared by adjusting the pD of D_2O (initial pD 4.4 ± 0.1) by adding the appropriate amount of 1 N DCl or 1 N NaOD. pD was calculated from the equation pD = pH + 0.45 (Covington et al., 1968). Lactose samples varying in pH (or pD) between 0.8 and 12.5 were prepared in screw-capped glass tubes by adding 20 g of lactose to 100 g of H_2O or 111.28 g of D_2O preadjusted to the desired pH (or pD). On a molar basis the samples contained 3.6 g of lactose/mol of H_2O or D_2O. The lactose solutions were kept in a 75 °C water bath and inverted several times until the lactose crystals were

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Table I. Experimental ^1H, ^2H, and ^{17}O NMR Parameters

nucleus	frequency, MHz	90° pulse width, μs	recycling time, s	no. of scans
^1H	300.06	5	2.02	4
^2H	46.06	35	2.82	4
^{17}O	40.68	35	0.206	2000
	(300.06)[a]	(71)[a]		

[a] ^1H decoupling frequency and pulse width.

Table II. Comparison of ^1H, ^2H, and ^1H-Decoupled ^{17}O NMR R_2 and R_1 Values for H_2O and D_2O

nucleus	sample	R_2, s^{-1}	R_1, s^{-1}	$\Delta(R_2 - R_1)/\pi$, Hz
^1H	H_2O	4.65	0.38	1.36
^2H	D_2O	4.23	2.70	0.49
^{17}O[a]	H_2O	155.89	149.26	2.11

[a] ^1H-decoupled ^{17}O NMR.

completely dissolved. The solutions were then cooled to room temperature (23 ± 1 °C) and transferred to NMR tubes.

Concentration Samples. Lactose and sucrose solutions were prepared by adding the appropriate amount of distilled H_2O or D_2O to lactose or sucrose crystals to give concentrations of 1.8–10.8 g of lactose/mol of H_2O or D_2O and 1.8–28.8 g of sucrose/mol of H_2O or D_2O. The sugar solutions made with H_2O were used for ^1H NMR and ^1H-decoupled ^{17}O NMR R_2 relaxation rate measurements, while those made with D_2O were used for ^2H NMR R_2 relaxation rate measurements. The solutions were prepared as described above and transferred to NMR tubes. The pH was 5.6 ± 0.1 for all sugar–H_2O solutions, and pD was 4.4 ± 0.1 for all sugar–D_2O solutions.

NMR R_2 Relaxation Rate Measurements. A GN 300NB multinuclear NMR spectrometer (General Electric Inc., Fremont, CA) was used for the ^1H, ^2H, and ^1H-decoupled ^{17}O NMR R_2 relaxation rate measurements. A multinuclear 10-mm probe was used. Single-pulse experiments were done in triplicate at 20 ± 1 °C. The experimental ^1H, ^2H, and ^1H-decoupled ^{17}O NMR parameters are given in Table I. The MLEV decouling sequence with a decoupling field of 3521 Hz was used for the ^1H-decoupled ^{17}O NMR measurements. The samples used for the ^1H and ^2H NMR measurements were placed in 5-mm NMR tubes and spun at 22 ± 1 Hz, while those used for the ^1H-decoupled ^{17}O NMR measurements were placed in 10-mm NMR tubes and spun at 12 ± 1 Hz.

Relaxation Rate Calculations. The line width (υ_{obs}) at half-height of each spectrum was obtained by using the computer line fit routine available on the GN 300 NIC 1280 computer software (General Electric). The transverse relaxation rate (R_2, s^{-1}) was then calculated from the line width by (Dwek, 1973)

$$R_2\ (s^{-1}) = \pi \upsilon_{obs}\ (s^{-1}) \quad (1)$$

The average experimental error associated with measuring ^1H, ^2H, and ^1H-decoupled ^{17}O NMR R_2 values was 0.6%, 0.3%, and 0.6%, respectively.

The net or differential transverse relaxation rate (ΔR_2, s^{-1}) was calculated by subtracting the line width of liquid H_2O (or D_2O) (υ_{free}) from the line width of the sample (υ_{obs}) before multiplying by π:

$$\Delta R_2\ (s^{-1}) = \pi(\upsilon_{obs} - \upsilon_{free})\ (s^{-1}) \quad (2)$$

The net transverse relaxation rates (ΔR_2, s^{-1}) were normalized by dividing by the transverse relaxation rate of bulk H_2O or D_2O (Table II) measured at 20 ± 1 °C.

