

Proton Nuclear Magnetic Resonance Measurements on Various Sugar Solutions

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The dependence of low-field proton longitudinal relaxation rate on concentration was used to investigate water associations in sucrose, lactose, fructose, and glucose solutions. Data were analyzed in terms of Zimmerman and Brittin's theory. Results indicated that, at low concentrations, all four sugars bind nearly the same amount of water by weight and present analogous structures of highly mobile hydrated molecules. At higher concentrations, above 0.7 g/g of water, data suggested the formation of sugar associations of decreased rotational mobility, more so for the disaccharides than for the monosaccharides. For highly concentrated solutions (above 1.4 g/g of water), results were attributed to the depletion of free water and the formation of highly developed structures within the solution.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is generally recognized as an effective method for probing the dynamic state of water in aqueous systems (Kumosinski and Pessen, 1989; Hills, 1991; Belton et al., 1991). Lioutas et al. (1986) employed oxygen-17 and deuterium NMR relaxation data to distinguish five different populations of water in hydrated lysozyme. Kakalis et al. (1990) studied soy protein hydration and quantified the amount of "bound" water in the system using proton and oxygen-17 relaxation measurements. Numerous researchers collected data probing the ¹H nucleus because of its high sensitivity, although problems in data interpretation due to chemical exchange (Berliner and Reuben, 1980) and cross-relaxation (Edzes and Samulski, 1978) have been discussed. Pessen et al. (1985) concluded that, although cross-relaxation contributes to the longitudinal relaxation process, especially at high frequencies, data are useful to monitor hydration changes or trends.

Padua et al. (1991) investigated the dependence of low-field proton longitudinal relaxation rate on concentration for aqueous whey protein preparations over a wide concentration range. Data showed three linear regions which were analyzed according to Zimmerman and Brittin's theory (Zimmerman and Brittin, 1957). Three states of water were identified associated with the protein. In the dilute region, the presence of bound and free water was established. In the medium-range concentration, three water states were found to coexist: free, bound, and trapped or capillary water. In the high-solids region, free water was no longer present; only the bound and trapped portions were found.

In this paper, a similar approach was taken to investigate the water associations in solutions of sucrose, lactose, fructose, and glucose. Several researchers have studied the structure and dynamics of water in sugar solutions through NMR. Tait et al. (1972) measured the oxygen-17 relaxation rate of glucose solutions and reported an increase in relaxation rate with increasing concentration, which

was attributed to viscosity effects. Richardson et al. (1987) determined the mobility of water in sucrose solutions by deuterium and oxygen-17 NMR relaxation measurements. Three regions of successively decreasing water mobility were found with increasing solute concentration from 5 to 80% (w/w). This behavior was probably due to an increasing development of hydrogen bonds from water to sucrose and water bridging between sucrose molecules. Mora-Gutierrez and Baianu (1989) measured proton NMR transverse relaxation rates of a series of carbohydrate solutions and concluded that structural differences among the carbohydrates give rise to differences in hydration behavior. Uedaira et al. (1989) measured oxygen-17 relaxation in aqueous solutions of carbohydrates and related their findings to the number of equatorial hydroxyl groups on the respective carbohydrate molecules. Hills (1991), using proton and deuterium NMR relaxation measurements in glucose solutions, presented a model in which each carbohydrate hydroxyl group is hydrogen bonded to two molecules of water. The bound water reorientational motion was described as anisotropic and characterized by two correlation times.

MATERIALS AND METHODS

Sucrose, crystalline analytical reagent grade, was from J. T. Baker, Phillipsburg, NJ. Lactose, refined (lactose monohydrate 99.7%, minerals 0.2%, protein 0.1%), was from Foremost Whey Products Inc., Baraboo, WI. Fructose, crystalline, was obtained from Sigma Chemical Co., St. Louis, MO. Dextrose, anhydrous analytical reagent grade, was from E. K. Industries, Addison, IL. Sugar solutions were prepared by adding the appropriate amount of distilled water to the sugar to give concentrations of 0.50-2.00 g of sucrose/g of water, 0.34-1.32 g of lactose/g of water, 0.14-2.00 g of fructose/g of water, and 0.60-1.74 g of glucose/g of water. Supersaturated samples were heated over boiling water to dissolve the sugars; losses through evaporation were monitored by weight. Solutions were cooled slowly to prevent crystallization.