The field inhomogeneity in the ^1H, ^2H, and ^1H-decoupled ^{17}O NMR measurements was determined by comparing the R_2 values (eq 1) for H_2O and D_2O to the R_1 values obtained by the inversion recovery method (Vold et al., 1968) at the same temperature (20 ● 1 °C) (Table II). The difference between the R_2 and R_1 values for each nucleus is an estimate of the field inhomogeneity value for that nucleus (Kumosinski and Pessen, 1982).

RESULTS AND DISCUSSION

pH/pD Effect on ^1H, ^2H, and ^{17}O NMR R_2 Measurements. The effects of pH (or pD) on the ^1H, ^2H, and ^{17}O NMR R_2 values for H_2O (or D_2O) and 3.6 g of lactose/mol of H_2O (or D_2O) solutions as a function of pH (or pD) are shown in Figures 1, 2, and 3, respectively. The ^1H NMR R_2 values for H_2O and the ^2H NMR R_2 values for D_2O remained constant at average R_2 values of 4.65 and 4.23 s^{-1}, respectively, over the pH (or pD) range studied (Figures 1 and 2). On the other hand, the ^1H and ^2H NMR R_2 values for the 3.6 g of lactose/mol of H_2O (or D_2O) solutions were not constant over the pH (or pD) range. The ^1H NMR R_2 values varied widely, exhibiting a maximum value of 55.50 s^{-1} around neutral pH (pH 6.6) and an average minimum value of 15.12 s^{-1} at the extreme pH values (Figure 1). The ^2H NMR R_2 values did not vary nearly as much as the ^1H NMR R_2 values; however, a slight increase in R_2 near neutral pD was observed (Figure 2).

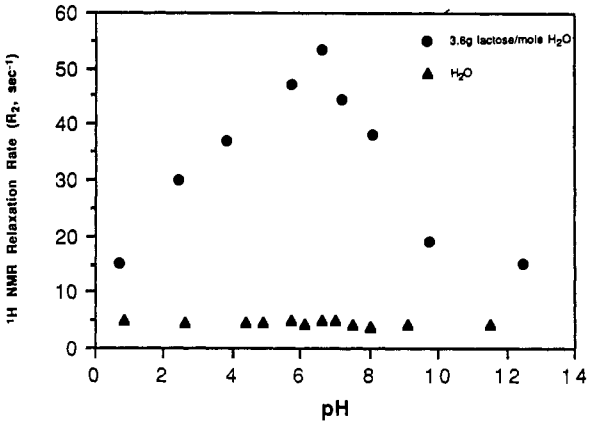

Figure 1. ^1H NMR transverse relaxation rates (R_2, s^{-1}) as a function of pH for H_2O and lactose–H_2O (3.6 g of lactose/mol of H_2O) systems.

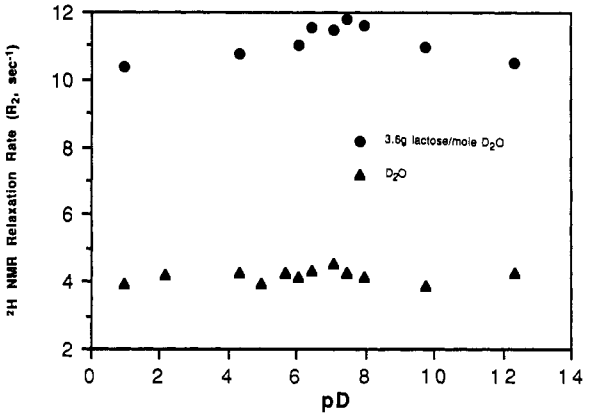

Figure 2. ^2H NMR transverse relaxation rates (R_2, s^{-1}) as a function of pD for D_2O and lactose–D_2O (3.6 g of lactose/mol of D_2O) systems.

These results can be explained by examining the contribution of chemical exchange to the transverse relaxation experienced by the ^1H and ^2H nuclei. In the case of the ^1H nucleus, the contribution of chemical exchange to the transverse relaxation rate is influenced by the rate of exchange between the water protons and the exchangeable hydroxyl protons on the lactose and the difference in the chemical shift between these proton sites (Hills et al., 1989b). When the rate of exchange is slow (near neutral pH), the contribution of chemical exchange results in a fast relaxation rate and thus a larger R_2 value. However, when the rate of exchange is fast (low and high pH values), the contribution of chemical exchange results in a slow relaxation rate and thus a smaller R_2. The effect of chemical exchange on the ^1H transverse relaxation rate

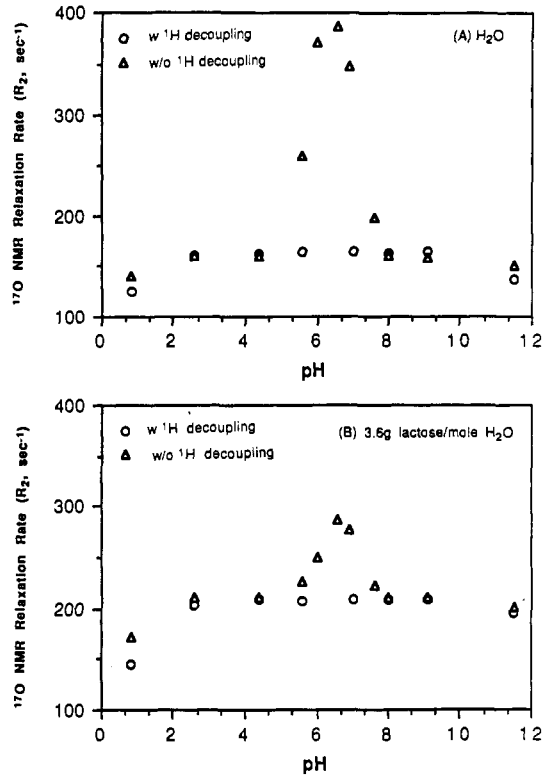

Figure 3. ^{17}O NMR transverse relaxation rates (R_2, s^{-1}), measured using a one-pulse experiment with and without ^1H decoupling as a function of pH for (A) H_2O and (B) a 3.6 g of lactose/mol of H_2O solution.

is discussed in detail by Hills and co-workers (Hills et al., 1989a,b; Hills, 1991).

The ^2H nuclei still suffer from the same problem of chemical exchange; however, since ^2H is a quadrupolar nuclei, the time scale for relaxation is much faster and the contribution of chemical exchange to the transverse relaxation rate is much smaller than in the case of the ^1H nuclei. Thus, R_2 is only slightly affected by variations in the rate of chemical exchange when the pD of the lactose–water system is altered. In addition, since ^2H has a lower gyromagnetic ratio, it is less sensitive than the ^1H nucleus to chemical shift differences between the different deuterion (or proton) sites, which also serves to explain the differences observed between the ^1H and ^2H R_2 relaxation data as a function of pD (or pH).

In addition to the relaxation enhancing mechanism of chemical exchange, cross-relaxation between water protons and lactose protons may also contribute to the ^1H relaxation rate, especially at the high magnetic field used in this experiment. However, cross-relaxation is thought to primarily affect the longitudinal rather than the transverse relaxation rate (Kumosinski and Pessen, 1989).

The ^{17}O NMR R_2 data for H_2O and lactose solutions without ^1H decoupling exhibited the characteristic maximum in R_2 around neutral pH (Figure 3). In the pH range 5.6–7.6, the proton-exchange rate was slow and the contribution due to proton-exchange broadening was largest, reaching a maximum of 226.7 (386.4 minus 159.7 s^{-1}) and 78.0 (288.5 minus 210.5 s^{-1}) s^{-1} at pH 6.6 for H_2O and lactose solutions, respectively. As the pH either decreased (pH <5.6) or increased (pH >8.5) from neutrality, the proton-exchange rate increased, and the effect of proton-exchange broadening became small to negligible. The average R_2 values at these pH values were approximately 159.7 and 210.5 s^{-1} for H_2O and lactose solutions, respectively.