Proton longitudinal relaxation rates (R_1 , s⁻¹) were determined at room temperature (22 °C) with a 10-MHz-pulsed proton spectrometer (PR-103, Praxis Corp., San Antonio, TX) using a 90°-T-90° pulse sequence. All samples showed an exponential relaxation. R_1 was calculated by a microprocessor connected to the spectrometer. Typically, 10 points of the curve, log amplitude vs time, were used in the calculations. The equation fitted to the data was

$$Y = C_0 \exp(-B_0 t)$$

where Y is the amplitude difference between the first and second

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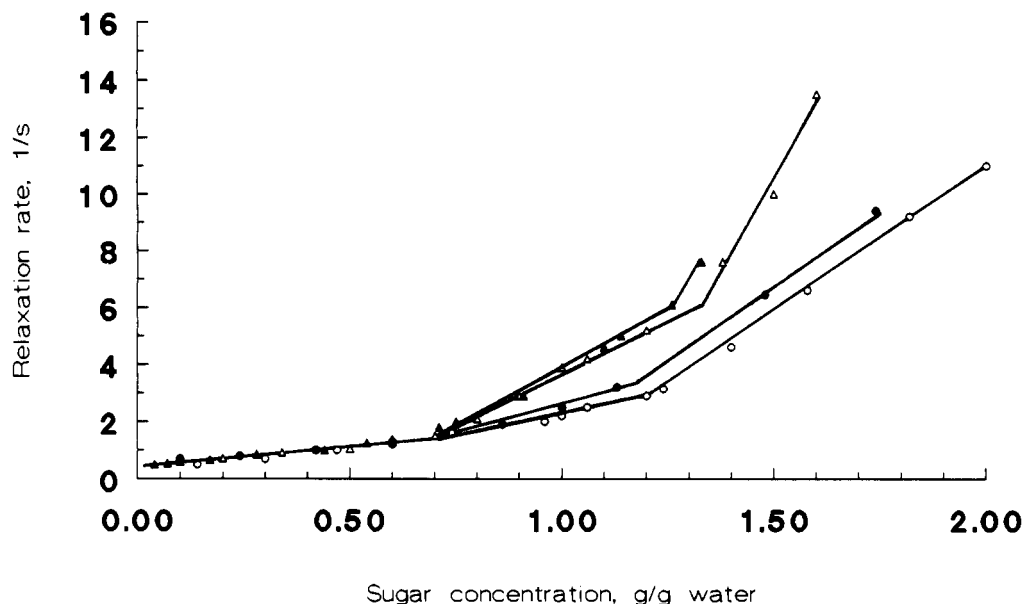


Figure 1. Proton longitudinal relaxation rate (R_1) dependence on concentration for sucrose (Δ), lactose (\blacktriangle), fructose (\circ), and glucose (\bullet) solutions.

pulses, C_0 is the amplitude of the first pulse, B_0 is the relaxation rate being evaluated, and t is time. Correlation factors were 0.995 or higher. Reported measurements are averages of four determinations.