A dramatic decrease in proton-exchange broadening was observed for the lactose solutions (78.0 s^{-1}) compared to the proton-exchange broadening effect on the H_2O (226.7 s^{-1}) at pH 6.6. Thus, the contribution due to proton exchange was greatly affected by the presence of the lactose. Halle and Piculell (1982), studying water–proton exchange in polymer solutions by ^{17}O NMR, found that the addition of even small amounts of either poly(acrylic acid) or poly(methacrylic acid) drastically reduced the maximum broadening of the ^{17}O absorption curve (234 s^{-1} for H_2O at pH 7.2 and 72 s^{-1} for the polyelectrolyte solutions at pH 7.4). They attributed this decrease in the broadening to the introduction of additional catalytic mechanisms that are much more effective for water–proton exchange than those operating in bulk water around neutral pH. On the basis of their hypothesis, it appears that the presence of lactose in water also provides for more effective mechanisms for water–proton exchange. In contrast to these findings, Richardson (1989) found that at pH 7.2 the contribution of broadening in cornstarch–water systems (66.67 g of dry starch/100 g of water) (200 s^{-1}) did not significantly differ from that in water (203 s^{-1}).

The ^1H-decoupled ^{17}O NMR R_2 data for both H_2O and lactose solutions showed no change with pH between 2.6 and 9.1. The average R_2 values of the H_2O and lactose solutions within this pH range were 161.8 and 208.4 s^{-1}, respectively. These values are similar to the average ^{17}O NMR R_2 values obtained without decoupling for the H_2O and lactose solutions in the acidic (2.6–5.6 pH) and basic (7.6–9.1 pH) pH ranges. The effect of decoupling the protons from the ^{17}O nuclei was to successfully remove the proton-exchange broadening effect (PEB) within this pH range (Earl and Niederberger, 1977; Richardson, 1989). However, at the extreme pH values (0.8 and 11.5) the R_2 values for both H_2O and lactose solutions were significantly lower than those in the 2.6–9.1 pH range. The R_2 values of the H_2O and lactose solutions were 124.4 and 145.4 s^{-1} at pH 0.8 and 137.0 and 200.3 s^{-1} at pH 11.5, respectively.

The proposed hypothesis to explain the observed decrease in R_2 at the extreme pH values, 0.8 and 11.5, was that the Cl^- and Na^+ ions from the HCl and NaOH decreased the water mobility of these systems at the high concentrations needed to reach the extreme pH values. To test this hypothesis, H_2O and lactose–H_2O samples (pH 5.6) were prepared with sodium chloride (NaCl) to approximate the high ionic condition of the 0.8 pH samples, without decreasing the pH. The ^1H-decoupled ^{17}O NMR R_2 values of the NaCl-containing H_2O and lactose–H_2O systems were 136.01 and 160.72 s^{-1}, respectively. These R_2 values were significantly lower than the ones for the salt-free samples at equivalent pH (159.7 and 210.5 s^{-1}, respectively), although not as low as the R_2 values for the extreme pH samples at equivalent pH (124.4 and 145.5 s^{-1}, respectively). These results suggested that the observed decrease in the R_2 values at the extreme pH values may be due to the effect of the ions used to adjust the pH on the mobility of water.

^1H, ^2H, and ^{17}O NMR R_2 vs Sugar Concentration and Type. The variation in the ^1H NMR ΔR_2 with increasing concentration showed two linear regions for both lactose and sucrose (Figure 4). The break point for lactose occurred at approximately 5.4 g of lactose/mol of H_2O, while that for sucrose occurred at approximately 10.8 g of lactose/mol of H_2O. It is interesting to note that the slope of the ΔR_2 vs concentration plot changed after the break point for both sugar–H_2O systems. At all concentrations measured, the ^1H NMR ΔR_2 of the sucrose–H_2O system was larger than that of the lactose–H_2O system.

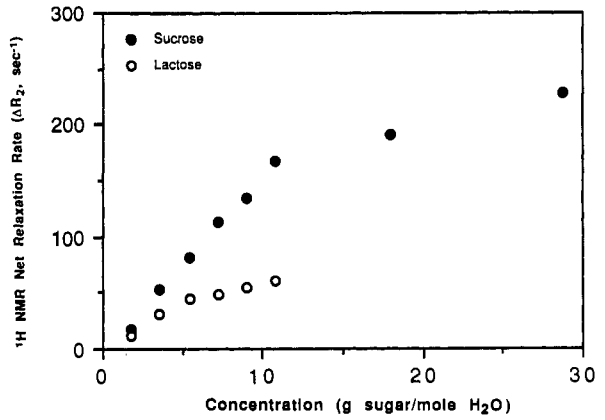

Figure 4. ^{1}H NMR net transverse relaxation rates (ΔR_2, s^{-1}) in lactose– and sucrose–H_2O systems as a function of sugar concentration.

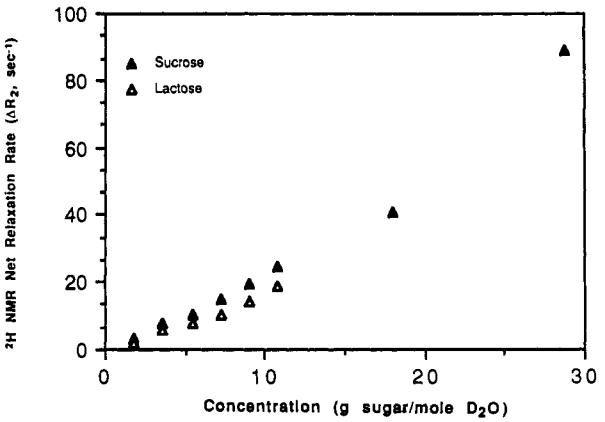

Figure 5. ^{2}H NMR net transverse relaxation rates (ΔR_2, s^{-1}) in lactose– and sucrose–D_2O systems as a function of sugar concentration.

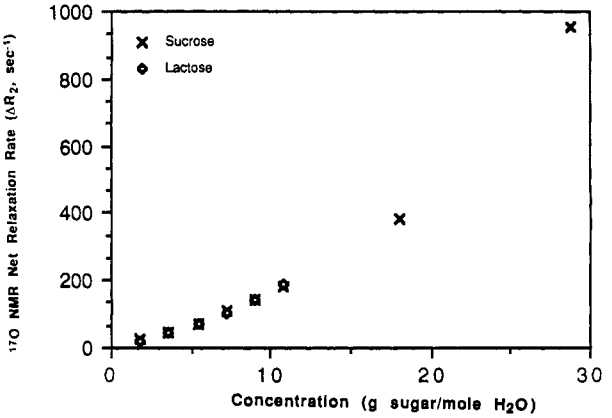

Figure 6. ^{1}H-Decoupled ^{17}O NMR net transverse relaxation rates (ΔR_2, s^{-1}) in lactose– and sucrose–H_2O systems as a function of sugar concentration.

Both the change in slope and the difference between the sucrose and lactose ΔR_2 values as a function of concentration may be attributed to the effect of chemical exchange.

The contribution of chemical exchange in the sugar systems studied here is mainly affected by the number, type, and availability of protons that can participate in the exchange process. At low sugar concentrations the sugar molecules are fully hydrated and all available sugar protons are participating in the chemical exchange process with the water protons. As the concentration of sugar increases, however, there is an increase in the competition for waters of hydration between the sugar molecules. As a result, some of the sugar molecules begin to hydrogen bond with each other (i.e., form of sucrose clusters) and the number of protons available to participate in the exchange process decreases. Thus, the contribution of chemical exchange to the relaxation rate decreases as the concentration of sucrose increases. For sucrose, these sugar–sugar associations are thought to occur between 30 and 40% sucrose on a total sample basis (Bowski et al., 1971; Allen et al., 1974; Mathlouthi and Luu, 1980; Mathlouthi et al., 1980; Flink, 1983). The break point in Figure 4 for sucrose of 10.8 g of sucrose/mol of H_2O corresponds to a sucrose concentration of 37.5% on a total sample basis. It is interesting to note that the increase in viscosity in the sugar–H_2O systems with increasing concentration does not appear to significantly affect the ^{1}H NMR ΔR_2 behavior at the higher sugar concentrations.

Chemical exchange effects can also be used to explain the differences observed in the ^{1}H NMR ΔR_2 values between the sucrose– and lactose–H_2O systems. The difference in the interaction of the two sugars with water is reflected in their very different solubility values: 199.4 g of sucrose/100 g of water for sucrose (Charles, 1960) and 19.25 g of lactose/100 g of water for lactose (Whittier, 1944), both at 20 °C. This greater degree of sugar–H_2O interaction in the case of sucrose results in a larger contribution of chemical exchange to the ^{1}H NMR ΔR_2 relaxation rate for sucrose over that for lactose.