RESULTS AND DISCUSSION

Figure 1 represents the dependence of R_1 on concentration for sucrose, lactose, fructose, and glucose solutions. The graph shows that up to 0.7 g of sugar/g of water the relaxation rate followed the same linear behavior for all four sugars. This observation follows the prediction of Zimmerman and Brittin's two-state model with fast exchange

$$R_{\text{obs}} = R_{\text{bound}}P_{\text{bound}} + R_{\text{free}}P_{\text{free}} \quad (1)$$

where R_{obs} is the observed relaxation rate and R_{bound} and R_{free} are the values for relaxation rates of the bound and free water states, respectively. P_{bound} and P_{free} represent the mass fraction of the water protons in the bound and free states, respectively, in a way that $P_{\text{bound}} + P_{\text{free}} = 1$. P_{bound} can be related to the solution concentration by the expression

$$P_{\text{bound}} (\text{g of bound water/g of water}) = \frac{C(\text{g of sugar/g of water}) \times X(\text{g of bound water/g of water})}{C(\text{g of sugar/g of water}) \times X(\text{g of bound water/g of water})}$$

where C is the solution concentration and X is the amount of water bound to the solute.

Linear behavior was observed before for aqueous whey protein dispersions of low solids content (Padua et al., 1991). However, the slope for the sugar solutions, in this study, was markedly smaller than the slope for whey protein dispersions under the same experimental conditions. This behavior suggests a higher rotational mobility of sugars and their bound water with respect to the hydrated protein. McCain and Markley (1986) measured carbon-13 NMR relaxation times for sucrose solutions and determined that at concentrations below 1 M the solution consists of individual solvated sucrose molecules of a conformation similar to that of the crystal form. The fact that R_{obs} varied with concentration in the same manner for all four sugars, regardless of their individual chemical properties, could be explained on the basis of the hydration model presented by Hills (1991) in which every carbo-

hydrate hydroxyl group binds two molecules of water. According to that model, 1 mol of glucose binds 10 mol of water, whereas 1 mol of sucrose binds 16 mol of water. Thus, sucrose binds only slightly less water (0.84 g/g of sugar) than glucose (1 g/g of sugar). On the other hand, sucrose molecules would have slightly slower rotational motion than glucose due to their larger size. Since at the same concentration the term $R_{\text{free}}P_{\text{free}}$ in eq 1 is the same for all sugar solutions, the combination of the two effects mentioned above would result in very close values of R_{obs} for all sugars. R_{bound} can be calculated from the experimental points, as described by Richardson et al. (1986), using eq 1 and the value for $R_{\text{free}} = 0.4 \text{ s}^{-1}$ taken from Padua et al. (1991). Since $P_{\text{free}} = 1 - P_{\text{bound}}$, then

$$R_{\text{obs}} = P_{\text{bound}}(R_{\text{bound}} - R_{\text{free}}) + R_{\text{free}} \quad (2)$$

The water bound to the sugar (X) was taken as 1 g of bound water/g of sugar; therefore, P_{bound} in grams of bound water per gram of water is numerically equal to the concentration (C) in grams of sugar per gram of water, and $(R_{\text{bound}} - R_{\text{free}})$ can be taken as the slope of the plot R_{obs} against concentration (Figure 1), which has a value of 1.43 (from linear regression analysis). From this result, R_{bound} was estimated as 1.83 s^{-1} . It is apparent that the value for R_{bound} is not very different from the value of R_{free} . These results indicate that the sugar molecule and its bound water exhibit a high degree of mobility with respect to free water.

Although additional relaxation measurements are required for a complete characterization of the system's hydration, the interest of this work was to investigate the usefulness of T_1 measurements to monitor hydration changes in sugar solutions. According to Lioutas et al. (1986), the observed transverse relaxation rate values for the different water populations identified in the lysozyme-water system keep the same relaxation among themselves as the correlation times, which indicates that R_2 may be used as an estimate of water mobility. Richardson et al. (1987) calculated correlation times for bound water molecules in the sucrose-water system based on oxygen-17 NMR transverse relaxation rates and concluded that the water bound to sucrose has a very high mobility. Also, Uedaira et al. (1989) reported that the correlation times