The ^{2}H NMR ΔR_2 increased with increasing sugar concentration for both the lactose– and the sucrose–D_2O system (Figure 5). At all concentrations measured, the ^{2}H NMR ΔR_2 of the sucrose–D_2O system was slightly larger than that of the lactose–D_2O system. As discussed previously, the effect of chemical exchange on the ^{2}H nucleus is much smaller than on the ^{1}H nucleus. This is reflected in the differences between the ^{1}H (Figure 4) and ^{2}H (Figure 5) NMR ΔR_2 data as a function of sugar concentration and type: (1) There is no change in the direction of the slope in the ^{2}H NMR ΔR_2 vs concentration plot. (2) There is a much smaller difference in the ^{2}H NMR ΔR_2 values for sucrose and lactose.

The ^{1}H-decoupled ^{17}O NMR ΔR_2 increased with increasing sugar concentration for both sugars (Figure 6). At all concentrations measured, the ^{1}H-decoupled ^{17}O NMR ΔR_2 of the sucrose–H_2O systems was the same as that of the lactose–H_2O systems. The ^{1}H-decoupled ^{17}O NMR ΔR_2 values are not affected by chemical exchange processes and can therefore be used to monitor the mobility of the sugar–H_2O systems. The ^{17}O data indicate that the water in the two sugar systems was equally mobile at equal sugar concentrations.

From the data presented above, it is apparent that the three nuclei do not monitor the same processes. Only the ^{17}O nucleus appears to directly monitor the mobility of the water. Additional comparison of the nuclei on a normalized basis is given in the next section.

Comparison of ^{1}H, ^{2}H, and ^{17}O NMR Relaxation Data. To compare the ^{1}H, ^{2}H, and ^{1}H-decoupled ^{17}O NMR R_2 relaxation measurements of the sugar systems, the ΔR_2 relaxation data were scaled or normalized to their respective rates in pure H_2O (or D_2O), $\Delta R_2/R_{2F}$ (Piculell and Halle, 1986; Kakalis and Baianu, 1988). Normalized ^{1}H, ^{2}H, and ^{1}H-decoupled ^{17}O NMR R_2 relaxation data as a function of sugar concentration for both lactose– and sucrose–H_2O (or D_2O) systems are shown in Figure 7.

In both sugar–H_2O systems, the normalized ^{1}H relaxation rates were the largest and the normalized ^{1}H proton decoupled ^{17}O relaxation rates were the smallest. The trend of relaxation rates vs concentration was dependent

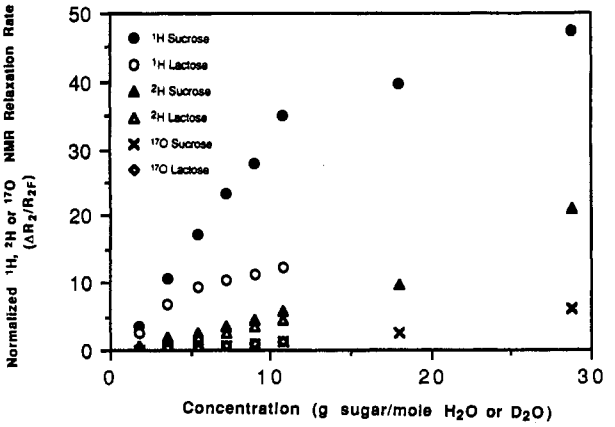

Figure 7. Normalized ^1H, ^2H, and ^1H-decoupled ^{17}O NMR transverse relaxation rates of lactose– and sucrose–H_2O (or D_2O) systems as a function of lactose concentration.

on the nucleus probed. This nucleus dependence was attributed to the contributions of additional mechanisms of relaxation to the ^1H and ^2H NMR relaxation rates. The ^1H NMR R_2 was influenced by chemical exchange and possibly cross-relaxation, and the ^2H NMR R_2 was only slightly influenced by chemical exchange, whereas the ^1H-decoupled ^{17}O NMR R_2 was unaffected by these additional mechanisms. Therefore, the three nuclei do not monitor the same molecular species in the two sugar–H_2O (or D_2O) systems studied here. Thus, only the ^{17}O (^1H decoupled) monitors directly the mobility of the water. Similar results have been reported by Piculell and Halle (1986) and Kakalis and Baianu (1988) for protein–water systems and Mora-Gutierrez and Baianu (1990) for maltodextrin systems.

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