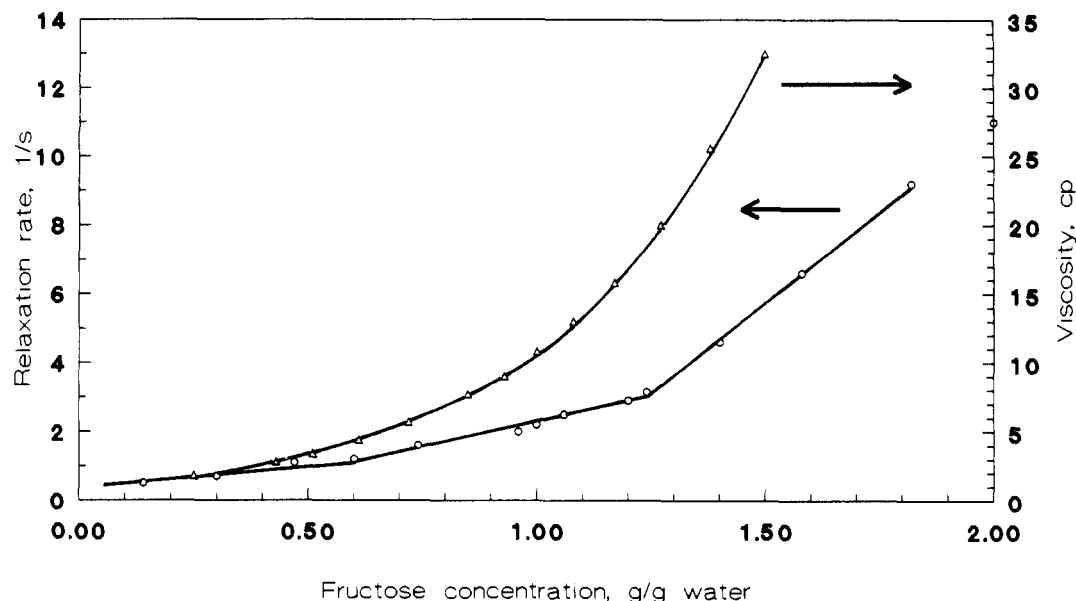


Figure 2. Proton relaxation rate (R_1) and viscosity dependences on concentration for fructose solutions.

of molecular reorientation were nearly the same for fructose, glucose, and sucrose solutions of low-solids content.

Above 0.7 g of sugar/g of water, the dependence of R_{obs} on concentration deviated from the original linear function. For concentrations up to 1.2 g/g of water of fructose and glucose and 1.4 g/g of water of sucrose and lactose, the behavior was fitted with linear segments of higher slope, 4.3 for fructose and glucose solutions and 7.7 for lactose and sucrose. This behavior could reflect, as suggested by Pidoux (1972), the formation of clusters of sugar molecules. The clusters would have a slower rotational motion than the single sugar molecules. For this case

$$R_{obs} = P_{bound}R_{bound} + P_{cluster}R_{cluster} + P_{free}R_{free} \quad (3)$$

where $R_{cluster}$ is the relaxation rate of the water protons in the clusters and $P_{cluster}$ is the mass fraction of the water protons in the clusters. $P_{cluster}$ is a function of P_{bound} , which can be related to solute concentration, and $P_{free} = 1 - (P_{bound} + P_{cluster})$.

Consistent with these results, Schneider et al. (1963) reported that viscosity measurements in sucrose solutions showed larger values than predicted by theory for concentrations above 40%. From those observations, a change in structure was proposed, from hydrated sucrose in water to sugar-water associations (Schliephake, 1965). Also, Yasunaga et al. (1964a,b) found the hydration values for glucose and sucrose to be virtually independent of concentration up to 1.2 M (35%) in sucrose and 2.4 M in glucose, indicating a change in structure of the solution above those concentration levels.

The R_{obs} dependence on concentration (Figure 1) showed a second change in behavior with respect to the original line. For fructose and glucose solutions the change occurred at about 1.2 g/g of water; the function remained linear but with a higher slope of 10.5. This point probably marked the depletion of free water and the formation of highly developed structures within the solution. These structures would have a much slower rotational motion than the single sugar units. On the basis of Zimmerman and Brittin's theory, the model proposed for this case is

$$R_{obs} = P_{bound}R_{bound} + P_{cluster}R_{cluster} + P_{structure}R_{structure} \quad (4)$$

where $R_{structure}$ is the relaxation rate of the water protons in the larger structures and $P_{structure}$ is the mass fraction

of those water protons. Again, $P_{structure}$ is a function of P_{bound} and $P_{cluster} = 1 - (P_{bound} + P_{structure})$.

Hills (1991) reported that the dependence of deuterium-exchange flux on composition for glucose solutions in D_2O changed markedly after 1.2 g of sugar/g of water. He concluded that the change was due to the lack of free water at this concentration level. The present results support this point of view.

Mora-Gutierrez and Baianu (1989) reported T_2 values for sucrose solutions that were significantly shorter than those for glucose and fructose. In turn, T_2 values for glucose solutions were shorter than those for fructose. Our observations followed the same pattern for solutions of concentrations above 0.7 g of solids/g of water.

For sucrose and lactose solutions, the second change of behavior in the dependence of R_{obs} on concentration was observed at about 1.4 g/g of water. Above this point, the shape of the functions was approximated with linear segments. The value of 1.4 g/g of water was considered to mark the depletion of free water for the disaccharide solutions. The concentration at which there was no longer free water was higher for lactose and sucrose than for glucose and fructose solutions. This observation is consistent with the previous conclusion that disaccharides bind less water by weight than monosaccharides, thus leaving more free water available at the same concentration level. An exponential fit was attempted (not shown) which approximated part of the data, in the concentration range 1.1–1.4 g of sugar/g of water, but overall gave a poor fit.

Figure 2 illustrates how the dependence of R_{obs} on concentration showed trends similar to those observed in the dependence of handbook viscosity values (Weast, 1984) on concentration for fructose solutions. The similarity suggests that the increased viscosity is due to aggregation of the sugar molecules into clusters of slower tumbling motion. At higher concentrations, the steep gradient of the curve may be due to the depletion of free water which would act as lubricant between larger structures and would facilitate flow. The similarities between the viscosity and the relaxation curves indicate that low-field R_1 measurements monitor actual physical changes in the structure of the solution.

CONCLUSIONS

The results presented indicate that low-field proton NMR relaxation measurements are a useful methodology to detect changes in the structure of sugar solutions. At low concentrations all four sugars were found to bind nearly the same amount of water by weight. This observation was in agreement with the hydration model that proposes two molecules of bound water for every carbohydrate hydroxyl group. The rotational motion of water was only slightly slowed down by its association with the sugars considered in this study, in contrast with the substantial restriction in mobility reported for the water associated with proteins.

Changes in the dependence of relaxation rate on concentration at about 0.7 g of sugar/g of water suggested structural changes in the solution. The model adopted considers the formation of clusters of sugar molecules which have a rotational motion much slower than the single sugar units. In this context, fructose and glucose clusters showed a faster rotational motion than those formed by sucrose and lactose.

Above approximately 1.2 g of sugar/g of water, a second change in the dependence of relaxation rate on concentration suggested the depletion of free water and the formation of highly developed structures within the solution. According to this model, free water was depleted sooner for the monosaccharides than for the disaccharides, and between 0.7 and about 1.2 g of sugar/g of water there was more free water in sucrose and lactose solutions than in solutions of fructose or glucose. At high concentrations, the NMR relaxation rate for sucrose solutions was considerably larger than for fructose solutions; this could be an indication of a more developed and rigid structure in sucrose than in fructose solutions.

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Registry No. Water, 7732-18-5; sucrose, 57-50-1; lactose, 63-42-3; fructose, 57-48-7; glucose, 50-99-7.